

THE UNIVERSITY OF OKLAHOMA

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PHYSIOLOGICAL CONSEQUENCES OF EXCESS AMINO ACID INTAKE:

A REVIEW

A THESIS

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in partial fulfillment of the requirements for the

degree of

MASTER OF SCIENCE

By

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Norman, Oklahoma

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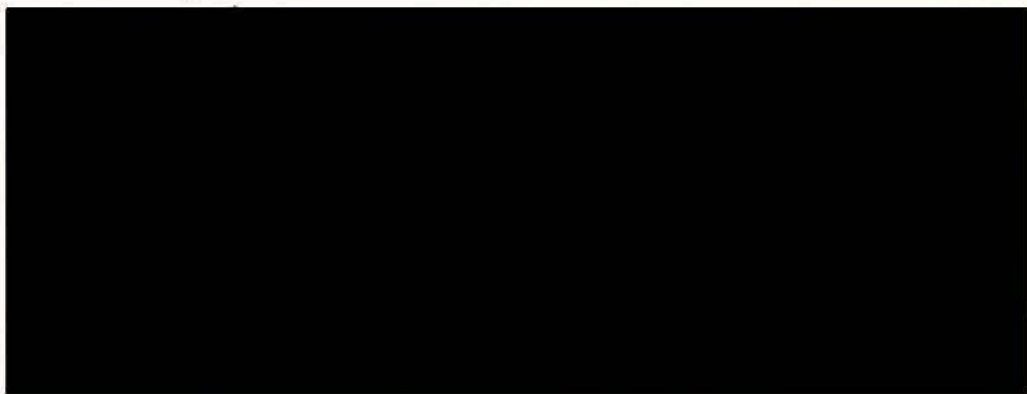
APPROVED FOR THE SCHOOL OF HUMAN DEVELOPMENT

I wish to express my appreciation to my entire committee, Dr. Matthew E. Kraynak, Dr. Allen W. Knehan, and Professor Harriet B. Turkington for their assistance during the development of this thesis. Special gratitude goes to Dr. Kraynak, as my thesis director, for his undying dedication and meticulous concern over this project. He has been a great inspiration to me, both academically and personally, throughout my graduate studies.

Additional thanks to Dr. Allen Knehan for encouraging me to complete this degree when my thoughts strayed to other endeavors and for serving as one of the finest professors I have encountered.

Finally, greatest appreciation to my children, Melissa and Jeffrey, for the sacrifices they made so that now could finish school, and to their father, Mark, for his continuing support to my many unpredictable endeavors.

By



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PHYSIOLOGICAL CONSEQUENCES OF EXCESS AMINO ACID INTAKE:

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maintenance, and repair of animal tissues and for numerous other metabolic functions, such as organic catalysts (enzymes), structural components of cells, messengers (peptide hormones), and antibodies. Proteins are formed by various combinations of twenty-two different amino acids. Of these twenty-two amino acids, eight are considered nutritionally essential in man since they cannot be synthesized in the body and must be supplied through the diet. Twelve of the amino acids are considered nutritionally non-essential and can be synthesized in the body providing the essential amino acids are present in sufficient quantities. Two amino acids are considered nutritionally semi-essential meaning they are not synthesized in sufficient quantities to support the growth of children and must be supplied in the diet; however, these amino acids are considered non-essential in adults (see table 1).

Since amino acids are food items and are involved in so many metabolic functions in the body, it has become popular to regard them as

"natural" and "safe" dietary supplements for use in various therapeutic measures.

PHYSIOLOGICAL CONSEQUENCES OF EXCESS AMINO ACID INTAKE:
CLASSIFICATION OF DIETARY AMINO ACIDS
A REVIEW

Essential	Non-essential	Semi-essential
-----------	---------------	----------------

Isoleucine	Alanine	Arginine
Leucine	Asparagine	Histidine
Lysine	Aspartic Acid	
Methionine	Cysteine	
Phenylalanine	Glutamic Acid	
Threonine	Glycine	
Tryptophan	Proline	
Valine	Tyrosine	

INTRODUCTION

Dietary proteins supply the amino acids required for growth, maintenance, and repair of animal tissues and for numerous other metabolic functions, such as organic catalysts (enzymes), structural components of cells, messengers (peptide hormones), and antibodies. Proteins are formed by various combinations of twenty-two different amino acids. Of these twenty-two amino acids, eight are considered nutritionally essential in man since they cannot be synthesized in the body and must be supplied through the diet. Twelve of the amino acids are considered nutritionally non-essential and can be synthesized in the body providing the essential amino acids are present in sufficient quantities. Two amino acids are considered nutritionally semi-essential meaning they are not synthesized in sufficient quantities to support the growth of children and must be supplied in the diet; however, these amino acids are considered non-essential in adults (see table 1).

Since amino acids are food items and are involved in so many metabolic functions in the body, it has become popular to regard them as

"natural" and "safe" dietary supplements for use in various therapeutic effects in animals as a direct consequence of dietary amino acid measures.

supplementation.

TABLE 1
CLASSIFICATION OF DIETARY AMINO ACIDS

Essential	Non-essential	Semi-essential
Isoleucine	Alanine	Arginine
Leucine	Asparagine	Histidine
Lysine	Aspartic Acid	
Methionine	Cysteine	
Phenylalanine	Cystine	
Threonine	Glutamic Acid	
Tryptophan	Glutamine	
Valine	Glycine	
	Hydroxyproline	
	Proline	
	Serine	
	Tyrosine	
	Ornithine	

(Taken from Ref. 1.)

The amino acids currently being promoted for supplemental use and reviewed in this thesis are: arginine, lysine, tyrosine, tryptophan, histidine, phenylalanine, glutamine, valine, leucine, isoleucine, cysteine, methionine, threonine, and ornithine.

Following a thorough search of the scientific literature concerning amino acid supplementation and the use of amino acids for therapeutic purposes, it has been concluded that the promoters of amino acid supplementation are advocating their products as very simplistic "cures" for very complex problems. Indeed, there is ample

well-documented scientific evidence which establishes adverse health effects in animals as a direct consequence of dietary amino acid supplementation.

CHAPTER II

STATEMENT OF THE PROBLEM

Due to the possibility of developing toxicities from ingestion of excess amino acids, it was deemed necessary to find out which amino acid supplements are currently being marketed, what therapeutic uses they are being promoted for, and investigate, through the scientific literature, the physiological consequences of amino acid supplementation.

The objectives of this study were to:

- (A) visit local retail outlets which market amino acid supplements and gather information regarding the sale and usage of these supplements;
- (B) review the faddist literature concerning amino acid supplements;
- (C) review scientific literature on each amino acid currently being sold as a dietary supplement to either find support for or disclaimers against the proposed therapeutic uses;
- (D) make judgments on the objectivity of these claims.

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example, methionine fed in large amounts to laboratory rats causes pancreatic and salivary gland damage as well as retarded growth and food intake (9). On the other hand threonine, although tolerated well in great excess, has been shown to cause moderate growth retardation and depressed food intake in rats (10).

CHAPTER III

BASIC CONCEPTS OF DISPROPORTIONATE AMINO ACIDS

Plasma amino acid concentrations, even during prolonged fasting, remain relatively constant (2) and rapidly return toward the standard value after they have been elevated from ingestion or injection of an excess (3, 4, 5). Therefore, it seems evident that plasma amino acid concentrations are homeostatically well regulated. However, adverse effects from disproportionate amounts of amino acids occur when these homeostatic mechanisms for the regulation of blood and body fluid amino acid concentrations are deficient or defective or are artificially overloaded (6).

Harper (7) has attributed the adverse effects of disproportionate amounts of ingested amino acids to either amino acid toxicities, antagonisms, or imbalances.

The term "amino acid toxicity" refers to a situation in which the adverse effect of an amino acid excess cannot be overcome by supplementation of another amino acid (8). This term has been used for adverse effects seen from ingestion of large quantities of individual amino acids. The effects of excessive intakes of each of the individual amino acids being sold to the public are discussed one by one in this thesis, but only two examples are given here to illustrate the extremes. For studies, development of fatty livers (8).

example, methionine fed in large amounts to laboratory rats causes pancreatic and salivary gland damage as well as retarded growth and food intake (9). On the other hand threonine, although tolerated well in great excess, has been shown to cause moderate growth retardation and depressed food intake in rats (10).

"Amino acid antagonism" refers to a pattern of dietary amino acids which causes growth depressions that can be overcome by supplementation with an amino acid structurally similar to the antagonist. For example, excess lysine fed to chicks depressed growth and caused symptoms similar to those seen in arginine-deficient chicks. Addition of arginine to the diet reversed the adverse effects (8).

The term "amino acid imbalance" refers to a pattern of amino acids in the diet that induces growth depressions which can be overcome by supplementation with the amino acid which is most limiting to the diet. For example, methionine supplemented to a low casein diet caused a methionine excess, thus resulting in an amino acid imbalance. This imbalance was manifested by depressed growth and food intake (8, 11). The term "amino acid imbalance" is also defined operationally. It is not defined by the degree of deviation from the recommended requirements but only by whether or not the disproportion of amino acids is great enough to cause adverse effects. Two requirements in defining an amino acid imbalance are that no amino acid is present in amounts sufficient enough to be considered toxic itself and that the adverse effect can be prevented by a supplement of the limiting amino acid. These adverse effects are primarily growth and food intake depressions and, in some studies, development of fatty livers (8).

The effects of ingestion of disproportionate amounts of amino acids depends on the type of diet, degree of disproportion, and the age and physiological health of the animal. Healthy animals tolerate disproportionate amounts of amino acids better than unhealthy ones. However, excessive intakes of individual amino acids, especially in young animals fed a low protein diet, can result in adverse effects ranging from moderate growth and food intake depressions to more toxic reactions (7).

The relative toxicities of various amino acids based upon Harper's rat studies concerning amino acid imbalances, antagonisms, and toxicities are listed in table 2.

TABLE 2
RELATIVE TOXICITIES OF AMINO ACIDS*

-
- | | |
|----|---------------|
| 1) | Methionine |
| 2) | Tryptophan |
| 3) | Tyrosine |
| | Histidine |
| 4) | Phenylalanine |
| 5) | Leucine |
| | Isoleucine |
| | Valine |
| 6) | Lysine |
| 7) | Threonine |
-

*Listed in decreasing order of toxicity, i.e., methionine is most toxic and threonine is least toxic (7).

Due to the current trend of human dietary supplementation with various amino acids for a number of claimed therapeutic purposes, it was deemed necessary to: (A) visit health food stores in the greater Oklahoma City metropolitan area to collect data on the types of amino acid

supplements being sold along with recommended dosages and any literature advocating use of specific amino acid supplements; (B) review the fad-dist as well as the scientific literature on the use of individual amino acids in order to compile the available literature into one source; and (C) attempt to document the rationale and validity of over-the-counter (OTC) amino acid supplement usage by humans.

Amino Acid

For comparative purposes throughout this thesis, the established

essential amino acid requirements for humans are listed in table 3.

Amino Acid	1-11 years	12-17 years	18 years and over
Histidine	27	7	7
Isoleucine	23	28	12
Leucine	13	27	16
Lysine	21	24	12
Total S-containing (methionine and cysteine)	42	22	10
Total aromatic (phenylalanine and tyrosine)	44	32	16
Threonine	21	24	8
Tryptophan	11	4	3
Valine	22	25	14

*When adequate amounts of all other amino acids of nutritive importance are also provided, alanine, arginine, aspartic acid, citrulline, glutamic acid, glycine, histidine, hydroxyproline, proline, and serine.

(Reference no. 1)

TABLE 3

ESSENTIAL AMINO ACID REQUIREMENTS FOR HUMANS

Amino Acid	Requirements*		
	(milligrams/kilogram body weight/day)		
	Infant (4-6 months)	Child (1-12 years)	Adult
Histidine	33	?	?
Isoleucine	83	28	12
Leucine	155	42	16
Lysine	99	44	12
Total S-containing (methionine and cysteine)	49	22	10
Total aromatic (phenylalanine and tyrosine)	141	22	16
Threonine	68	28	8
Tryptophan	21	4	3
Valine	92	25	14

*When adequate amounts of all other amino acids of nutritive importance are also provided (alanine, arginine, aspartic acid, citrulline, glutamic acid, glycine, histidine, hydroxyproline, proline, and serine).

Over the counter (OTC) arginine is usually sold in 500 milligram tablets with dosage recommendations of one tablet per day. Supplemental

arginine supposedly increases the immune response, prevents or ameliorates arthritis, reduces existing atherosclerotic plaques, induces weight loss, combats flu, prevents cancer and strokes, and speeds wound healing (12).

Each of these proposed uses will be discussed along with current research to support or dispute the claims.

Pearson and Shaw (12) claim that immune system failure is responsible for arthritis, cancer, atherosclerotic plaques and other

CHAPTER IV

"diseases of aging." It is claimed that if the immune system were kept in the physiological condition of very early adulthood, a person could

ARGININE

reach a Arginine, a nutritionally semi-essential amino acid in humans, is involved in many metabolic reactions. Among these are creatinine synthesis, melanocyte-stimulating hormone synthesis in the pituitary, and the formation of alpha-ketoglutarate.

Arginine is considered to be semi-essential due to the fact that it is synthesized at rates inadequate to support growth of children (11). However, in adults, arginine is non-essential (dispensable) in that the body can produce nutritionally adequate amounts without additional dietary intake. Although arginine supplementation to the diet is not required in normal, healthy humans, some faddists propose its use in various therapeutic or preventative measures. While arginine supplements have been shown to be beneficial in treating some disorders in the clinical setting (i.e., hepatic encephalopathy, hyperammonemia), its beneficial use and safety for the average consumer as a dietary supplement remains questionable.

