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The Importance of Dispensable Amino Acids in Protein Nutrition

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Since the classic work of Rose and co-workers in the middle of the last century (Rose, 1938) amino acids have been classified as Dispensable and Indispensable or alternately, Essential and Non-essential. During the last couple of decades it has become apparent that there is not a clear distinction in these categories. Indeed, Rose himself predicted this when he defined arginine as an essential amino acid for the rat and dispensable for humans (Borman et al, 1946):

> "The classification of arginine as dispensable or indispensable is purely a matter of definition. At the present time, we define an indispensable dietary component as one which cannot be synthesized by the animal organism, out of materials *ordinarily available* to the cells (Cox and Rose (2)), at a speed commensurate with the demands for *normal* growth. Under this definition, arginine must be classified as indispensable, although it alone of its group may be excluded from the food without occasioning a *loss* in weight. As additional information comes to light concerning the role of the amino acids in other functions such as reproduction and detoxication, redefinition of the term "indispensable" may become necessary. As currently used in this laboratory it refers to growth alone. Furthermore, one must always denote the species in classifying a dietary component as dispensable or indispensable since important species differences have already been recognized and doubtless are destined to become more numerous."

Metabolically, why are some amino acids essential and others dispensable? When one examines the biosynthesis and role of the essential and dispensable amino acids it is apparent that organisms (the plant kingdom) that synthesize the essential amino acids require 6~10 enzymes (there is some overlap so the total number of enzymes required to synthesize the 9 essential amino acids is 58) to accomplish this purpose while for most dispensable amino acids only one is necessary (the exceptions are: three enzymes for serine synthesis and two for cysteine synthesis) from metabolites present in the cell during intermediary metabolism. Since all animals, from single cell animals to the largest of the whales and elephants, require the same 9 essential amino acids (and most also require arginine) it is apparent that there is a metabolic advantage for animals not to have to have

all of the enzymes necessary to synthesize the 10 essential amino acids. Alternately, it has been suggested that the dispensable amino acids are so important and quantity (flux) needed was so high in the intermediary metabolism of nitrogen compounds that sustaining of life is dependent on the continuous flow of these amino acids. Probably, both suggestions are true. For example, the flux of nitrogen through glutamine is high to provide all of the nitrogen compounds needed for cell replication and nitrogen detoxification that its biosynthesis is essential to life. Indeed, the regulation of glutamine biosynthesis is allosterically regulated by a large number of different metabolic end products.

The amino acids present in most proteins that are essential in all animals are:

Leucine	Lysine	Phenylalanine
Isoleucine	Histidine	Tryptophan
Valine	Threonine	Methionine

Arginine is essential in most animals, but dispensable in most primates (including humans) and ruminants.

The amino acids present in most proteins that are dispensable in most animals are:

Glutamate	Glutamine	Proline	Alanine
Aspartate	Asparagine	Cysteine	
Glycine	Serine	Tyrosine	

It is important to note that cysteine and tyrosine are "unique" dispensable amino acids since they can only be made from the essential amino acids methionine and phenylalanine, respectively, and cannot be made in an animal from ammonia and intermediary metabolism products apart from those of the parent essential amino acids. Nevertheless, both are needed for protein synthesis and several other metabolic needs. Thus, it is appropriate to say, cysteine spares methionine and tyrosine spares phenylalanine, but it should never be said that methionine spares cysteine or phenylalanine spares tyrosine since there is no dietary requirement of cysteine or tyrosine in the normal animal. Indeed, there is an obligatory synthesis of cysteine and tyrosine in the catabolism of methionine and phenylalanine. Both of these dispensable amino acids can thus be considered conditionally essential if their parent essential amino acids are limiting. In addition, there are conditions such as parenteral nutrition and the premature infant when they also become conditionally essential.

Of the other 8 dispensable amino acids, glycine / serine is essential in birds, reptiles and a number of other animals, but neither of these amino acids has been reported to be essential in mammals. Alanine is a central metabolite in carrying nitrogen to the liver from the intestine, kidney and periphery, but it is easily synthesized from glutamate in virtually

all tissues and has never been shown to be "conditionally essential" . It is an appropriate and "safe" amino acid to use for the nitrogen need for any animal and no toxicity, even at very high dietary concentrations, has ever been reported. Although glutamate and aspartate are intimately involved in a lot of intermediary metabolism of nitrogen, they are not necessary in the diet, and indeed excesses of either of these amino acids in the diet cause neurological problems, probably because at high concentrations they chelate calcium and thus interfere with proper neurotransmission. The adverse neuro-effect of excess dietary glutamate is commonly referred to as the "Chinese Restaurant Syndrome" . Proline is known to spare arginine in the rat and a number of species and will be discussed below with glutamine and arginine. Asparagine is not involved in many reactions but is required for protein synthesis. Its synthesis is much more limited than that of glutamine, glutamate and aspartate. Although the response in plasma to the deletion of dietary asparagine is similar to that of an essential amino acid, the only report of it being conditionally essential is that of small growth responses which have been reported in early weaned rats to dietary asparagine (Breuer et al, 1964; Rogers and Harper, 1965; Rogers et al., 1970).