Over the counter (OTC) arginine is usually sold in 500 milligram tablets with dosage recommendations of one tablet per day. Supplemental arginine supposedly increases the immune response, prevents or ameliorates arthritis, reduces existing atherosclerotic plaques, induces weight loss, combats flu, prevents cancer and strokes, and speeds wound healing (12).

Each of these proposed uses will be discussed along with current research to support or dispute the claims.

Pearson and Shaw (12) claim that immune system failure is responsible for arthritis, cancer, atherosclerotic plaques and other "diseases of aging." It is claimed that if the immune system were kept in the physiological condition of very early adulthood, a person could reach a life span of over 800 years. Pearson and Shaw (12) continue to explain how, after puberty, the thymus "shrivels up and ceases to function at the high level of activity it had in the youthful years before puberty." They claim the thymus instructs certain white blood cells (T cells) what and when to attack. "The vital function of the thymus gland was not recognized until the past fifteen years or so. Many doctors still do not know about this." Pearson and Shaw claim that immune system performance can be improved by using the right nutrients. Arginine is one of these proposed nutrients that is "readily available and safe" (12).

Pearson and Shaw (12) state that one of the most important factors leading to immune system decline is the greatly reduced rate of release of growth hormone (GH) by the pituitary as we age. Since the thymus requires GH to function properly decreased hormone output is a "severe blow" to the immune system. Therefore, "since arginine (5-10 grams daily) taken on an empty stomach at bedtime causes growth hormone release, it is helpful in delaying immune system aging" (12).

For many years, it was believed that GH was secreted primarily during the period of growth, but then disappeared at adolescence. However, we now know this to be untrue. After adolescence, GH secretion continues at a rate about two-thirds as great as in childhood (2). The

fact that adults have nearly as much GH as children does not lend credence to the "severe blow" to the immune system due to decreased GH output with age, proposed by Pearson and Shaw.

Although there were no precautions mentioned in Pearson and Shaw's book concerning excess arginine, per se, there were several notations throughout the book concerning inappropriate use of "growth hormone releasers." It was cautioned that they should not be used by persons who have not completed their long-bone growth. Also, excess GH will cause skin to grow so rapidly it becomes coarser and thicker. Excessive GH over an extended period of time can cause irreversible enlargement of joint diameter ("which is unsightly, but not dangerous") and lowering of vocal pitch due to larynx growth. What Pearson and Shaw do not explain is what constitutes excessive GH or the dosage of GH-releasers which can be considered excessive (12).

It has been conjectured that the normal stimulus for GH release is low blood glucose levels. When blood glucose levels decrease (as during fasting), the pituitary secretes increased quantities of GH, which increases catabolism of fat and prevents the entry of glucose into tissues by blocking the action of insulin. The net result is conservation of glucose for use in the nervous system which must have it for metabolic needs. Other tissues can effectively use fatty acids for their energy needs. However, reliance upon large quantities of fatty acids for energy presents the risk of developing ketoacidosis.

Several recent studies support the concept of arginine supplementation and increased immunity. In 1980, Barbul et al. (13) studied the role of arginine supplementation on T-lymphocyte function in

injured and uninjured rats. The feeding of one percent supplemental dietary arginine for six days resulted in increased thymic weight and cellularity, increased immunologic function, and minimized deleterious effects of trauma on host immunity. In 1981, the same researchers (14) studied the effects of supplemental arginine on immune response in twenty-one healthy humans. For seven days, the subjects were given a supplement of 30 grams arginine-HCL. Results indicated that arginine significantly increased stimulation indices of peripheral blood lymphocytes (PBL). It is known that in vitro assays of lymphocyte transformation can provide a viable means of assessing immunologic deficiency states and the effects of various immunoenhancing therapies. None of the volunteers in this study were immunodeficient, but arginine supplementation increased their lymphocyte mitogenic blastogenesis by at least 200 percent indicating a greater ability to respond to antigenic challenges. Cells harvested post-arginine ingestion did not have enhanced viability or survival. Therefore, arginine induced greater blastogenic responses by an equal number of cells, not by making more cells. Side effects of either diarrhea or mild nausea were experienced by five of the 21 subjects.

Seifter et al. (15) suggested that the immune effects of arginine were due to its secretagogue release of pituitary and pancreatic hormones. In 1983, Barbul et al. (16) more specifically showed that wound-healing and thymotrophic effects of supplemental dietary arginine could not be demonstrated in hypophysectomized rats, suggesting that an intact hypothalamic-pituitary axis was essential for arginine's effect.

Barbul et al. (14) stated: "We believe that supplemental arginine may provide a safe, nontoxic, nutritional means of boosting the immune response."

Barbul et al. (17) also investigated the immunostimulatory actions of supplemental arginine on impaired cellular response in ob/ob (genetically obese) mice. One percent dietary arginine supplementation was given to mice. Supplemental arginine partially prevented the abnormally high weight gain and blood glucose levels of these mice. This did not occur in lean mice (which had normal glucose levels). In both ob/ob and lean mice, supplemental arginine (one percent) significantly increased thymic weight, number of thymic lymphocytes per gland, and thymic lymphocyte immunoreactivity in vitro.

It is interesting to note that not only does it seem that arginine stimulates insulin secretion and thus GH secretion, but has been shown to enhance the release of somatostatin, gastrin, and glucagon (20). Therefore, it appears the "beneficial effects" of arginine's use in stimulating GH release may be counteracted by its ability to also increase somatostatin release. This would appear to be a regulatory mechanism which would ensure physiologically stable levels of GH and somatostatin.

There is accumulating evidence that arginine has an effect on L-enkephalins (21). Enkephalins are peptides with powerful opiate-like effects that are 18-30 times more potent than morphine on a molar basis. Opiates have been reported to enhance the release of insulin and glucagon from isolated perfused dog pancreas (22) and to also stimulate insulin secretion in isolated rat islets (23). Sachse et al. (24) found

that an arginine infusion (2.5 mM/minute) into isolated perfused rat pancreas produced a significant decrease of L-enkephalin levels. In addition, enteral and parenteral administration of arginine suppressed plasma L-enkephalin levels.

Pearson and Shaw (12) include arginine in their list of immune system stimulants which can prevent arthritic attacks. No documentation of this claim was cited. Further investigation of current scientific literature failed to validate their claim.

The rationale behind claims of arginine's ability to reduce existing atherosclerotic plaques involves its T-cell stimulating ability. Pearson and Shaw (12) stated that vitamin C alone and in combination with vitamin B-6 caused regression on 4 atherosclerotic plaques in rabbits. "Other T-cell stimulants can also be expected to be effective in reducing existing plaques, e.g., arginine, ornithine, vitamins A and E, selenium, cysteine, and zinc" (12).

While there is no scientific evidence that increasing dietary arginine will reduce atherosclerotic plaques, recent research has shown that the ratio of lysine to arginine may be a determining factor in a protein's cholesterolemic and atherosclerotic qualities (25, 26, 27, 28). In most of these experiments, the effects of vegetable and animal protein were compared: the replacement of casein with soybean or cottonseed protein reduced plasma cholesterol (27, 28). The lysine:arginine ratio in casein is higher than in plant proteins; the higher this ratio, the more severe the atherosclerosis.

When arginine was added to casein to lower the ratio to levels similar to that of plant proteins, serum cholesterol was lowered as was

existence of atherosclerotic plaques in rats and rabbits (28). Glycine and alanine addition to casein had the same effect as arginine on hypercholesterolemia, possibly due to increasing the proportions in casein to equal plant protein proportions. Katan et al. (28) concluded that the beneficial effects of these amino acids could just as well be explained by the presence of an excess of other amino acids in casein (amino acid imbalance) as by an absolute shortage of glycine, arginine, or alanine.

More recently, Gibney (29) concluded that the arginine:lysine ratio of the diet is not the major determinant of the cholesterolemic properties of a protein, but that the overall amino acid composition is primarily responsible. He determined this by feeding rabbits milk or soya and found that, as expected, serum cholesterol was higher in milk-fed than soya-fed rabbits, but this was not influenced by reversing the lysine:arginine ratio in the foods.

Explanations for the possible connection between amino acid proportions in proteins and atherosclerosis are under investigation. For example, it was suggested that the high proportion of lysine in animal proteins may inhibit liver arginase, which would make more arginine available for synthesis of the arginine-rich lipoprotein that is associated with increased atherosclerosis in rabbits (30, 31). If this is so, it would appear that intakes of arginine over and above amounts needed to equilibrate lysine:arginine could actually contribute to atherosclerosis rather than deter it as Pearson and Shaw suggested and some research supports.

Park and Liepa (27) have suggested that dietary protein may play a role in cholesterol metabolism via an effect on lecithin:cholesterol

acyltransferase (LCAT). This enzyme has been shown to affect serum lipoprotein concentrations in humans and experimental animals (32). LCAT is also associated with the transfer of tissue cholesterol to the liver for possible removal from the body (27).

The feeding of animal protein to rats increased serum LCAT activity and increased the level of serum cholesterol beyond that observed with cottonseed protein. When arginine was added to casein, LCAT activity was lowered. Thus, if the LCAT activity is lowered with added arginine, reduced amounts of tissue cholesterol would be excreted from the body, thereby increasing blood cholesterol.

In 1982 Sugano et al. (25) were unable to demonstrate a reduction in serum cholesterol by addition of arginine to casein. The results indicated that arginine:lysine ratio in soybean and casein seemed to have little effect, if any, on serum cholesterol levels. However, serum triglycerides were found to be dependent on the arginine:lysine ratio. In general, serum triglycerides were inversely related to the arginine:lysine ratio. In addition, arginine supplementation of casein caused a dose-dependent increase in plasma glucagon. Therefore, it appears that hormones may also influence the effect of various proteins on hypercholesterolemia.

KAL Series #5, the information provided by many health food stores, suggests that arginine's ability to increase secretion of GH from the pituitary can aid in weight loss by burning fat and building muscle tissue (33). Pearson and Shaw (12) recommend the daily ingestion of 20 grams of arginine for weight loss.

While it appears to be true that arginine stimulates GH secretion from the pituitary, its safe use as a weight-reduction aid is questionable.

GH is known to have several basic metabolic effects upon the body (34): In 1961 Isidori et al. (35) conducted a study in humans to

(A) GH stimulates overall protein synthesis with a pronounced increase in nitrogen (N) and phosphorous (P) retention. In muscle, GH can stimulate protein synthesis by increasing the transport of amino acids into the cells. It also contributes to increased synthesis of DNA and RNA.

(B) In muscle, GH antagonizes the effects of insulin. Therefore, glycolysis impairment as well as glucose transport inhibition can be seen with GH administration. Prolonged administration of GH results in enhanced release of insulin from the pancreas during glucose stimulation. Thus, there may be a diabetogenic action of GH due to increased circulating glucose, fatty acids, and ketones.

(C) GH accelerates the release of free fatty acids from adipose tissue and increases plasma free fatty acid concentration by increasing the rate of lipolysis of triacylglycerol stores by activating the hormone-sensitive lipase. For optimum effect, most of these lipolytic processes require the presence of glucocorticoids and thyroid hormones. On its own, the lipolytic hormones, GH included, do not increase lipolysis markedly, but act in a facilitory capacity with respect to other lipolytic endocrine factors.

There are several dangers associated with the increased secretion of GH, especially its ketogenic effect. One of the consequences of increased fatty acid mobilization is a greatly increased use of fatty acids for energy. Therefore, large amounts of ketone bodies are formed in the liver and transported in the blood. Over a period of time, ketoacidosis would result. In addition, increased GH could lead to fatty liver due to the increased fatty acid release from adipose tissue (34).

Another complication of arginine's use as a GH stimulator is the diabetogenic effect of GH. Since GH causes a decrease in carbohydrate utilization for energy, glycogen begins to be stored in the cells.

Eventually, the cells become saturated, after which the cellular uptake of glucose becomes greatly depressed. As a result, blood glucose concentration rises above normal much the same as in diabetes (34).

Pearson In 1981 Isidori et al. (35) conducted a study in humans to evaluate the secretion of growth factors following stimulation by oral amino acids: a single dose of 2400 milligrams amino acids (1200 milligrams arginine plus 1200 milligrams lysine-HCL) was given on an empty stomach. Blood samples were taken at 30, 60, 90, and 120 minutes.

Results showed that GH and insulin secretion increased with amino acid supplementation, but only when the two amino acids were used in combination and not when administered separately (1200 milligrams arginine or 1200 milligrams lysine).

Although there is no evidence that arginine supplementation can safely and effectively "burn fat and build muscle," there is evidence that various amino acids taken in excess can cause a depression in growth, a decrease in food intake, and in some cases, a change in the total protein RNA and DNA contents in the liver (36). Contrary to the claimed body composition changes with arginine supplementation, Kobayashi and Otsuka (36) found no changes in whole body composition of rats fed increasing levels of arginine.

It has been shown that various amino acids, including arginine, taken in excess or in proportions to cause imbalance, will lower food intake and slow growth in rats (36). Itoh et al. (37) speculated that stomach distension resulting from the osmotic effect of amino acids may be responsible for the reduced food intake. In this sense, it is plausible to imagine a person losing weight by eating less.