At the organismal level, dietary protein is required for two reasons: 1) to provide the essential amino acids that they cannot synthesize, and 2) to provide the nitrogen to make all of the other nitrogenous compounds necessary for life such as the dispensable amino acids, purines, pyrimidines, heme, creatine, etc. At the cellular level, all of the amino acids must be present at the same time for protein synthesis, thus metabolically, all of the amino acids are essential. Only certain tissues are capable of specific steps in the synthesis of certain amino acids and / or in their catabolism, thus malfunctioning of these tissues or excessive production of catabolic hormones (e.g., certain cytokines) caused by severe trauma or sepsis causes a disruption of amino acid metabolism which may result in a need for an exogenous source of a given amino acid that is normally dispensable. The requirement of the amino acid under these conditions for that particular amino acid is called a "conditionally essential" amino acid. Thus, in renal disease the kidney is incapable of convertting citrulline to arginine so arginine becomes "conditionally essential" . Likewise, in phenylketonuria, tyrosine is conditionally essential because without phenylalanine hydroxylase, phenylalanine cannot be converted to tyrosine so tyrosine becomes a dietary essential. Under severe stress, glutamine is "conditionally essential" to maintain optimal anabolic reactions where glutamine is involved in cell replication (Lacey and Wilmore, 1990; Ziegler et al., 2000). A growing number of conditions are being reported in which dispensable amino acids become "conditionally essential" (see Table 1 for a limited list).

Before considering more fully the dispensability of tyrosine and arginine, it is important to point out that historically in most animals, including humans, amino acid requirements have been determined in the young using weight gain and/or nitrogen retention, the latter

Table 1. Conditionally Essential Amino Acids in Animals or Humans

Amino Acid	Condition
Arginine	Kidney or Liver Disease, Premature infant, Parenteral Nutrition Burned Patients
Asparagine	Early weaning
Cysteine	Premature infant, Cystathionine Synthase Deficiency (when methionine is limiting)
Glutamine	Severe Stress, Sepsis, Bone Marrow Transplants, Chemotherapy
Tyrosine	Premature Infant, Phenylketonuria (when phenylalanine is limiting)
Proline	Spares arginine when arginine is limiting

generally being considered the gold standard for the dependent variable. However, as an understanding of intermediary metabolism has developed, more metabolic variables have become important and indeed have often been more sensitive indicators of the quantitative need of the organism for the exogenous source of the essential amino acids than nitrogen balance. Thus, adequate choline synthesis (to prevent fatty liver) and optimal hair growth from cysteine are important variables for determining methionine/total sulfur amino acid requirements while prevention of anemia (optimal hemoglobin synthesis) is an important outcome of providing adequate histidine. When there are needs secondary to protein synthesis for growth, most often more of the amino acid is needed to optimize the secondary need than for protein synthesis (i.e., protein synthesis has the highest priority). This occurs because the Km for the amino acids of the amino acyl synthases for the charging of the tRNAs are considerably lower (usually 10^{-5} 10^{-6} molar) as compared to the Kms for the same amino acids for the catabolic enzymes or for other enzymes involved in the biosynthesis of other compounds (usually 10^{-4}–10^{-3} molar, Rogers, 1976). For example, if there is no niacin in the diet, most animals can make plenty from tryptophan. However, if there is not enough niacin in the diet and trytophan is limiting, then the animal gets pellagra (metabolically, a niacin deficiency) rather than exhibiting the serotoninergic syndrome. Metabolically this results from a lack of serotonin synthesis when sufficient niacin is present in the diet and tryptophan is inadequate. In each situation, the tryptophan concentration is dropped below that of the capability of the enzyme system to make niacin or serotonin because protein synthesis proceeds to the point of depleting plasma and tissue tryptophan below the concentration that allows an adequate rate of synthesis of niacin or serotonin.

Abnormal physiological conditions often increase the anabolic need for certain dispensable amino acids as well as increase the catabolism of the same amino acid, thus causing a metabolic deficiency which may be partially provided by providing an exogenous source of the amino acid, thus making it conditionally essential. Such conditions are usually extreme, causing a number of cytokines to be released contributing to the extreme metabolic cata-

bolic state. These conditions include severe trauma, sepsis, burns, etc. As might be expected, amino acids central to intermediary anabolic (e.g., glutamine) and regulatory (e.g., arginine) processes are the amino acids most commonly involved. Deficiencies of other dispensable amino acids may cause observable effects, but less important to the health of the animal (e.g., cysteine deficiency causing poor hair growth or tyrosine deficiency causing low melanin synthesis with the resulting bleaching or reddening of black hair) while deficiencies of other normally dispensable amino acids may become life-threatening (hyperammonemia resulting from arginine deficiency in premature infants, during parenteral nutrition or in liver disease). Below I will discuss in more detail the clinical consequences and quantitative differences in the requirement of arginine and tyrosine for optimal growth (protein synthesis) versus that required for non-protein synthetic functions. Since a conference on the nutritional importance of glutamine has recently been published (Wilmore and Rombeau, 2001), this topic will not be discussed further in this paper.

Arginine: Arginine is present in proteins and has an important function in providing a strongly cationic side chain for the secondary structure of proteins. In addition, arginine is an important intermediate in the synthesis of urea and provides allosteric activation of acetylglutamate synthase which accelerated the synthesis of acetylglutamate which in turn is an activator of carbamyl phosphate synthase, the first enzyme involved in the sythesis of urea from ammonia. It has been reported that in a variety of disease states that arginine supplementation improves the immune response, reduces bacterial translocation across the intestinal mucosa, enhances wound healing, reduces the adverse effect of various types of severe trauma and influences other endocrine activity. One common mechanism for many of these effects is arginine as a substrate for the synthesis of nitric oxide, a compound known for enhancing the killing of bacteria in macrophages as well as being a neuro-active compound, among other things causing vasodilatation.

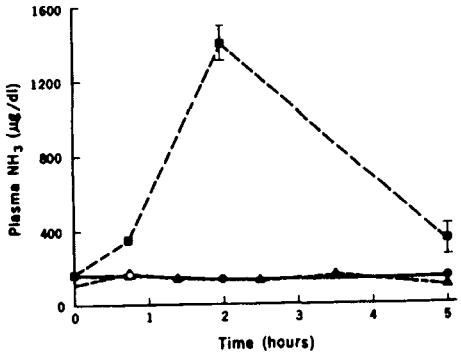

Figure 1. Plasma ammonia of adult cats fed a single meal of an arginine-free diet. Triangles = + orn circles = + arg (Taken from Morris and Rogers, 1978).

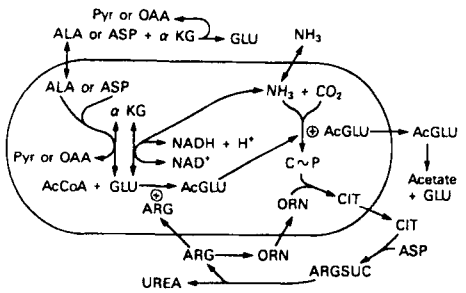

Figure 2. Regulation of urea synthesis.

The effect of arginine deficiency on ammonia detoxification is most easily seen in animals that cannot readily synthesize arginine. In the domestic cat, the ingestion of an arginine-deficiency diet causes such severe hyperammonemia (Figure 1) that it is life-threatening (Morris and Rogers, 1978a, 1978b). This negates the often suggested idea that the presence of the urea cycle is evidence for the dietary dispensability of arginine. As the requirements across various animal species are examined, birds and fish show the highest requirements. Fish, especially carnivorous fish, appear to have a high requirement of all of the essential amino acids (2.4 % arginine in the diet for Salmon), perhaps to support the needed gluconeogenesis, whereas for chicks and poults, the higher the growth rate the higher the arginine requirement (0.8 vs. 1.8 % of diet for low and high requirement chicks, respectively, Hutt and Nesheim, 1966). Also, in rats (Milner and Visek, 1978) and cats (Taylor et al., 1997) it has been shown that the arginine requirement increases (10~20 μ) with increasing dietary protein.