Pearson and Shaw (12) presented an undocumented claim that arginine or ornithine was able to block formation of tumors in mice inoculated with Maloney sarcoma virus (MSV), a cancer-causing virus. Pearson and Shaw (12) cited this as an example of arginine's role in cancer prevention. Without proper documentation, it was difficult to locate their source of information. References were made to an article by Barbul et al. (16) which dealt with arginine and its thymotropic and wound-healing properties and to an earlier study (38) of arginine's influence on the incidence, size, and regression of tumors in mice inoculated with the MSV solution. Neither report mentioned a role for arginine in cancer prevention.

There is, however, an accumulating body of experimental evidence which indicates that arginine supplementation does have an effect on certain cancer cells in their early stages. In 1976 Pryme (39) reported prevention of tumor formation in rats maintained on arginine-supplemented drinking water and subsequently injected subcutaneously with single-cell suspensions of myeloma cells. A follow-up study in 1978 (40) reported that the presence of arginine-HCL in the drinking water was able to prevent formation of subcutaneous tumors in response to single-cell injections but had no effect when tumor fragments or cell aggregates were injected. It was suggested that perhaps an early event in tumor cell-host interaction was inhibited by the increased dietary arginine concentration. In order to test this possibility, arginine-supplemented mice were inoculated with ascites tumors. Since ascites tumors do not have host cells . . . they grow in suspension . . . it was expected that arginine would have no effect upon tumor growth. As expected, arginine

was unable to prevent formation or slow down ascites tumor development (40).

On the contrary, growth of Ehrlich ascites tumor cells in mice was inhibited by a three or five percent dietary arginine supplement (41). It was suggested that arginine inhibited cellular replication.

Recent evidence suggests a positive correlation of polyamine concentrations and the rate of cellular or tissue growth. Increased ornithine decarboxylase (ODC), a key enzyme in pyrimidine biosynthesis, activity

in addition to the more general complications associated with favors more rapid cellular growth. Arginine supplementation reduced ODC activity and thus retarded tumor growth. Therefore, arginine supplements may slow tumor growth by reducing pyrimidine biosynthesis (41).

Arginine supplements have been reported to actually reduce the size of mammary tumors. Daily injections of L-arginine (50 milligrams per kilogram body weight produced a highly significant decrease in arginine-HCL/200 gram rat/day) into rats bearing primary 7,12-dimethylbenzanthracene (DMBA) tumors consistently produced growth inhibitions and reduced tumor size to 80 percent of the initial size. Tumor growth inhibition was dose-dependent. No toxic effects were observed in these animals. Growth arrest by arginine was accompanied by an increase in cellular cyclic adenosine monophosphate (cAMP) content. This suggested a specific role for arginine in the cAMP-mediated inhibition of mammary tumors. The mechanism by which this occurs is not yet understood (42).

A similar in vitro study was conducted on growth of human breast cancer cell cultures. Growth was inhibited by a daily addition of L-arginine (1 milligram/milliliter) to the culture media. Arginine acted synergistically with cAMP to enhance growth inhibitory effect by arresting cell replication within two days after treatment (43).

In conclusion, claims that arginine supplementation can prevent or slow neoplastic growth by boosting the immune system were not scientifically supported. However, research continues to explore the use of arginine supplements for cancer therapy. The suggestion that arginine is a safe supplement which should be used by all is a frivolous, non-documented claim.

Potential Hazards of Arginine Supplementation

In addition to the more general complications associated with amino acid excesses covered previously, several specific complications associated with arginine supplementation have been shown. Massara et al. (44) studied arginine's effect on plasma phosphorous and potassium levels and that these phenomena were not due to alterations of parathyroid hormone secretion. It was found that an arginine infusion of 0.5 grams arginine per kilogram body weight produced a highly significant decrease in functional renal reabsorption of calcium. Intraperitoneal arginine loading (1.5 millimoles/kilogram) in diabetic subjects in an effort to delineate the roles of insulin and glucagon. Insulin-dependent diabetics showed a significant decrease in phosphorus following arginine infusion much the same as normal subjects. The diabetics' increase in blood potassium was much greater than that of normals and rose to pathological levels in more than 50 percent of the subjects. Therefore, it was concluded that arginine-induced hyperkalemia was exacerbated by low blood insulin (45). Clinically, persons with low insulin secretion due to stress or diabetes run the risk of pathological hyperkalaemia if subjected to high levels of arginine.

A more recent study (46) showed that arginine (12 millimoles/kilogram/hour) infused through the jugular vein of rats at 1.2 milliliters

per hour significantly increased urinary calcium excretion by almost six times normal value. Since this phenomenon was not seen in streptozotocin-induced diabetic rats, the increase was attributed to an arginine-induced increase in plasma insulin. In addition, arginine infusion increased urine flow rate in both normals and diabetics. In humans, the arginine-induced calciuria was not caused or compensated by an increased intestinal absorption of calcium; thus, a negative calcium balance is another consequence of arginine supplementation (47, 48).

Gollaher et al. (49) confirmed the facts that arginine infusion markedly increased urine volume, urine calcium, and glomerular filtration rate and that these phenomena were not due to alterations of parathyroid hormone secretion. It was concluded that arginine infusion impairs functional renal reabsorption of calcium. Intraperitoneal arginine loading (1.5 millimoles/kilogram) in rats produced an increase in liver levels of arginine and, to a greater extent, ornithine (50). The relatively larger increase in ornithine concentration resulted in an increase in the ratio of ornithine to arginine from 8.86 to 15. Therefore, secondary complications resulting from amino acid imbalances could occur. In addition, the plausibility of high levels of the very toxic ornithine must be considered in the use of arginine supplements.

Arginine's ability to increase plasma glucagon and insulin in man presents another potential complication of arginine supplementation (6). The ability of glucagon to impair glucose tolerance has been amply demonstrated (51, 52, 53). Stimulation of glucagon secretion increases

hepatic glucose production and fasting glucose levels. Mackes et al. (51) demonstrated that arginine-induced intermittent endogenous glucagon secretion resulted in plasma levels of glucose in normal man approximating those seen in diabetics postprandially. Since these abnormal glycemic profiles occurred in spite of increases in insulin, presumably hyperglucagonemia could impair postprandial glucose tolerance in diabetics in whom insulin secretion is deficient. Consequently, excess arginine intake could affect glucose tolerance in normal man as well as diabetics and should, therefore, be used with caution.

Rizza et al. (52) found that in both normal and diabetic subjects, each hourly episode of arginine-induced hyperglucagonemia increased plasma glucose levels. In normal subjects, glucose increased moderately during infusion and returned to base line between infusions. In diabetics, plasma glucose increased to a greater extent than in normals and did not return to normal between infusions. Therefore, indications were that hyperglucagonemia can cause sustained hyperglycemia in man when pancreatic beta-cell function is limited.

Results of chronic arginine and/or aspartate administration on sleep-endocrine relationships in humans show that with an intake of 250 milligrams arginine per kilogram body weight per day for seven days, growth hormone and, to a lesser extent, prolactin secretion during the slow-wave sleep cycle increased. There was no alteration in the total duration of sleep or in percentages of stages III, IV, or REM sleep. Several theories to explain the mechanism of the hormonal alteration during sleep were offered by Besset et al. (54). These include serotonergic, catecholaminergic, or GABAnergic systems playing a possible

role. The physiological effects of sleep endocrine alterations is presently unknown.

One of the most recent studies (55) concerning arginine intake shows that pancreatic damage can ensue from excess arginine. Rats given daily injections of arginine (500 milligrams/100 grams body weight) exhibited destruction of pancreatic acinar cells with no damage to islets

of Langerhans. Within 24 hours after injection, loss of basophilia,

zymogen degranulation, vacuolar and necrotic changes of acinar cells were noted. After three days, fibroblastic activity and atrophy of pancreatic lobuli were seen. The adverse effects may be ascribed to amino acid imbalance subsequent to decreased protein synthesis in the acinar cells. In addition, fat necrosis and leucocyte infiltration were observed in adipose tissue in peripancreatic, epididymal, omental, and retroperitoneal areas. This correlated with marked necrosis of the

pancreas. There was also an increase in blood lipoprotein lipase level (49). Surprisingly, Pearson and Shaw (12) did not include information concerning supplemental lysine use in their book. However, lysine is

widely promoted for the treatment of herpes viral infections (57, 58, 59,

60). Consumer product information published by the General Nutrition Corporation (37) claims lysine inhibits herpes growth. Their information is based upon a study by Griffith et al. of Indiana University School of Medicine (61). The KAL Series #6 (58) recommends dosages of one gram per day for maintenance and up to four grams per day during outbreaks of herpes. This publication claims that extra lysine forces the virus to the human diet is not and cannot be recommended at this time.

lie dormant in nerve cells. "Benefits are that herpes outbreaks are more mild when they do occur and if regular daily supplements of one

gram or so of lysine is continued, the outbreaks will occur very

infrequently . . . perhaps never" (58). As in other KAL publications, documentation for their claims is vague.

In addition to herpes treatment, lysine is also reportedly associated with a reduced incidence of dental caries. KAL Series #6

CHAPTER V

cites Goodhart and Shills' textbook to support the claim (62). No such claim could be found in a review LYSINE textbook. Lysine is available OTC in 500 to 1100 milligram tablets.

Lysine is a nutritionally essential amino acid which is involved in a variety of metabolic functions in addition to protein synthesis. In 1964 Tankersley (63) reported that arginine encouraged and lysine antagonized herpes virus replication. In 1974 Kagan (64) For instance, lysine serves as a precursor for carnitine, as a nitrogen donor for glutamate production in the liver (56), enhances calcium absorption (56), and, through oxidative deamination, is involved in collagen formation and function (6). Lysine is abundant in meat, dairy products, beans and seeds, and is limited in vegetables and grains.

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In 1964 Tankersley (63) reported that arginine encouraged and lysine antagonized herpes virus replication. In 1974 Kagan (64) observed clinically the efficacy of lysine as suggested by Tankersley. A 1978 study by Griffith et al. (61) followed 45 patients who suffered from herpes viral infections for two months to three years. These patients were kept on a daily maintenance dose of 312 to 500 milligrams oral L-lysine (capsules or tablets). Therapeutic dosage during overt infection was 800 to 1000 milligrams L-lysine. It was reported that the pain disappeared abruptly overnight in virtually every instance, new vesicles failed to appear and recovery from overt skin eruptions was quicker than without lysine supplementation.

Most encouraging was the reduction in frequency of recurrence. Patients were maintained infection-free while on lysine but found that when lysine supplementation was discontinued, lesions returned within one-to-four weeks. Therefore, it was concluded that lysine works as a suppressor of herpes virus, not as a cure. It was found that a maintenance dosage of 500 milligrams or more per day kept the majority of herpes victims lesion-free (61).

The ability of excess lysine to counter herpes was attributed to an alteration in the arginine:lysine ratio of the virus. It was suggested that excess arginine promoted viral growth whereas excess lysine inhibited growth. The rationale for lysine's efficacy was attributed to lowered arginine concentration due to competition for the same transport mechanism across the mucosal wall (65, 66, 67) and to renal tubular reabsorption (68). The net result of oral lysine supplementation to chicks was a reduction of arginine absorption, an increased urinary excretion of arginine and accelerated arginine catabolism since lysine induces arginase production (69). It was interesting to note that the same reactions which inhibit herpes virus growth were also the reactions which inhibit arginine absorption.

Griffith (65) confirmed his 1978 (61) study by demonstrating that increasing the concentration of arginine in herpes virus-infected monkey kidney tissue culture antagonized the inhibitory action of lysine. This suggested that herpes viral activity may be stimulated by either a reduced level of lysine or an excess level of arginine. Griffith suggested that treatment of viral infections should include supplementary lysine along with a reduction of dietary arginine which could be accomplished by limiting the intake of cereals, seeds, nuts, and chocolate (65.)

In contrast, DiGiovanna and Blank (70) reported in 1984 no difference in frequency, duration, or severity of herpes episodes between ten lysine supplemented (500-1000 milligrams per day) patients and ten placebo patients. Earlier controlled studies also failed to demonstrate any beneficial effects of lysine supplementation on herpes viral infections (71, 72). pyrimidine biosynthesis, both in vivo and in vitro. As early as 1945, Hunter and Downs (73) observed competitive inhibition of

Potential Hazards of Excess Lysine

Accumulating evidence shows possible detrimental consequences of excess lysine intake. Many studies have suggested that excess lysine antagonized arginine producing an amino acid imbalance which repeatedly inhibited growth in laboratory animals (69, 73, 74). The imbalance was attributed to the competitive inhibition of arginine absorption from the gut by excess lysine, increased excretion of arginine in the urine, the induction of arginase activity by lysine and, lysine's entrance into the transport system of tissue cells decreasing the intracellular content of arginine (65, 73, 74). It was interesting to note that the same reactions which support claims of herpes treatment were also the reactions used to explain the effects of lysine toxicity.

Another possible complication of lysine supplementation concerns its ability to interfere with the availability of selected minerals (75, 76, 77). In 1975 Voelter et al. (77) showed that copper binds with the alpha-amino and the carboxyl groups of lysine which are necessary for collagen formation and function. More recently (1983) addition of 2.1 percent L-lysine to a 10 percent casein diet in rats produced a 53 percent reduction in hepatic copper and a significant reduction in hepatic iron. The addition of 0.7 percent or 2.1 percent lysine to the basal diet also lowered plasma levels of copper and zinc. This suggested that excess lysine interfered with mineral availability by either reducing tissue utilization or promoting excretion or both (75).