Nearly all mammals require dietary arginine despite the presence of the urea cycle, thus the various laboratory mammals that have been examined (rat, guinea pig, rabbit etc.) all have a dietary requirement for arginine. The mouse may be an exception since Bauer and Berg (1943) and Bell and John (1981) reported that the mouse does not need dietary arginine to achieve optimal growth. However, Milner et al (1975), using an amino acid diet have shown that mice fed an arginine-free diet do not grow maximally and exhibited orotic aciduria. The difference may lie in the difference in the dispensable amino acid composition of the diets since glutamic acid and proline spare arginine in some species (Rogers et al, 1970; Austic, 1976). Several herbivores are known to have a relatively high arginine requirement (e.g., rabbit) whereas others require none (e.g., lamb). There is no evidence that indicates that ruminants (even young suckling ruminants) require dietary arginine, and even though the rate of biosynthesis has not been examined, the circulating level of citrulline in plasma is high even in the pre-ruminant lamb which has been shown to grow well without any dietary arginine (Morris and Rogers, 1987). Neither infants nor adult humans require dietary arginine (Snyderman, 1959; Carey et al, 1987), although it is considered "conditionally essential" because it is needed in certain disease states (e.g., renal disease)(Laidlaw & Kopple, 1987; Visek, 1984,1986) and when total parenteral nutrition is used (Fahey, 1957,1958).

In order to understand the metabolic basis of the arginine requirements among various species it is important to understand the functioning of the urea cycle and where the various enzymes that are involved are located (Fig. 2). The liver is the only organ that contains high levels of all of the urea cycle enzymes. In fact, arginase is so high in the liver that the concentration of arginine in liver is only about 5~25 percent of that in the systemic circulation. Since the arginine transport system functions to transport arginine

into the liver across a concentration gradient, any arginine made cannot get out of the liver but is either used within the liver or is continually converted to urea. The K_m of arginine aminoacyl synthase is about 10^{-6} molar so even though the concentration is in the range of 10~20 molar, the enzyme is still nearly saturated, so protein synthesis in the liver is maintained. Also, citrulline is trapped inside the cell so when it increases, argininosuccinate synthase, which is the limiting enzyme when carbamylphosphate synthase is fully activated, can increase in activity, thereby increasing the rate of urea synthesis. Normally, the rate of urea synthesis is limited by carbamylphosphate synthase activity which is not fully activated because of the low concentration of acetylglutamate, an absolute allosteric activator which is continually diffusing out of the mitochondria and being degraded by a cytoplasmic acetylase. Acetylglutamate concentration is dependent upon the concentration of glutamic acid and the activity of acetylglutamate synthase which in turn is allosterically activated by arginine. Adequate exogenous arginine (or ornithine directly) also is necessary to provide adequate ornithine via the high hepatic arginase, to provide adequate urea cycle intermediates to sustain an optimal rate of synthesis of citrulline. Thus, exogenous arginine, citrulline, or ornithine act anaplerotically to stimulate urea synthesis. An influx of ammonia not only acts as a substrate for carbamylphosphate synthase, but if present in excess also increases glutamic acid since glutamic acid dehydrogenase is a very active enzyme in liver and is reported to be at equilibrium (Williamson et al, 1967). When ammonia is in excess, argininosuccinic acid synthase becomes the limiting enzyme so citrulline accumulates and eventually carbamylphosphate builds up inside the mitochondria to the point it leaks out. An increase in carbamylphosphate in the cytoplasm causes an increase in orotic acid synthesis because the normal feedback inhibition of cytoplasmic carbamylphosphate synthase II is bypassed. The increased orotic acid is free to diffuse out of the liver and eventually ends up in the urine. Although this urinary orotic acid is a good indicator of the limitation of the urea cycle and is a metabolic indicator of an arginine deficiency, the total quantity of nitrogen excreted as orotate is negligible.

When arginine is left out of the diet of the young rat orotate increases in the urine 40~50 fold and although weight gain is somewhat improved with added proline or glutamic acid the quantity of urinary orotate is not decreased. The severity of the orotic aciduria decreases with age (Milner and Visek, 1973), since the demand for arginine decreases as growth decreases toward adulthood and arginine can then be used for ornithine synthesis in the liver. Thus, using urinary orotic acid as the key variable, arginine would be indispensable in the young rat and dispensable in the adult rat.

It has often been assumed that arginine is dispensable, based on the presence of a functioning urea cycle. We found nearly 30 years ago when we tried use ^{14}C-citrulline to label some liver proteins with ^{14}C-arginine that dietary citrulline did not act as a good

precursor for arginine for hepatic proteins, whereas citrulline served as a good precursor for other body proteins, with the kidney having an especially high specific activity (Rogers et al, 1972). This led to a series of experiments (Featherston et al, 1973) which clearly showed that the kidney was the primary organ that made arginine from citrulline in the rat.