Evidence indicates that excess lysine affects urea cycle operation and pyrimidine biosynthesis, both in vivo and in vitro. As early as 1945, Hunter and Downs (78) observed competitive inhibition of

arginase activity by lysine in vitro; similar inhibitions in rat livers have also been reported (50). In 1981 Ulman et al. (79) reported shown increased creatinine excretion per body weight in rats after lysine supplementation. The major structural component of creatinine is derived from arginine. If excess lysine does, in fact, inhibit arginase activity, an accumulation of liver arginine could account for increased creatinine production and excretion.) conjectured that lysine supplementation may

have indu Current studies support the ability of lysine to alter nitrogen metabolism to a greater extent than most amino acids by its ability to increase the rate of orotic acid biosynthesis (80). In addition, rats fed a high lysine diet had an increased excretion of ammonia and orotic acid, but a decreased excretion of urea (79,80). These effects could be attributed to an inhibitory effect of lysine upon either arginase or ornithine transcarbamylase (OTCase) activities producing depressed rate ureagenesis (79). Lysine inhibition of (OTCase) could similarly cause an accumulation of ornithine and, therefore, reduced ammonia detoxification resulting in hyperammonemia. their liver lipid profiles were identical to

Elevated plasma lysine levels produced weakness, tremors, and poor feathering in chicks (81), and neurological disturbances in man (6). Since lysine excess may produce ammonia intoxication (6, 50, 79, 80) concern plus the fact that the level of blood ammonia parallels the severity of mental dysfunction (6), it is not surprising that lysine excess could result in neurological disturbances. It is also important to realize that disorders of the urea cycle caused by excess lysine could produce symptoms of toxicity such as episodic vomiting, lethargy, convulsions, and coma (6).

Excess lysine is also responsible for lipid metabolism alterations. Rat studies conducted by Hevia et al. (82, 83) have shown that addition of five percent lysine to a 15 percent casein diet produced fatty liver while serum lipids remained normal. By contrast, lysine feeding of rabbits was associated with normal liver lipids but a sharp increase in serum cholesterol (84).

Hevia et al. (82) conjectured that lysine supplementation may have induced an orotic acid excess as well as arginine deficiency which were associated with the development of fatty livers. Regarding serum lipids and lysine, a recent study with chicks fed four percent lysine suggested that lysine-induced hyperlipidemia was not mediated by the antagonistic effect of lysine intake. Rather, it was suggested that excess lysine stimulated cholesterol biosynthesis (85).

Interestingly, Hevia et al. (83) reported fatty livers in rats fed a five percent lysine diet for two weeks but no fatty livers in rats fed the same diet for six weeks. Apparently, rats adapted to five percent lysine within six weeks since their liver lipid profiles were identical to those fed no supplemental lysine.

In view of the present information concerning the various metabolic alterations caused by lysine excess and the inconclusive evidence concerning lysine supplementation for herpes treatment, it remains to be seen if the benefits of supplemental lysine use outweigh the risks.

Since tyrosine is a dispensable amino acid, no recommended daily allowance (RDA) exists for this amino acid alone. However, the RDA for total aromatic amino acids (phenylalanine and tyrosine) is 16 milligrams per kilogram body weight per day. In addition to its endogenous production from phenylalanine, tyrosine is widely distributed in proteins of plant and animal origin (87).

Supplemental tyrosine is most commonly marketed in 500 milligram tablets. Dosage recommendations on the bottle are one or two tablets daily.

Tyrosine is being promoted for the following therapeutic purposes: control of anxiety and depression (12, 59, 60, 88), weight control (12, 59), memory improvement (12, 59), treatment of allergies (59), sleep aid (59), as an anti-aging factor (12), and as a treatment for sexual dysfunction (12). These claims are all based upon tyrosine's precursor role in catecholamine synthesis.

CHAPTER VI

TYROSINE

Pearson and Shaw (12) state that NE is one of the brain's neurotransmitters which is important in primitive drives and emotions like sex as well as memory and learning. When the level of NE in the brain is too low, people become depressed and their immune systems do not function normally (12). It is claimed that the immune system boost is due to NE causing the brain to release growth hormone (GH), which is essential to proper immune system function. Thus, supposedly, an increase in tyrosine supplied through supplementation of the diet, would increase NE synthesis and enhance all NE-related biochemical functions. In addition to the claim of tyrosine increasing NE synthesis, of tyrosine molecules form the thyroid hormones thyroxine (T4) and triiodothyronine (T3) (11).

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In addition to the claim of tyrosine increasing NE synthesis, thus relieving depression, KAL Series #4 (88) offers an additional biochemical pathway to depression control. They claim that phenylalanine and/or tyrosine is a precursor to phenylethylamine (PEA); too little PEA in the brain's cerebellum may produce depression. Therefore, phenylalanine or tyrosine supplementation may relieve depression by elevating cerebellar levels of PEA (88).

Tyrosine's promotion as an anti-aging factor is based upon its catecholic precursor status thus being an "effective free-radical quencher" (12). Presumably, free-radicals "attack" the body and break it down resulting in aging; therefore, those nutrients which "quench"

free-radicals will slow aging. No specific mechanism nor documentation for this action was cited.

Tyrosine's use in weight control is related to its NE precursor status in which NE presumably functions as an appetite inhibitor (12). Lee (60) claims NE acts in two ways to help control appetite. First, it makes people feel better about themselves and better able to resist uncomfortable situations. Secondly, it helps stimulate cholecystokinin (CCK) which convinces the brain the stomach is full.

Concerning tyrosine's treatment of sexual dysfunction, Pearson and Shaw (12) make a connection between depression and loss of sexual interest. "When people are depressed, they almost always lose interest in sex." They recommend doses of 100-500 milligrams per day for two weeks which they state will relieve depression "and, of course, sexual interest may be revived."

Pearson and Shaw promote tyrosine supplementation for use not only as an anti-free radical scavenger to combat aging, but also implicate tyrosine in age-related loss of hair pigment. They claim the pigment loss of hair is due to free radical damage to melanocytes in the hair follicles and that these melanocytes are subject to free radical damage by the internal polymerization of L-DOPA and L-tyrosine. Thus, on the one hand, tyrosine is claimed to be a free radical "quencher" to prevent aging and on the other hand, it, in combination with L-DOPA, produces free-radicals (12).

No explanations were given for tyrosine's use as a sleep aid. It was stated that tyrosine in combination with tryptophan may be a better sleep aid than tryptophan alone (60).

Caution was given by Pearson and Shaw (12) throughout the book for the use of tyrosine supplements. They stated overdose symptoms of irritability, insomnia, and elevated blood pressure. Recommendations to those with high blood pressure were to start with very low doses of 100 milligrams and check their blood pressure frequently as they increase the dosage. In addition, persons taking monamine oxidase inhibitors for depression or hypertension were warned to refrain from supplemental tyrosine use due to the possibility of an extremely hazardous and possibly fatal hypertensive crisis. An additional warning was given to people with malignant melanoma in which the black melanin pigment is produced by the autoxidation of tyrosine, phenylalanine, and L-DOPA. Therefore, those afflicted with this disorder were advised against using supplemental tyrosine (12).

In mammals, dopamine and NE synthesis occurs in the brain from the amino acid precursor, tyrosine (89, 90). The initial and rate-limiting step in NE and dopamine synthesis is the hydroxylation of the aromatic ring of tyrosine by tyrosine hydroxylase (91). It was once thought that tyrosine hydroxylase was normally saturated, thereby excluding any possibility that an increase in tyrosine could affect catecholamine (CA) synthesis (89). However, in 1978, Carlson and Lindqvist (92) estimated that tyrosine hydroxylase was only 75 percent saturated. This initiated speculations that changes in brain tyrosine concentration could cause parallel changes in CA synthesis (86, 93). Several investigations have found this to be true (89, 94, 95, 96).

In 1982 Badawy and Williams (95) reported that rat brain CA synthesis was enhanced by intraperitoneal injections of up to

50 milligrams tyrosine per kilogram body weight but not by doses of 50 milligrams or more. In 1983 Benedict et al. (97) reported that dietary tyrosine supplementation (7.5 grams per day) in man caused an increase in brain catecholaminergic activity which subsequently led to a decrease in peripheral sympathetic activity as a result of a decrease in plasma CA. The changes in blood pressure and heart rate in man were small despite the significant increase in circulating NE (97). Other researchers have observed a similar lack of blood pressure and heart rate response in normal rats. Spontaneously hypertensive rats, on the other hand, exhibited a marked decrease in blood pressure (98) and heart rate (99) after tyrosine administration.

Human and animal studies indicate that tyrosine administration increases urinary CAs (100, 101, 102, 103). A single oral dose of 100 or 150 milligrams per kilogram body weight of tyrosine to humans caused significant increases in urinary NE, dopamine, 3-methoxy-4-hydroxyphenylglycol, vanilmandelic acid, and homovanillic acid during the first two hours after ingestion (101). Since urinary CAs are derived from central nervous system (CNS) and peripheral sources, it is possible that tyrosine administration may increase CA synthesis and release from cells throughout the body (100, 101, 102, 103).

In 1971 Benkert et al. (104) indicated that peripheral tyrosine metabolism and uptake into the brain may be altered in depressed patients. Kishimoto and Hama (105) found decreased levels of blood tyrosine in depressed patients as compared to normals. Gaillard and Tissot (106) postulated that decreased transport of tyrosine into the brain may correlate with depression, whereas Moller et al. (107) concluded that the

ratio of tyrosine to other large, neutral amino acids may be responsible for the depression. In 1980 Gelenberg et al. (108) and Goldberg (109) both reported improvement of depression following oral doses of 100 milligrams tyrosine per kilogram body weight per day to human patients. The only adverse effect reported was epigastric distress which was relieved by ingestion of the tyrosine supplement with food.

Various behavioral functions have been associated with dopamine transmission. One of these is regulation of motor activity (110). In 1982 Gibson et al. (111) demonstrated increased motor activity in rats following tyrosine administration. Another function associated with CAs is regulation of moods (112). More recent evidence fails to support tyrosine's role in mood alterations (113, 114). However, when tyrosine administration was combined with monamine oxidase inhibitors, intense behavioral stimulation was observed in rats (hyperactivity, sniffing, chewing, walking backwards, and splayed hindlimbs), which were attributed to increased production of meta-tyrosine (95, 96, 115). Judging from these studies, it appears that tyrosine supplementation, whether alone or in conjunction with a monoamine-oxidase inhibitor, may have potential as a treatment for depression. However, no conclusions have been reached on a recommended safe dosage of tyrosine supplementation; neither have adverse side effects been established.

Tyrosine's role as an aid to weight loss is based upon its stimulation of NE and cholecystokinin (CKK) synthesis with a resulting decrease in feeding. Throughout the last decade, research on regulation of food intake has favored a neurochemical approach. However, the exact mechanisms attributed to each neurotransmitter in food intake regulation have not been identified.

Chemical stimulation of the brain with NE may inhibit or initiate food intake (116, 117). Dopamine may play an indirect role in feeding by acting as a "gate" to permit the expression of eating or by altering the rate of feeding (116). In addition, it has been found that the plasma tyrosine-to-phenylalanine ratio positively correlates with long-term energy consumption in rats (116, 118).

Although it is unlikely that amino acid precursors of CCK influence its synthesis and release due to genetic control, it is possible that CCK's presence and release is relevant to the possible role of amino acids in the regulation of food intake. This may be due, in part, to the fact that amino acids (in this discussion, tyrosine), trigger its release (56). CCK has been shown to inhibit feeding (56, 119).

Stunkard (116) offered four precautions to adopting the neurotransmitter theory as a means of weight loss and control: a) a number of other brain chemicals such as acetylcholine, gamma-amino-butyric-acid (GABA), and certain peptides may influence food intake also by direct action or by modulating the amine systems; b) the connection between the metabolic information and neuronal activity is unclear; c) the mechanisms of translation or neurotransmitter status into behavior is vague; d) the relationships between various transmitter systems such as NE and dopamine, NE and serotonin, for example, can result in quite different behavioral aspects (116).

Results of a recent study by Johnston et al. (120) offer an additional consideration concerning tyrosine supplementation in the treatment of obesity. They concluded that urinary CA metabolite production is related to body composition and the amount of tyrosine intake in

the treatment of obesity. They concluded that urinary CA metabolite production is related to body composition and the amount of tyrosine intake in normal weight women. However, these relationships are altered in the obese, suggesting an association between obesity and abnormal CA metabolism. It was suggested that in the obese, tyrosine hydroxylase, the rate-limiting enzyme for NE synthesis, may be saturated, therefore, unable to respond to increases in tyrosine concentration (121). An alternate explanation may be the presence of altered availability of sup-tyrosine in the free amino acid pool (120).

Another interesting consideration involves the observation that the obese maintain a low tryptophan:neutral amino acid ratio compared to leans. This ratio may result in reduced synthesis of the neurotransmitter 5-hydroxytryptamine in the obese (122) which Waldbillig et al.

Potential Hazards of Excess Tyrosine
(123) have shown to be associated with overeating in animal models of obesity. Tyrosinemia is a metabolic disorder of tyrosine catabolism that is characterized by elevated plasma tyrosine levels. The common clinical findings include hepatosplenomegaly, cirrhosis of the liver, abnormal decrease tryptophan transport into the brain, thus decreasing the synthesis of tyrosine and methionine metabolism, p-hydroxyphenyllactic acidosis of 5-hydroxytryptamine which stimulates overeating. This theory contradicts tyrosine's appetite-suppressing capabilities claimed by promoters of supplemental tyrosine for weight control.