Table 2. Synthesis of ornithine, citrulline and proline by the small intestine[1]

	^{14}C-Glu	^{14}C-Gln
Product	% of total	% of total
CO_2	64	55
Pro	4.1	5.8
Cit	3.2	4.4
Ala	3.2	4.0
Orn	1.1	2.1

[1]Adapted from Windmueller and Spaeth (1975)

It was the elegant work of Windmueller and coworkers, however, that showed that it was the intestine that made ornithine, proline, and citrulline de novo, using glutamate (or in vivo, primarily glutamine, Windmueller and Spaeth, 1975). Indeed, they showed that glutamine is a major energy source for the small intestine and that during the metabolism of glutamine significant amounts of ornithine, proline and citrulline are formed (Table 2). The enzymic basis of these findings was provided in 1983, when Wakabayashi and Jones demonstrated that pyrroline-5-carboxylate (P-5-C) synthase, a mitochondrial enzyme complex that phosphorylates the -carboxyl of glutamic acid, then using NADPH reduces the -glutamyl phosphate to -glutamic semialdehyde(GSA), releasing inorganic phosphate. The GSA cyclizes nonenzymatically, forming water and P-5-C, which is quite stable (Fig 3). P-5-C can then be reduced to form proline or GSA can be transaminated to ornithine, which can be further converted to citrulline, all within the small intestinal mucosal mitochondria. It appears that only in intestinal mitochondria are these enzymes all present at high enough levels to form enough ornithine and citrulline to allow them to escape for use by the rest of the body. The equilibrium constant for ornithine transamination greatly favors the formation of GSA, so only in the intestine, where P-5-C dehydrogenase is absent, can P-5-C build up to sufficient concentrations to allow conversion to ornithine. In other tissues, especially in the liver and kidney, ornithine transaminase is high, but P-5-C dehydrogenase is also high, resulting in the conversion of P-5-C to glutamic acid. The limited activity of ornithine transcarbamylase in the intestine results in some ornithine being left over (and therefore some net synthesis), but also means that dietary ornithine cannot increase the synthesis of citrulline and therefore ornithine does not improve growth when given to an arginine deficient animal.

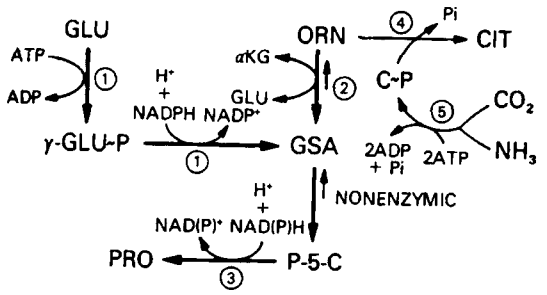

Figure 3. Pathway of synthesis of proline, ornithine and citrulline by intestinal mucosa: (1) P-5-C synthase; (2) ornithine delta amino transferase; (3) P-5-C reductase; (4) ornithine transcarbamylase; (5) carbamyl phosphate synthase I (Taken from Rogers and Phang, 1985)

Figure 4. Photograph of an 18 hour food-deprived cat after feeding one meal of an arginine-free diet.

Exogenous ornithine can, however, during an arginine deficiency, be used by the liver to accept carbamyl phosphate and help maximize urea synthesis (and thereby prevent the hyperamonemia of arginine deficiency). In animals proline spares arginine, not by providing an intermediate of arginine synthesis, but by preventing the need for proline synthesis from P-5-C which originated from the ornithine which originally came from dietary arginine. Thus, dietary ornithine will not spare arginine, whereas dietary citrulline will. The kidney converts the circulating citrulline to arginine for itself and the rest of the body (Windmueller and Spaeth, 1981) and it has been shown that dietary citrulline will substitute for dietary arginine in all species that require arginine. Furthermore, if the synthesis of citulline is inhibited in the intestine of rats, growth is severely retarded (Hoogenraad, et al, 1985).

The nutritional interactions among arginine, proline and glutamic acid in the rat are illustrated in Table 3. Note that maximal weight gain can be achieved without any proline (gp 6) or glutamic acid (gp 7) if arginine and other dispensable amino acids are high enough, whereas even if proline and glutamic acid are very high, a minimum of about 0.25% dietary arginine is required for maximal growth. It takes about one percent dietary arginine in the diet to support maximal growth if proline and glutamic acid are not present at high levels (Rogers and Harper, 1965). Whether or not mammals require dietary arginine appears to depend on the activity of P-5-C synthase (Jones, 1985; Rogers and Phang, 1985) in the intestine. In most species the citrulline forming enzymes in the small intestine appears to be constitutive. However, in the mouse (which can just about make enough arginine for maximal growth if enough other dispensable amino acids are provided, Bell and

John, 1981), Riby et al (1990) and Hurwitz and Kretchmer (1986) have shown that these enzymes go up at birth and then decrease with age toward adulthood as the need for arginine diminishes. Proline is known to be essential in the chick (Graber et al, 1970) and, on a biochemical basis, it has been suggested that it is essential in the piglet (Ball et al. 1986), but the nutritional basis for its essentiality has not been clearly shown.