As early as 1946, Niven et al. (127) demonstrated growth retardation and external lesions in rats resulting from the addition of one percent dl-phenylalanine and one percent l-tyrosine to a 10 percent efficacy. Wheeler et al. (124) investigated the adjuvant properties of casein diet. They suggested that since phenylalanine is converted to L-tyrosine in guinea pigs and rats. Various allergens adsorbed to tyrosine in the animal, it may add to the effect of the excess tyrosine. The addition of relatively large amounts of l-tryptophan alleviated the

attributed to a delay in the bioavailability of the allergenic materials rather than a direct effect upon immunocompetent cells.

Tyrosine has been added to skin-tan-promoter products in the belief that topically administered tyrosine penetrates skin cells' metabolic pool increasing the concentration of melanin precursors and enhances skin pigmentation. Agin et al. (125) investigated this claim by treating mice with ultraviolet irradiation and 0.01 percent tyrosine either as a dietary supplement or topically. Four weeks of tryosine supplementation had no influence on melanogenesis but, in fact, may cause direct or indirect inhibition of pigmentation. Pawelek and Lerner (126) have shown that pigmented cells can be killed by exposure to excessive tyrosine.

Potential Hazards of Excess Tyrosine

Tyrosinemia is a metabolic disorder of tyrosine catabolism that lesions in skin, bone, pancreas, liver, kidney, and testes of rats (128), is characterized by elevated plasma tyrosine levels. The common clinical findings include hepatosplenomegaly, cirrhosis of the liver, abnormalities of tyrosine and methionine metabolism, p-hydroxyphenyllactic aciduria, defects in renal tubular reabsorption, rickets, hyperphosphaturia, proteinuria, and aminoaciduria (11).

As mentioned earlier, tyrosinemia, an inborn error of metabolism in humans, is characterized by a variety of clinical findings, some of which are life-threatening. In addition, numerous animal studies support the potential toxicity of excess tyrosine. In view of the available evidence, there is no clear-cut consensus establishing the validity or desirability of supplemental dietary tyrosine for the purported therapeutic purposes. Once again, the potential risks outweigh the purported benefits. As early as 1946, Niven et al. (127) demonstrated growth retardation and external lesions in rats resulting from the addition of one percent dl-phenylalanine and one percent L-tyrosine to a 10 percent casein diet. They suggested that since phenylalanine is converted to tyrosine in the animal, it may add to the effect of the excess tyrosine.

The addition of relatively large amounts of L-tryptophan alleviated the

deleterious effects of excess L-phenylalanine and L-tyrosine demonstrating an antagonism between tryptophan and phenylalanine and tyrosine.

Intraperitoneal injections of 0.5 millimoles of tyrosine daily for ten days in mice produced arteriolar lesions (128). Excess tyrosine altered protein synthesis and degradation in liver, muscle, and brain; polysome disaggregation and decreased protein synthesis was observed in brain and muscle; increased protein degradation was observed in liver (129).

Excess tyrosine reportedly induces riboflavin and/or nicotinic acid deficiencies (127, 130). The severity of growth depression in rats or chicks resulting from a one-to-twenty percent increase in tyrosine concentration was directly correlated with the concentration of tyrosine excess (6). Other toxic symptoms observed in animal studies include hip and knee joint discoloration, increased blood pressure (131, 132), lesions in skin, bone, pancreas, liver, kidney, and testes of rats (128, 133, 134, 135), and kidney and liver damage in rabbits (136) and dogs (137).

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Therefore, the rate of tryptophan entry into the brain is strongly influenced by the presence of competing LNAA (56).

Tryptophan is naturally occurring in most protein foods and is most abundant in legumes, nuts, and seeds. The RDA for tryptophan in adults is three milligrams per kilogram body weight per day (1).

CHAPTER VII

TRYPTOPHAN

Tryptophan is being promoted as a depression fighter (59, 60), sleep aid (12, 59, 60, 138), treatment for migraine headaches (59), immunity enhancer (59), and weight control (12). Tryptophan is a nutritionally essential heterocyclic amino acid which is ketogenic because, through catabolism, it produces acetoacetyl CoA and acetyl CoA (11). Although a large proportion of tryptophan is

incorporated into proteins, a substantial fraction appears in various functions as a serotonin precursor. Lee (60) stated that "tryptophan catabolites.

helps form a neurotransmitter called serotonin, which can alter reactions to stress and mood swings, changing excitement and confusion to calm and understanding. . . . when phenylalanine and tyrosine act to increase manufacture of one milligram niacin in vivo (62). Hydroxylation of tryptophan to 5-hydroxytryptophan (5-HT) followed by decarboxylation forms the neurotransmitter 5-hydroxytryptamine (serotonin) (11). One percent of tryptophan is normally converted to serotonin (62). No dosage recommendations were presented in Lee's literature.

Tryptophan is the only amino acid which is significantly bound by plasma proteins. In the fed state, 80 to 90 percent of the total circulating tryptophan is bound to albumin (90). Tryptophan is transported across the blood-brain barrier (BBB) by the same carrier of large neutral amino acids (LNAA) tyrosine, valine, leucine, isoleucine, histidine, and threonine. Therefore, the rate of tryptophan entry into the brain is strongly influenced by the presence of competing LNAA (56). transport competition concerns carbohydrate versus protein intake.

Garrison (59) explained that by eating a complex carbohydrate (CHO) meal

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Many faddist claims exist regarding supplemental tryptophan's use as a therapeutic agent. It is marketed most commonly in 500 milligram tablets with recommended dosages of one to two tablets per day.

Tryptophan is being promoted as a depression fighter (59, 60), sleep aid (12, 59, 60, 138), treatment for migraine headaches (59), immunity enhancer (59), and weight control (12).

The claim that tryptophan is a depression fighter arises from its function as a serotonin precursor. Lee (60) stated that "tryptophan helps form a neurotransmitter called serotonin, which can alter reactions to stress and mood swings, changing excitement and confusion to calm and understanding . . . when phenylalanine and tyrosine act to increase excitement as a means of combating depression, tryptophan is more of an emotion stabilizer that fights depression while producing none of the side effects sometimes experienced with drugs." No dosage recommendations were presented in Lee's literature.

Enhanced serotonin production is supposedly responsible for tryptophan's use as a sleep inducer. Pearson and Shaw (12) recommend two grams of tryptophan at bedtime on an empty stomach as a sleep aid. Most promoters of tryptophan's use do point out the necessity of taking tryptophan on an empty stomach in order to prevent the amino acid uptake (transport) competition at the BBB. Another mechanism to decrease transport competition concerns carbohydrate versus protein intake. Garrison (59) explained that by eating a complex carbohydrate (CHO) meal

with little or no protein as the last meal of the day and then taking the tryptophan supplement, little competition into the brain will exist. Lee (60) suggested taking the tryptophan supplement with carbohydrate. "Starches and other CHOs stimulate the body's insulin production, which pulls most of the other amino acids out of the bloodstream except for L-tryptophan, enabling more of this essential amino acid to cross the BBB."

Claims of tryptophan's use for migraine headaches is based on findings that a drop in serotonin levels in the blood coincides with migraines. Therefore, tryptophan supplements may raise serotonin blood levels, thus relieving headaches (59).

The claim of tryptophan's use to enhance immunity is based on reports that a deficiency of tryptophan inhibits the antibody-forming response. It was implied that tryptophan supplementation would prevent this inhibition (59). Although no statements as such were found, it is possible that tryptophan stimulates the release of growth hormone (GH) accounting for increased immunity.

Pearson and Shaw (12) suggested the use of one or two grams tryptophan at bedtime for weight control. Their rationale presumes that since tryptophan stimulates GH release from the pituitary, GH preferentially causes one to catabolize fat and form muscle tissue.

Several precautions were offered in promotional literature accompanying OTC tryptophan supplements. KAL Series #7 (138) warned against the regular use of any sleep-inducer. "If you need 'something extra' to help you get to sleep, this is usually an indicator that some other nutritional deficiency or potentially serious illness may be at

work" (138). Pearson and Shaw (12) stated that too much tryptophan could cause headaches, sinus congestion, or constipation. Tryptophan and oxygen (1) Tryptophan is absorbed into the bloodstream where 10-to-20 percent circulates as the free amino acid; the remainder is bound to serum albumin within two hours after ingestion (139, 140). Ingested tryptophan also increases tryptophan and 5-hydroxyindoleacetic acid (5-HIAA) levels in cerebrospinal fluid (CSF) which indicates that tryptophan hydroxylase may not be fully saturated in humans (140, 141). Due to the competition from other LNAA, the amount of tryptophan that actually crosses the BBB depends upon the ratio of tryptophan to other LNAA (122, 142).

Fernstrom and Wurtman (143) demonstrated that physiological amounts of tryptophan increased plasma and brain tryptophan and brain serotonin levels in rats along with an increase in 5-HIAA levels indicating increased serotonin metabolism. The same researchers also showed that insulin, or a high carbohydrate (CHO) meal which would stimulate insulin release, lowered plasma levels of most LNAA, but did not affect the albumin-bound tryptophan (144, 145); thus, as tryptophan increased, brain serotonin levels increased (144). Colmenares et al. (146) showed that the increase in brain serotonin was due to increased synthesis of serotonin. Gillman and associates (141) reported increased tryptophan concentration in frontal cortex, ventricular CSF, and lumbar CSF following infusion of 15 milligrams per hour of tryptophan into patients undergoing neurological operations. In addition, 5-HIAA concentration was significantly increased in both ventricular and lumbar CSF.

In the brain, tryptophan is hydroxylated by tryptophan hydroxylase into 5-hydroxytryptophan (147) which is rapidly decarboxylated

to form serotonin (5-hydroxytryptamine) (148). Tryptophan hydroxylase is normally unsaturated with respect to its substrates tryptophan and oxygen (149). Therefore, it was speculated that an increase in substrate would increase amounts of serotonin produced (150). The major catabolite of serotonin metabolism in brain is 5-hydroxyindoleacetic acid (5-HIAA) (151).

The use of tryptophan as an anti-depressant remains controversial. Several investigators support the theory that endogenous depression may be due to a defect in central serotonergic neurotransmission (152, 153, 154). Glassman and Platman (157) established that tryptophan in combination with monoamine oxidase inhibitors can relieve some depressions. However, test results using tryptophan alone on depressive patients were inconclusive (155, 156). While some investigations report an anti-depressant effect, others find no benefit from tryptophan administration. Recent evidence suggests there may be an optimal dose range for tryptophan. Young and Sourkes (156) reported that a mean dosage of six grams per day tended to be anti-depressive while a mean dose of 8.7 grams per day was ineffective. Beitman and Dunner (159) suggested an effective dosage range of two-to-twelve grams per day.

Moller and coworkers (107) measured the ratio of plasma tryptophan to its neutral amino acid (NAA) competitors in 32 patients with depression. Eighty percent of the patients had decreased plasma tryptophan ratios; twelve patients improved during tryptophan ingestion. DeMeyer et al. (160) concluded that the plasma tryptophan ratio was inversely correlated with the severity of depression: the lower the ratio, the greater the depression.

Moller et al. (161) recently suggested that depression involves a decreased sensitivity of serotonin receptors. In addition, anti-depressive therapy may require normalization of receptor sensitivity rather than merely enhancing the amount of serotonin.

There is considerable evidence to support the hypothesis that serotonin plays a role in regulation of pain sensitivity. An increase in plasma tryptophan increases serotonin levels which correlate with elevations in pain threshold (162). In animals, consumption of a tryptophan-poor diet increased pain sensitivity (163). It has been reported (164) that tryptophan administration was beneficial in relieving patients with chronic pain; tryptophan at three grams per day plus a high CHO diet decreased human pain sensitivity (163). Lieberman et al. (114) concluded that tryptophan at 50 milligrams per kilogram body weight in humans may act as a mild analgesic, similar to aspirin or acetaminophen.

Current research favors tryptophan's use in weight control. However, contrary to Pearson and Shaw's (112) theory that tryptophan's ability to increase GH accounts for its use in combating fat, many researchers attribute tryptophan's use in weight control to its appetite-suppressant qualities (117, 152). The experimental use of tryptophan in appetite control is based on the theory that serotonergic neurons act as sensors for plasma amino acid concentration (165, 166). Various workers have suggested that serotonin may play a general inhibitory role in feeding and induce feelings of satiety (167, 168). In general, the serotonin precursors tryptophan and 5-hydroxytryptophan enhance the synaptic activity of serotonin and bring about inhibitions of feeding (169, 170). Blocking synaptic serotonin activity leads to overeating

and obesity. Synaptic serotonin activity can be blocked by parachlorophenylalanine (PCPA) which inactivates tryptophan hydroxylase or 5,6-dihydroxytryptamine which is believed to cause degeneration of nerve terminals (171, 172).

Serotonin may also control the selection of protein in the diet (117, 173, 174). Wurtman and Wurtman (175) gave drugs to rats that enhanced serotonergic transmission and found that they decreased CHO and calorie consumption without affecting protein intake. Anderson (176) observed an inverse relationship between the quantity of protein the rat selected and the plasma ratio of tryptophan to LNAA.

In general, treatments which lead to increased availability of 5-HT in the synaptic cleft or which activate 5-HT receptors reduce food consumption; procedures which decrease 5-HT receptor activation increase food consumption (171).

An alternate theory regarding tryptophan's inhibition of feeding involves cholecystikinin (CCK). Although tryptophan probably does not influence CCK synthesis, it has been shown to be a strong stimulator of CCK release (177); CCK is a potent appetite suppressant.

Weekely et al. (172) recently suggested the possibility of serotonin involvement in the development of obesity. Their hypothesis was based upon confirmation of previous studies that reductions in brain 5-HT result in hyperphagia in rats (178, 179). It also has been reported that the obese strain of Osborne-Mendel rats exhibit lower brain 5-HT levels than do normal strains (172).

It appears that tryptophan may play a role in feeding behavior. However, exact mechanisms are yet to be defined, as are proper (safe)

dosages required to induce feeding inhibition. Tryptophan's use by the general public for weight control should be discouraged due to incomplete research on the subject and the possible side effects involved with excess tryptophan intake. *et al.* (191) studied the relationship between dosage and Serotonin is believed to be important in the induction and maintenance of sleep (180). Hence, many researchers have evaluated L-tryptophan as a possible hypnotic agent in the treatment of insomnia. Results have been contradictory (181, 182, 183, 184, 185). Lindsley *et al.* (186) suggested that serotonin was effective in some sleep disorders but not in others. Due to the large array of sleep disorders (187) and research pointing to several neurotransmitter systems that work together to initiate and organize sleep (188, 189, 190), it is suspected that more than one neurotransmitter may be responsible for sleep or insomnia. Data suggest the possibility that different sleep disorders arise from very distinct systems. For example, tryptophan may act only on those with a disturbance along the tryptophan/serotonin/5-hydroxyindoleacetic acid pathway, but be ineffective in other types of sleep disorders (186). In a study on 54 male and female insomniacs fed one gram tryptophan daily, a variance in results was seen. Lindsley *et al.* (186) suggested that chronic insomniacs were separable into tryptophan responders or non-responders on the basis of a subjective report on sleep maintenance difficulty. These subjective reports were based on the subjects' opinions reflecting their overall experience with the treatment, day-to-day rating of their sleep experiences, and open-ended comments solicited on daily questionnaires. The non-responders were those either with a complaint of interrupted sleep or a complaint of

dozing rather than deep, quality sleep. These data suggest an explanation for the conflicting results of tryptophan supplementation in previous studies.

earlier reports (196, 197, 198) that either oral or intravenous tryptophan. In 1979 Hartman et al. (191) studied the relationship between dosage and efficacy of tryptophan to induced sleep maintenance in humans.

The subjects ate a casein meal with either no tryptophan or one-fourth, one-half, one, or four grams of tryptophan added. Four grams of L-

Potential Hazards of Tryptophan Excess
Dietary tryptophan can be decarboxylated in the gut by intestinal bacteria to produce the toxic amines indole and skatole (11). Skatole produced a small trend in the same direction, whereas lower doses were not different from the control. Several investigators have recently reported that 50 to 500 milligrams of tryptophan have increased fatigue and decreased vigor and alertness (114, 192, 193). Infants fed a single dose of tryptophan (33 milligrams per deciliter in a ten percent glucose solution) entered quiet sleep sooner and spent more time asleep than with a single dose of valine (50 milligrams per deciliter in a five percent glucose solution) (194).

There is overwhelming evidence that L-tryptophan has a positive effect on sleep. These effects include increased sleepiness, reduced

sleep latency and decreased waking time (192). The mechanisms for these effects have yet to be established.

Evidence has accumulated that nitrosation of a variety of amines, amides, ureas, and guanidines can occur and yield potentially cancerous

nitrosos compounds (201, 202, 203). In 1983 Gatehouse and Wadd (201) demonstrated that tryptophan, tryptamine, and 5-hydroxytryptamine were directly mutagenic to *S. typhimurium* TA 1537. In 1982, Matsushima et al. (204) suggested that certain L-tryptophan metabolites may be involved in urinary bladder carcinogenesis.

of tryptophan present a very complicated picture of tryptophan's efficacy as a sleep aid. None of these variations are mentioned in the literature accompanying tryptophan's sale.

No support for tryptophan supplementation to increase the immune response, per se, was found. However, in 1982, Charney et al. (195) confirmed earlier reports (196, 197, 198) that either oral or intravenous tryptophan administration increased GH in six of the ten subjects studied. A discussion of GH and its role in immunity was presented in chapter IV.

Potential Hazards of Tryptophan Excess

Dietary tryptophan can be decarboxylated in the gut by intestinal bacteria to produce the toxic amines indole and skatole (11). Skatole has been shown to be involved in arthritic injury in rabbits (199, 200). It is conceivable that skatole and indole might initiate inflammatory processes in the joints of man when present in high concentrations, either by increased formation in the intestinal tract, or from decreased detoxification of skatole in the liver. It was suggested that these agents interact with the lipid components of cell membranes and interfere with metabolic processes and/or release of lysosomal contents from the cells. Lysosomes may ultimately be responsible for the inflammation in arthritis (199).

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Tryptophan supplementations of one-and-one-half-to-ten grams have resulted in growth depressions in rats (6). Excess tryptophan has also produced central nervous system disturbances in man (201, 205), cataracts in rats (10), and pulmonary emphysema in cattle (206). In 1984 Thomas and Rubin (207) reported acute behavioral and neurologic symptoms following tryptophan administration in a patient taking phenelzine, an anti-depressant. This toxicity developed while the subject was taking 90 milligrams of phenelzine and six grams of L-tryptophan daily. Recently, Baloh and associates (208) described myoclonic movements and ocular oscillations in a 26-year-old woman who took two grams of tryptophan in addition to 20 milligrams of phenelzine daily.

L-tryptophan (100 milligrams per kilogram body weight) administered orally to normal subjects and adult-onset diabetics caused elevations in plasma insulin (209). Therefore, tryptophan supplementation could be especially hazardous for diabetics. Lloyd and associates (210) have reported on tryptophan's ability to induce hypoglycemia in rats. Tryptophan supplementation has also caused increased fatty acid synthesis in rat liver which could lead to fatty livers (11, 211).

Histidine shares a transport system across the blood-brain barrier (BBB) with other large, neutral amino acids: phenylalanine, tyrosine, tryptophan, valine, leucine, isoleucine, and threonine. All are transported at similar rates (56).

Histidine is available in 500 milligram tablets with a recommended dosage of one tablet daily. It is being promoted for a limited number of ailments. One of these is sexual dysfunction. This claim is based on the fact that histidine is a precursor of histamine

CHAPTER VIII

and histamines are released during sexual excitation. Pearson and Shaw

(12) promote niacin supplements along with histidine-containing foods

to enhance sexual function. They claim the combination of these two

Histidine is considered nutritionally semiessential because it is a nutrient causes the mucous membranes of the mouth and sexual organs to be synthesized at rates inadequate to support the growth of children (11).

secrete mucus in response to sexual activity. This concept was promoted. Like lysine and arginine, histidine is a basic amino acid (20).

to maintain sexual capacities or to possibly enhance a depressed sexual

Histidine is biosynthesized from the condensation of ATP and drive. Neither recommended dosages of histidine nor specific references phosphoribosylpyrophosphate (20). It is converted to glutamic acid and

to substantiate the claims were given. subsequently enters the Krebs cycle as alpha-ketoglutarate. Histamine, a

Another proposed use of histidine supplements is in the treatment of powerful vasopressor (11), is synthesized from histidine by histidine of arthritis. The basis for these claims involves histidine and histidine-decarboxylase (2.2).

minerals as chelators of trace elements such as copper and zinc. "In cer-

Several investigators have shown that brain histamine levels may be increased by oral histidine administration (213, 214). In addition,

heavy metals. Because of the chelating properties of histidine, it is both brain histamine and histidine levels are elevated when plasma his-

tidine levels are increased (215). Brain uptake of histidine correlates

with the ratio of histidine concentration and the competing neutral amino

acid concentration (216). Histidine is involved in regulation of urea

production; it is ammoniogenic, i.e., it produces ammonia rather than

aspartate during catabolism (56, 217).

references were given.

Histidine shares a transport system across the blood-brain barrier

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Another proposed use of histidine supplements is in the treatment of arthritis. The basis for these claims involves histidine and histamines as chelators of trace elements such as copper and zinc. "In certain forms of arthritis, there is a tissue overload of copper and other heavy metals. Because of the chelating properties of histidine, it is sometimes used in the treatment of arthritis to remove these heavy metals" (60). Lee (60) stated that L-histidine has been under investigation as a possible treatment for rheumatoid arthritis ever since it was found that arthritics have approximately one-fourth as much histidine in their blood as non-arthritics. Once again, no dosage recommendations nor references were given.

Lee (60) also suggested L-histidine use in the treatment of allergic diseases ". . . studies have shown that as levels of dietary histidine increase, the concentration of histamine decreases. Histamine

is a biochemical which occurs normally in the soft tissue of the body and is released during trauma and other stressful situations" (60). Lee postulated that since histamine is released during trauma and stress, then increasing histidine would lower histamine and result in less trauma and stress and stressful situations. This postulate is in direct contrast to Pearson and Shaw's (12) view that increased histidine intake will increase histamine which will prove beneficial to sexual functioning.

No evidence was found supporting histidine's use for treatments of sexual dysfunction, per se. However, there is evidence that increased dietary histidine results in increased histamine synthesis (218, 219). Namsoo et al. (218) reported that as dietary histidine increased in rats, tissue concentrations of free histidine and histamine increased in all tissues analyzed; the greatest increase was seen in brain and stomach. By contrast, chick studies have shown that tissue histamine concentrations decreased as histidine intake increased (220).

No evidence was found supporting supplemental histidine use to treat arthritis. What is known, however, is that histidine is a chelator of copper and zinc which presupposes that arthritis is the result of copper and/or zinc excess.

Henken et al. (221) reported that large daily doses of L-histidine (32 to 64 grams) could temporarily reduce smell and taste acuity. In contrast, Geliebter et al. (222) and Schechter and Prakash (223) found no relationship between histidine supplementation and taste and smell alterations. Several studies have shown that histidine doses of eight grams daily deplete the human body of zinc and produce anorexia

(222, 224, 225, 226). L-histidine strips zinc from its binding sites on albumin and is subsequently excreted in the urine bound to histidine (224, 227, 228).

Although Lee's (60) postulate that increased histidine decreases histamine during stressful situations does not coincide with current research, some support is available for the use of histidine during trauma and stress (218, 229).

Fitzpatrick and Fisher (229) have reported a relationship between carnosine (beta-alanyl-L-histidine a dipeptide that is thought to act as an histamine reservoir), histidine, and wound healing. They found that histidine (one milligram per 100 grams body weight per day) given intraperitoneally for seven days after wounding increased tissue-free histidine concentrations and brought skin-breaking strength and collagen deposition up to "normal" in animals fed low histidine diets. No improvement of healing was seen in rats fed histidine-sufficient diets. The results suggested an interaction between carnosine and stress and implicated carnosine as a histamine reserve during trauma (229). It was also concluded that wounding and other types of stress increase histamine synthesis thus creating an increased requirement for histidine, the precursor of histamine.

Potential Hazards of Histidine Excess

Several detrimental effects of excess histidine have been reported. In 1970 Harper et al. (230) noted that histidine-imbalanced diets resulted in reduced brain levels of histidine which was associated with depressed feeding. Menon et al. (231) provided evidence that histidine

histidine decarboxylase inhibitor decreased food and water intake. More recently, Tobin and Boorman (232) using histidine and lysine infusions suggested that excesses of single amino acids affect food intake which could be attributed to a sensitive area in the brain. Apparently, the brain detects deranged amino acid patterns resulting from imbalance and attempts to maintain homeostasis by decreasing food intake (232, 233).

Several investigators have reported various toxicities attributable to excess histidine intake (128, 222, 234, 235). Lesions were produced in both soft and hard tissue when mice were intraperitoneally injected with excess histidine (128). Growth depressions in rats and chicks varying from slight to severe depending upon dosages of histidine (1.6 to 10 times normal) have been documented (130, 234, 235, 236, 237, 238). Kim and Park (234) recently reported depressed food intake and growth retardation in three groups of mice fed a five percent dietary supplementation of L-histidine, L-arginine-HCl, or L-lysine. Of the three amino acids, L-histidine-HCl decreased food intake and growth the most.

In 1961, Miyazaki et al. (239) found that DL-methionine, DL-tryptophan, L-phenylalanine, and L-histidine were the most toxic of the essential amino acids for rats in that these four caused greater growth depressions than any of the other essential amino acids. Muramatsu et al. (240) confirmed the findings. More recently, Giliebter et al. (222) reported symptoms of painful sensations in the eyes and difficulty focusing, anorexia, mental confusion, and depression in the human adult volunteers given 64 grams histidine per day for two and 1/2 days. The presence of excess histidine in the blood of pregnant women

was reported to be associated with decreased cranial volume and decreased mental development in fetuses (241).

An inborn error of metabolism, histidinemia, exists due to inadequate activity of liver histidase which is responsible for the conversion of histidine to urocanic acid (11). Reduced histidase activity is responsible for elevated levels of histidine in blood and urine and increased urinary excretion of imidazolepyruvic acid (11). Histidinemia manifests itself in liver, spleen, and kidney damage and decreased speech development and soft, friable bones (128, 242, 243).