Table 3. MINIMUM REQUIREMENT OF ARGININE, PROLINE AND GLUTAMIC ACID FOR THE GROWING RAT[1]

	Dietary amino acid (% of diet)			
Group	Arg	Pro	Glu	Wt gain g/2 wk
1	.16	2.0	8.0	79.9 ± 3.1
2	.25	2.0	8.0	93.3 ± 1.2
3	.33	2.0	8.0	95.0 ± 2.1
4	.41	2.0	8.0	93.8 ± 2.7
5	0.5	2.0	5.5	95.8 ± 3.3
6	1.7	0	8.0	99.0 ± 2.1
7[2]	1.7	2.0	0	98.1 ± 2.6

[1] Adapted from Rogers et al. (1970) [2] other dispensable amino acids increased

Arginine deficiency in carnivores causes severe clinical signs of ammonia toxicity which can be life-threatening (Morris and Rogers, 1978ab). In the cat the clinical signs of an arginine deficiency include emesis, tetanic spasms, sialorrhea (Figure 4), depression, ataxia, hyperesthesia, dyspnea and sometimes death. No other nutrient deficiency is so life threatening within two-four hours of first ingesting a deficient diet. The hyperammonemia seen in cats fed an arginine deficient diet is illustrated in Figure 1. Note that either arginine or ornithine prevents the hyperammonemia. Metabolically, the cats had hyperglycemia, low plasma ornithine, citrulline, and arginine (Morris and Rogers, 1978b). It was clear that there was little if any arginine or ornithine synthesis de novo in the cat. It appears probable that all the enzymes involved in the synthesis of citrulline in the small intestine are very low. Rogers and Phang (1985) and Costello et al (1981) (Table 4) have shown that both P-5-C synthase and ornithine δ-amino transferase are only about 4~5 percent as high (per kg body weight) in the cat as that found in the rat and as mentioned earlier the rat does not make enough arginine for maximal growth even if large amounts of glutamate and proline are provided in the diet. The cat, similar to other species, can utilize citrulline, but not ornithine for growth (Morris et al, 1979). As might be expected from work done in other mammals, urinary orotic acid excretion increases when cats are given an arginine deficient diet. It appears to take more dietary arginine to minimize urinary orotic acid excretion in the growing kitten than to maximize growth (Costello et al, 1980). Also it appears that although citrulline may replace dietary arginine it is not as efficiently utilized.

Other carnivores also show rather severe clinical signs of arginine deficiency. The young ferret shows more severe hyperammonemia, but it does not appear to show as severe clinical signs (Deshmukh and Shope, 1983; Thomas and Deshmukha, 1986). The puppy also shows mild clinical signs (sialorrhea) of hyperammonemia when given an arginine deficient diet (Ha et al, 1978).

Although published reports on nitrogen retention in infants, children and adults (Snyderman et al,1959; Nakagawa et al, 1963; Rose et al, 1954) support the view that arginine is a dispensable amino acid for humans, the lack of metabolic evidence that hyperammonemia or orotic aciduria did not occur when humans were fed an arginine-free diet was lacking until Carey et al (1987) published results showing that neither hyperammonemia nor orotic aciduria occurred when normal adults were fed a low or high protein diet containing no arginine (Figure 5). Nevertheless, when amino acids are administered in a peripheral vein arginine is required to prevent hyperammonemia (Fahey, 1957). Visek and Shoemaker (1986) have reported an abnormally high excretion of orotic acid in the urine of patients with hepatic cirrhosis which was responsive to alcohol detoxification, thus it is apparent that under certain physiological or disease states, there may be a role for dietary arginine, even in humans (Laidlaw and Kopple, 1987, and Visek, 1984 and Visek and Shoemaker, 1986).

Table 4. Activities of P5C synthase[1] and ornithine delta amino transferase[2] in the rat and cat mucosa

	Rat	Cat	Cat/Rat x100
P5CS[1]			
nmols/min/g tissue	2.9	4.2	18.3
nmols/min/kg BW	308	16	5.1
OAT[2]			
μmols/min/g tissue	1.9	0.3	15.8
μmols/min/kg BW	25.6	1.1	4.5

[1] Rogers and Phang (1985)
[2] Costello et al., (1981)

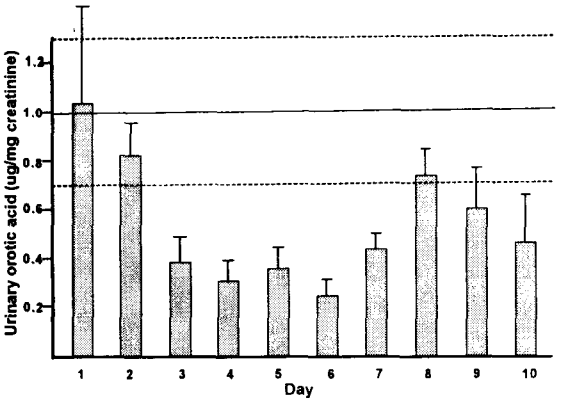

Figure 5. Effect of ingestion of an arginine-free diet in adult humans on urinary orotic acid excretion. Days 3-5, low protein; Days 6-8, high protein. (Taken from Carey et al., 1987)

Tyrosine: Tyrosine is present in proteins and among its important functions is providing the substrate for crosslinking peptides in collagen. In addition tyrosine provides the sub- strate for the catecholamines and for melanin synthesis. As mentioned earlier, tyrosine spares phenylalanine so is an important dispensable amino acid. The content of phenyla- lanine and tyrosine is similar in most proteins so if tyrosine is not present in the diet, about twice as much phenylalanine is required than if they are in equal concentrations. When more than enough tyrosine is present in the diet to meet the tyrosine needs of the growing kitten for protein synthesis (maximal growth), and the phenylalanine is marginally

limiting, plasma phenylalanine concentrations is about 15~20 nmols/mL. If no tyrosine is present in the diet and phenylalanine is marginally limiting, plasma phenylalanine is about 60~70 nmols / mL. Tyrosine concentrations for these two dietary treatments are about 40~50 and 10~15 nmols / mL, respectively (Williams et al., 1987). These results indicate that tyrosine is metabolically limiting when none or considerably less is present in the diet than phenylalanine, whereas, phenylalanine is metabolically limiting when plenty of tyrosine is present in the diet but not enough phenylalanine is present for maximal growth. Nevertheless, if enough phenylalanine is present in the diet no dietary tyrosine is required. Recently, it was discovered that a metabolic deficiency of tyrosine, even when enough phe/tyr was present to sustain maximal growth, would cause a graying or reddening of black hair in the cat (Yu et al., 2001). Reddening of black hair has been reported in cats and dogs sporadically for many years but despite the knowledge of a variety of environmental and nutritional factors that cause this effect no nutritional cause had previously been identified. Since no previous nutritional factor had been implicated the role of diet in this phenomena has been called a myth (Case et al., 2000). Indeed, sunlight, certain insecticides and other factors, including copper or lysine deficiency has been implicated in graying or reddening of black hair in a variety of species. Seasonally induced hair growth and these various factors complicated studies on the reddening of black hair for many years. The effect of diet was clarified when Yu et al. (2001) found reproducible gray or reddish hair after inadvertently feeding cats with black hair a gelatin-based purified diet sub-clinically deficient in tyrosine. After the diet was shown to be low in tyrosine (shown diagnostically to be deficient by post prandial plasma tyrosine of about 12 nmol/mL (about 1/5 normal) further work showed that although these diets met the NRC Requirements for all known nutrient requirements of the cat (NRC, 1986),including phenylalanine plus tyrosine and supported maximal growth, metabolically tyrosine limited the synthesis of eumelanin, the black melanin, and replaced it with phaeomelanin, the yellowish-red-brown melanin. The most surprising finding (Yu et al., 2001) was that the quantity of phenylalanine plus tyrosine required to maximize the black hair color was about twice that required for maximal nitrogen retention (1.6 % vs 0.8 %, respectively). No other report of such a large increase in the quantity of an essential amino acid to meet a secondary metabolic need over that required for maximal nitrogen retention (maximal protein synthesis) is known.