Excess histidine has also been implicated in hypercholesterolemia (244, 245, 246, 247, 248). Harvey et al. (244) found a 131.8 percent elevation of plasma cholesterol concentration in rats fed a stock diet supplemented with eight percent L-histidine. They suggested that the hypercholesterolemia was caused by changes in copper status since the effect could be abolished by simultaneous feeding of copper. Eichelmann et al. (248) suggested that the hypercholesterolemia was caused by an inhibitory effect of excess L-histidine on rat liver acetyl-CoA carboxylase and fatty acid synthetase. Although the exact mechanism of the alterations in lipid metabolism remains obscure, excess histidine is known to cause increased blood levels of cholesterol, triglycerides, and phospholipids in rats and monkeys (244, 249).

As mentioned earlier, histidine has been shown to be ammonigenic (6, 217). Therefore, excessive intakes of histidine could result in hyperammonemia and thereby, alter the efficiency of ureagenesis.

Considering the current evidence on histidine metabolic properties and potentials in addition to the undocumented claims of its benefits, it appears necessary to await further investigation before histidine supplementation can be considered safe for OTC use by consumers.

CHAPTER IX

PHENYLALANINE

Phenylalanine is available most commonly in 500 milligram tablets and is recommended for various therapeutic uses in dosages of one or two tablets daily. It is a nutritionally indispensable, aromatic amino acid and is also the precursor of the amino acid tyrosine (11). Phenylalanine is naturally occurring and widely distributed in most proteins of plant and animal origin (87).

Food faddists believe that phenylalanine has therapeutic value for weight control, learning and memory improvement, arthritic pain control, and depression control (12, 59, 60, 88).

The rationale behind phenylalanine's use as an anti-depressant and an enhancer of learning and memory is based upon the fact that phenylalanine is the precursor of tyrosine which is the precursor of norepinephrine (NE) (12, 88). Pearson and Shaw (12) stated that when brain NE levels are low, people become depressed. The citation for this statement referred to an undocumented study by Dr. J. M. Weiss who allegedly plunged rats into very cold water to produce a state of shock. A correlation was reported between NE brain levels and the rats' ability to escape. Results showed depleted NE levels in those rats who displayed helplessness.

Pearson and Shaw (12) concluded that "if NE depletion is significantly

involved in the biochemical events of learned helplessness, then phenylalanine or tyrosine should be effective in relieving human depression from a variety of causes." They recommend dosages from 100 to

100 milligrams per day taken on an empty stomach. KAL Series #4 (88)

CHAPTER IX

states, "supplemental dosages of 100-300 milligrams taken on an empty

stomach have been shown to help sharp and spirits up." No documentation was cited for these claims:

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They are required for the ultimate conversion of phenylalanine to NE (12). Pearson and Shaw (12) concluded that "if NE depletion is significantly KAL Series #4 (88) suggests that NE, "made from phenylalanine," causes

involved in the biochemical events of learned helplessness, then phenylalanine or tyrosine should be effective in relieving human depression from a variety of causes." They recommend dosages from 100 to 500 milligrams per day taken on an empty stomach. KAL Series #4 (88) states, "supplemental dosages of 100-500 milligrams taken on an empty stomach have been shown to keep our wits sharp and spirits up." No documentation was cited for these claims.

Phenylalanine's role in weight control was also attributed to its precursor status in NE formation (12, 60). Lee (60) stated that NE works in two ways in appetite control: first, for those who overeat due to emotional stress, NE "makes people feel better about themselves and and more able to resist uncomfortable situations"; secondly, NE helps to stimulate cholecystokinin (CKK) which "convinces the brain that the stomach is full and needs no more food." In addition, phenylalanine is claimed to promote weight loss by helping in the manufacture of thyroxine, a hormone needed to regulate the metabolic rate of the body, i.e., "how fast food is burned up" (60).

Pearson and Shaw (12) reported that the brain uses phenylalanine to make NE which is needed for communication between certain nerve cells that are involved in appetite regulation. A distinction was made by Pearson and Shaw (12) between the D and the L forms of phenylalanine. They claimed that the L form was more effective in weight loss while the D form was more effective in pain control. In addition, they recommended supplemental vitamins C and B-6 along with phenylalanine since they are required for the ultimate conversion of phenylalanine to NE (12). KAL Series #4 (88) suggests that NE, "made from phenylalanine," causes

indicates that phenylalanine supplement promoters may have a much too simplistic view of its properties. It is a fact that phenylalanine catabolism gives rise to tyrosine (250). It is also true that tyrosine is involved in NE and dopamine production. However, the deduction that increased phenylalanine intake will increase neurotransmitter function needs further investigation.

Phenylalanine is claimed to be an effective pain reliever (12, 60). D-phenylalanine supposedly inhibits the enzymes that catabolize the brain's natural morphinelike pain killers, enkephalins and endorphins (12).

Lee (60) explained that the brain manufactures endorphins which closely

resemble the narcotic pain reliever morphine. "It is speculated they factors influenced the brain content of an amino acid are its plasma concentration and the concentrations of its competitors (251, 252). There-kind was very young and needed to ignore pain while escaping a life-threatening situation" (60). However, now, enzymes are released to destroy endorphins. Lee (60) promotes use of the DL form of phenylalanine to inhibit such enzyme release, thus leaving the body's own pain relievers free to act.

Several precautions were offered by Pearson and Shaw (12)

In fact, elevations in brain phenylalanine and tyrosine could concerning supplemental phenylalanine. They warned hypertensive subjects that phenylalanine supplementation should be used with caution and under the supervision of a physician. Phenylalanine supplements should not be used by those with phenylketonuria or with pre-existing pigmented melanomas. In addition, they stated the possibility of phenylalanine supplementation producing irritability, headaches, or insomnia and recommended lowering the dosage if this occurred. "Continuous use of large doses of phenylalanine on an empty stomach to increase its availability to the brain, has not been tested for chronic toxicities. Such use must be considered experimental" (12).

A closer look at the biochemistry involved in the conversions of phenylalanine to tyrosine and subsequently to NE, dopamine, and CCK

indicates that phenylalanine supplement promoters may have a much too simplistic view of its properties. It is a fact that phenylalanine catabolism gives rise to tyrosine (250). It is also true that tyrosine is involved in NE and dopamine production. However, the deduction that increased phenylalanine intake will increase neurotransmitter function needs further investigation.

For instance, it has been shown in normal rats that the main factors influenced the brain content of an amino acid are its plasma concentration and the concentrations of its competitors (251, 252). Therefore, the increases seen in brain content and function of ingested amino acids depends in part upon the presence of other amino acids and their plasma concentrations. Brain uptake of tyrosine and phenylalanine might be enhanced as a result of: (a) increased plasma levels or (b) lack of competition by branched-chain amino acids (BCAA) (212).

In fact, elevations in brain phenylalanine and tyrosine could cause substrate inhibition of tyrosine hydroxylase, thereby slowing catecholamine synthesis. Excess amino acids could be diverted to decarboxylation, generating phenylethylamine and tyramine (212). Catecholamines are allosteric inhibitors of their own synthesis at the level of tyrosine hydroxylase. Thus, when the hormones are mobilized rapidly, synthesis is reduced accordingly (11).

Duration of amino acid intake could also affect their function. Anderson (176) found that a comparison between long-term and short-term feeding studies indicated that while the short-term effect of a high protein meal was to increase brain tyrosine and NE turnover, chronic high protein feeding actually decreased brain tyrosine levels.

The effectiveness of phenylalanine in treating depression is controversial. D-phenylalanine at mean dosages of 350 milligrams per day have been reported to be comparable to imipramine in its effectiveness against depression (111, 253, 254). Administration of D-phenylalanine has also been reported to increase brain phenylethylamine concentration in animal experiments, suggesting that this might be the mechanism of anti-depressant action (253, 255).

In contrast, Mann et al. (253) found no evidence for the anti-depressant effect of D-phenylalanine in the dosages reported in previous literature (350 milligrams per day). Mann et al. (253) also questioned the validity of studies supporting phenylalanine efficacy due to inconsistent dosages claimed to be effective (253, 254, 256, 257).

Phenylalanine supplements are also promoted as learning and memory enhancers. The basis for this claim is, once again, the connection between phenylalanine and the production of brain NE (88). "NE is a neurotransmitter. This means it carries messages from one nerve cell in the brain to another. Since NE is related to our clarity of thought, memory, and positive feelings of being in control, we can see that a lack of NE destroys alertness and leads us into depression" (88).

While it is established that phenylalanine affects brain chemistry, a close investigation of the literature does not provide as positive a view as promoters of amino acid supplements lead us to believe. In direct contrast to claims of "mind improvement," researchers found permanent learning impairment and abnormal learning patterns in monkeys with high phenylalanine levels (258, 259, 260, 261).

Much of the investigative work on phenylalanine excess involves phenylketonuria (PKU), a metabolic disease characterized by elevated serum levels of phenylalanine producing brain damage, mental retardation (262, 263), and behavioral abnormalities (258, 264). Experimental hyperphenylalanemia (HPA) was induced in newborn Rhesus monkeys by feeding three grams of L-phenylalanine per kilogram body weight for one year after birth resulting in abnormally high blood phenylalanine levels. The HPA monkeys were more withdrawn, more aggressive, less playful, and more emotionally reactive than controls; alterations in brain serotonin was suggested as an indirect cause of the abnormal behavior (258). Taylor et al. (265) found 50 percent decreases in serotonin and 5-hydroxyindoleacetic acid (5HIAA) due to increased blood phenylalanine levels in rats. Dillehay et al. (262) presented evidence that increased concentrations of phenylalanine in mouse cell cultures inhibited growth, was a potent inhibitor of protein synthesis, and decreased the initial rate of uptake and steady state levels of several amino acids.

Various neurological alterations were attributed to inhibition of protein synthesis (266, 267, 268, 269). High phenylalanine concentrations led to decreased myelination of the brain in experimental animals (270, 271, 272, 273). Inhibition of brain protein synthesis was attributed to inhibition of protein initiation and elongation (270, 273) as well as the release of lysosomal ribonucleases and other lytic enzymes into cerebral cytosol (269, 274). In addition, brain polyribosomal disaggregation results from high serum phenylalanine concentrations (273). Roberts and Morelos (269) suggested that high concentrations of phenylalanine altered cerebral ribosomal phosphorylation which could selectively

influence the ability of the ribosomal population to translate certain messenger RNA species in the brain.

Hogan and Coleman (268) postulated that the mental retardation resulting from HPA may be due to alterations of dendritic structure. Experimental HPA in rats produced fewer, shorter, and less dense dendrites than in control animals (266, 267, 268). Hogan and Coleman (268) suggested that HPA in humans, which is not severe enough to produce classical PKU, may produce alterations in the morphology of cortical dendrites and brain dysfunctions.

Phenylalanine's relationship to weight control is another unsubstantiated claim from phenylalanine promoters. Claims have been made that since phenylalanine is a precursor of tyrosine and NE and GH production, increasing the dietary intake of phenylalanine will increase NE, GH, and CCK production, thereby increasing fat catabolism and muscle anabolism (12, 60, 88). Epinephrine, NE, glucagon, adrenocorticotrophic hormone, and GH do, in fact, increase the rate of lipolysis from adipose tissue. However, on their own, these particular hormones do not enhance lipolysis markedly, but act in a facilitatory or permissive capacity with respect to the glucocorticoids and thyroid hormones (11).

The ability of phenylalanine to "convince the brain that the stomach is full" (12) appears to have some credence. Smith and Gibbs (119) have shown that CCK does inhibit feeding. It was claimed that phenylalanine helps stimulate the production of CCK (12). Martin et al. (11) stated that it is very unlikely that amino acid precursors of CCK influence its synthesis due to genetic controls. However, it has been

established that both tryptophan and phenylalanine are strong stimulators of CCK release (11, 177) and that CCK is a potent appetite suppressor (11, 119).

Pain control by use of phenylalanine supplementation is another undocumented claim by phenylalanine promoters. The basis for the claims discussed earlier involved DL-phenylalanine's presumed ability to inhibit enzymes which destroy endorphins and enkephalins, thus allowing these hormones to exhibit their analgesic properties. This information was supposedly derived from an unpublished report by Ehrenpreis, a pharmacologist at the University of Chicago, who detailed results of the clinical use of D-phenylalanine for various pain conditions (60).

Potential Hazards of Phenylalanine Excess

In addition to the previously mentioned neurological disturbances resulting from high serum phenylalanine concentrations, several other metabolic alterations have been reported following excess phenylalanine intake. Alterations in amino acid balances in the brain, blood, and liver have been seen following injection of a phenylalanine load into rats (275, 276). The net transport of valine, methionine, leucine, and isoleucine across the BBB was reduced 50 percent intracerebrally after phenylalanine intake (277, 278, 279).

Excess phenylalanine has also induced intracerebral lipid depletion in rats (280, 281, 282). The synthesis and transport of carbohydrate for incorporation into cerebral lipids was inhibited by increased phenylalanine levels (283). Excess phenylalanine may cause growth depressions due to niacin deficiency (284) or enzymatic disturbances (285).

Oral phenylalanine loading in man (0.6 millimoles per kilogram body weight) has been shown to increase serum insulin and glucagon possibly leading to a hypoglycemic reaction (275). Phenylalanine may promote melanoma growth (286).

Finally, evidence exists that high phenylalanine intakes produce a tyrosine deficiency which impairs catecholamine synthesis in the nervous system and melanin formation (287). Reduced melanin formation would result in lightening of the hair and decreased skin pigmentation.

As with many of the other amino acids, the safety and efficacy of phenylalanine supplementation has not been established. In view of the numerous abnormalities associated with excess phenylalanine and the tentative nature of the purported therapeutic advantages, dietary supplements of phenylalanine cannot, at this time, be recommended.