Optimizing dietary Dispensable Amino acids: The optimal ratio of essential amino acids to total nitrogen has been of interest to nutritionists since the last essential amino acid was discovered. The optimal ratio (or range) involves both meeting the essential amino acid needs and the nitrogen needs. One question this relates to is after the essential amino acid needs are met, what pattern of amino acids is both fully adequate and yet non-toxic at various levels of dietary nitrogen (Taylor et al., 1996; Rogers et al., 1998). Each amino acid becomes aversive (toxic) if enough is fed, the toxic level differing from

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2	.25	2.0	8.0	93.3 ± 1.2
3	.33	2.0	8.0	95.0 ± 2.1
4	.41	2.0	8.0	93.8 ± 2.7
5	0.5	2.0	5.5	95.8 ± 3.3
6	1.7	0	8.0	99.0 ± 2.1
7[2]	1.7	2.0	0	98.1 ± 2.6

[1] Adapted from Rogers et al. (1970) [2] other dispensable amino acids increased

Arginine deficiency in carnivores causes severe clinical signs of ammonia toxicity which can be life-threatening (Morris and Rogers, 1978ab). In the cat the clinical signs of an arginine deficiency include emesis, tetanic spasms, sialorrhea (Figure 4), depression, ataxia, hyperesthesia, dyspnea and sometimes death. No other nutrient deficiency is so life threatening within two-four hours of first ingesting a deficient diet. The hyperammonemia seen in cats fed an arginine deficient diet is illustrated in Figure 1. Note that either arginine or ornithine prevents the hyperammonemia. Metabolically, the cats had hyperglycemia, low plasma ornithine, citrulline, and arginine (Morris and Rogers, 1978b). It was clear that there was little if any arginine or ornithine synthesis de novo in the cat. It appears probable that all the enzymes involved in the synthesis of citrulline in the small intestine are very low. Rogers and Phang (1985) and Costello et al (1981) (Table 4) have shown that both P-5-C synthase and ornithine -amino transferase are only about 4-5 percent as high (per kg body weight) in the cat as that found in the rat and as mentioned earlier the rat does not make enough arginine for maximal growth even if large amounts of glutamate and proline are provided in the diet. The cat, similar to other species, can utilize citrulline, but not ornithine for growth (Morris et al, 1979). As might be expected from work done in other mammals, urinary orotic acid excretion increases when cats are given an arginine deficient diet. It appears to take more dietary arginine to minimize urinary orotic acid excretion in the growing kitten than to maximize growth (Costello et al, 1980). Also it appears that although citrulline may replace dietary arginine it is not as efficiently utilized.

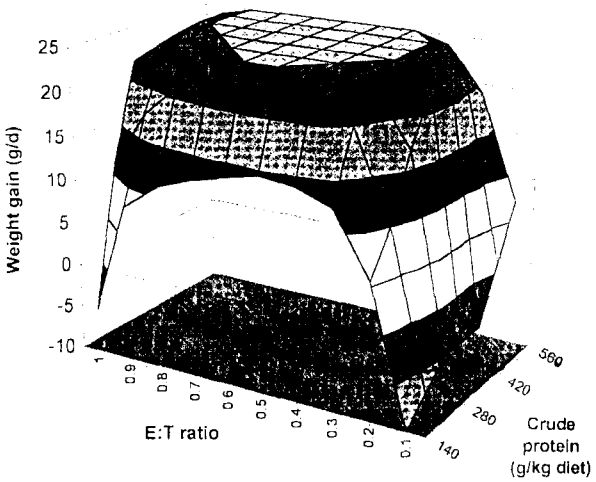

Figure 6. Effect of dietary crude protein and E:T ratio On weight gain of kittens (Redrawn from data of Taylor et al., 1998).

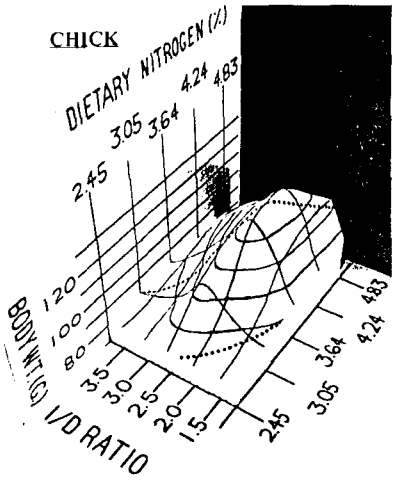

Figure 7. Effect of dietary nitrogen (crude protein) and indispensable:dispensable amino acid ratio (I/D) on weight gain in chicks (From Stucki & Harper, 1961).

As the level of dietary protein increases, the need for arginine increases. As crude protein increases in the diet it is important to limit proteins that may provide excess methionine. During clinically abnormal conditions requiring enteral or parenteral nutrition (e.g., severe stress, sepsis, trauma, premature birth, renal or liver disease) particular attention needs to be given to providing certain the dispensable amino acids, especially glutamine and arginine (and possibly cysteine and tyrosine). Clinical research is needed and indeed some is underway to provide the needed information for optimal amino acid nutrition for various life stages and recovery from a number of disease states.

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