Glutamine is a major nitrogen donor in the biosynthesis of non-essential amino acids and a variety of compounds such as carbamoyl phosphate, glucosamine-6-phosphate, and adenosine monophosphate. About one-half of the glutamine released from peripheral tissues is transformed to alanine in the liver where it provides a substrate for gluconeogenesis (36). Glutamine appears to play a key role in the overall exchange of amino acids between various tissues.

Glutamine is being promoted for use in the treatment of alcoholism, schizophrenia, ulcers, as a memory and intelligence enhancer, depression fighter, antifatigue agent, and to control sweet cravings. It was pointed out in the literature accompanying the sale of glutamine that glutamine should be used instead of glutamic acid since the latter

CHAPTER X

GLUTAMINE

Glutamine, the amide form of glutamic acid, is a nutritionally non-essential amino acid which is of fundamental importance for amino acid biosynthesis in all forms of life. Synthesis of glutamine is catalyzed by glutamine synthetase in which ammonia is transferred to glutamic acid to form glutamine in a reaction requiring ATP (11). Liberation of the amide nitrogen from glutamine occurs by the hydrolytic removal of ammonia catalyzed by glutamase.

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Glutamine is being promoted for use in the treatment of alcoholism, schizophrenia, ulcers, as a memory and intelligence enhancer, depression fighter, antifatigue agent, and to control sweet cravings. It was pointed out in the literature accompanying the sale of glutamine that glutamine should be used instead of glutamic acid since the latter

does not pass through the blood-brain barrier (BBB) as readily as glutamine. Recommended dosages range from one-to-ten grams daily depending upon the particular "treatment" desired (59, 60, 138). OTC glutamine is marketed in 500 milligram tablets with recommended dosages of one-to-four tablets daily.

According to Garrison (59) and Lee (70) the use of glutamine in the treatment of alcoholism is based upon observations by R. J. Williams (noted for his contributions to the structure and synthesis of thiamin) that L-glutamine protected rats against the poisonous effects of alcohol and also stopped the craving for it. KAL Series #7 (138) reports that the craving for alcohol by alcoholics may be due to an increased need for glutamic acid in the brain. "Researchers theorize that alcohol may improve transport of glutamic acid or glutamine across the BBB or speed absorption of glutamic acid by brain cells." According to this theory, if sufficient dietary glutamine were supplied, the increased brain needs would be met and alcohol would no longer be required as a solvent to carry glutamic acid across the BBB into the cells.

The purported use of glutamine supplements for memory and intelligence improvement, anti-depressant, and as an anti-fatigue agent all stem from its ability to be used as fuel by the brain (59, 60, 138). Lee (60) reported that the brain barrier is designed to protect the brain from substances that might be injurious. In some cases, this protective feature may work against the "well-being of the brain." He explained that the brain uses glucose and glutamic acid for fuel. Unfortunately, glutamic acid penetrates the BBB poorly. "Fortunately, by using a bit of nutritional magic, you can give the brain the fuel it needs and help

increase its activity, including long and short-term memory" (60). This "magic" is glutamine since it easily crosses the BBB (60). The rationale, therefore, is to supply the brain with more fuel which will presumably improve brain functions. KAL Series #7 (138) stated dosages of 10 grams have been used to improve the IQ of children and that "dosages of one-to-ten grams appear to be quite safe." No documentation was provided for either claim.

No explanation nor documentation was given for use of glutamine supplementation for schizophrenia, ulcers, or sweet cravings.

Scientific studies on glutamine, per se, were sparse. No supporting evidence was found for the proposed uses of glutamine supplements. One possible remote exception, however, may involve the use of glutamine in suppressing inflammatory reactions. A study conducted in 1981 demonstrated anti-inflammatory and analgesic properties of glutamine (288). Rats were administered an oral solution of glutamine at 0.3 to 1.0 milliliters for every 100 grams body weight. Several experimental inflammatory reactions were initiated. It was established that L-glutamine was effective in suppressing various inflammatory reactions and did not invoke any gastric irritation. It was suggested that L-glutamine may interfere with the action or synthesis of prosta-glandins.

L-glutamine has been used in the treatment of human cystinuria with conflicting results (289, 290, 291, 292). While Miyagi and colleagues (290) reported positive results from use of eight grams of L-glutamine per day, they felt this was too large a dose to use for long-term purposes. Korein (292) disagreed, stating dosages of 30-to-60 grams per day had been used for periods of months to three years with minimal

side effects (elevations in blood urea nitrogen and creatinine). These side effects subsided with decreased dosages plus an intake of one liter of water per day.

Potential Hazards of Glutamine Excess

Although L-glutamine is one of the least toxic amino acids, it has been known for some time that L-glutamine can destroy neurons in the mammalian central nervous system (293, 294). Olney et al. (295) suggested that the neurotoxic action of excitatory amino acids was related to their ability to depolarize neurons thus causing excessive firing. Nadler et al. (293) injected 148 to 2957 millimoles L-glutamine into rat hippocampus and found it to be weakly neurotoxic as compared to other excitatory amino acids such as kainic acid, aspartate, and homocysteate.

Davis and Cherkin (296) have shown that intravenous injections (6.0 micromoles of D-glutamine) produced seizures and amnesia in chicks. L-glutamine, under the same conditions, had no comparable effect.

An excess intake of glutamine by pregnant women caused decreased mental development of the fetus (241). Glutamine has also been shown to increase glucagon and insulin release in the dog (297) which suggests caution for glutamine use in diabetics.

In summary, there is no clear evidence in the scientific literature to support the use of supplemental glutamine in the treatment of alcoholism, schizophrenia, ulcers, memory and intelligence enhancer, depression fighter, anti-fatigue agent, or to control sweet cravings.

Potential Hazards of Valine Excess

Excess valine ingestion can produce several physiological alterations. In maternal blood, elevated valine predisposes the fetus to decreased birth weight (6). Excess valine has resulted in growth depression in rat studies (10, 299, 300). Valine excess competitively

CHAPTER XI

VALINE

Valine is a nutritionally essential branched-chain amino acid which is glucogenic as a result of its catabolism to succinyl CoA. As with other branched-chain amino acids, valine is involved in muscle amino acid metabolism (56). Valine can also be oxidatively deaminated to its corresponding alpha-ketoacid which can replace valine in the diet (11). The recommended dietary allowance for valine is 14 milligrams per kilogram body weight per day (1).

As with isoleucine, valine does not appear to be one of the more popular food faddist amino acid supplements. Virtually no information was obtained from the retailers of valine concerning its use as a supplement. One publication did mention valine briefly as a promoter of mental vigor, muscle coordination and calm emotions. It was also stated that a valine deficiency could lead to nervous ailments such as nail biting, sleeplessness, and generally poor mental health (60). No documentations, recommended dosages, or explanations were given for these claims. However, OTC valine is marketed in 500 milligram tablets with recommended dosages of one tablet per day.

Potential Hazards of Valine Excess

Excess valine ingestion can produce several physiological alterations. In maternal blood, elevated valine predisposes the fetus to decreased birth weight (6). Excess valine has resulted in growth depressions in rat studies (10, 299, 300). Valine excess competitively inhibits the transport of the neurotransmitter precursors tyrosine and tryptophan across the BBB, thereby altering neurotransmitter synthesis (56).

Excess valine is also involved in two rare metabolic diseases: maple syrup urine disease (branched-chain keto-aciduria) and hypervalinemia. In maple syrup urine disease, an oxidative decarboxylase in the white blood cells is missing. Therefore, the carboxyl groups cannot be removed, thereby allowing the amino acids and their keto acids to accumulate in the blood. Symptoms include lethargy, seizures, and mental retardation (87).

Hypervalinemia is characterized by elevated valine in plasma which is attributed to an inability to oxidatively deaminate valine to alpha-ketoisovalerate. The symptoms resulting from increased plasma valine include vomiting, weight loss, and hyperkinesis (11).

Growth inhibitions as a consequence of antagonism between related amino acids have been verified many times (301, 302, 303, 304). For example, valine inhibited growth of barley embryos (305) and cultures of tobacco tissue cells or protoplasts (306, 307, 308). Tatum (304) showed that *Escherichia coli* K-12 failed to grow in a minimal medium containing valine. These findings suggested that growth inhibition by amino acids may occur as a result of competition at the level of either

biosynthesis or utilization among amino acids having structural, biosynthetic, or regulatory relationships (301).

Valine inhibits synthesis of isoleucyl transfer ribonucleic acid (tRNA) by forming a complex with isoleucyl-tRNA synthetase (309). This phenomenon might contribute to the severity of valine action. Cohen (310) reported that in *E. coli* K-12, exogenous valine was incorporated into proteins in place of other amino acids, thus producing faulty proteins.

Leucine is a neutral, branched-chain amino acid which is nutritionally essential for humans (11). It is widely distributed in animal and plant proteins. Leucine is the only common amino acid that of a block of alpha-aceto-alpha-hydroxybutyrate synthesis (301).

In spite of reported consequences of valine excess, valine does not appear to be highly toxic (311). Kolski et al. (311) concluded that a wide range of tolerance for leucine and valine intakes exists when

adequate amounts of other amino acids were simultaneously consumed. It is unclear why leucine supplements are being promoted OTC or what they supposedly prevent or cure. Only two references to leucine supplementation could be found in area retail outlets. One reference by Pearson and Shaw (12) stated briefly that D-leucine retards the breakdown of endorphins and enkephalins. A vague correlation was made between the release of endorphins and rate of weight gain. Primarily, if endorphins were suppressed, the level of obesity would decrease (12).

A second reference to leucine briefly stated, "leucine is a stimulant to the upper brain, helping people to be more alert" (60). One publication distributed with the sale of amino acid supplements simply gave information on food sources of leucine, function of leucine, and metabolic disorders concerning leucine metabolism (59). No mention was made regarding the benefits of dietary leucine supplementation. Leucine

is available OTC in 500 milligram tablets with recommended dosages of one tablet per day.

Potential Hazards of Leucine Excess

CHAPTER XII

LEUCINE

Leucine is a neutral, branched-chain amino acid which is nutritionally essential for humans (11). It is widely distributed in animal and plant proteins. Leucine is the only common amino acid that is purely ketogenic. After deamination, it is catabolized by the same reactions as fatty acids resulting in the formation of acetyl CoA and acetoacetic acid (11, 20).

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is available OTC in 500 milligram tablets with recommended dosages of one tablet per day.

Potential Hazards of Leucine Excess

Although no evidence of scientific research supporting beneficial uses for supplemental leucine was found, research documenting detrimental effects was abundant.

One of the most prevailing detriments of excess leucine ingestion involves leucine's pellagrigenic action (312, 313, 314, 315, 316, 317). Current evidence supports the view that excess leucine affects the oxidative metabolism of tryptophan leading to a reduced synthesis of nicotinamide nucleotides thus producing niacin deficiency and pellagra (312, 314, 416, 317).

Several explanations for the mechanism of decreased nicotinamide nucleotide syntheses were identified by various investigators: (a) Ghafoorunissa and Rao (313) found that animals fed diets containing as much as 30 grams L-leucine per kilogram body weight had increased tryptophan oxidation to carbon dioxide thus providing less tryptophan for NAD synthesis; (b) Krishnaswamy and Bapu Rao (318) suggested that excess leucine might lead to decreased activity of quinolate phosphoribosyltransferase which would contribute to decreased nicotinamide nucleotides from tryptophan; (c) Magboul and Bender (319) suggested that an inhibition of kynureninase by excess leucine might explain the ultimate pellagrigenic effect.

In 1982 Sakakibara et al. (316) showed that hepatic nicotinamide adenine dinucleotide (NAD) content was significantly lower in rats fed L-leucine supplemented diets. The NAD reduction was due to competitive

inhibition of intestinal absorption of tryptophan by excess leucine rather than to an effect of accumulated leucine on tryptophan-niacin metabolism.

Kubota et al. (320) suggested that the lowered NAD content of liver in the presence of excess leucine may be attributed to increased NAD degradation. Rats fed for two weeks on a 10-percent casein diet supplemented with five percent leucine had decreased hepatic NAD content with increased NAD glycohydrolase activity (320). Excess leucine ingestion increased beta-hydroxybutyric acid and acetoacetic acid levels in the blood (334); Yamada et al. (321) concluded that this elevation in ketone bodies was responsible for inhibition of NAD biosynthesis.

Excess leucine has also been implicated in several hepatic enzymatic alterations in the tryptophan-niacin pathway (320, 333, 323). Current evidence supports excess leucine's ability to increase hepatic tryptophan oxygenase, 3-hydroxyanthranilate oxygenase, and leucine aminotransferase. The effect of excess leucine and undernutrition (50 percent food reduction) was additive in induction of these enzymes. In severe undernutrition (25 percent of normal daily intake) excess leucine seemed to repress enzymatic activity (322). Induction or repression of enzyme activity obviously depends upon nutritional status while consuming excess leucine; this fact cannot be ignored by leucine supplement users.

Excess leucine may also be involved in decreased immunity. It is known that due to the antagonistic qualities of the branched-chain amino acids (BCAA), leucine, isoleucine, and valine, an excess of one BCAA will induce a deficiency of the other two. For instance, it was reported that an excess dietary intake of leucine induced a secondary

deficiency in valine and isoleucine which was attributed to either excessive activation of BCAA catabolic enzymes or inhibition of BCAA biosynthetic enzymes (324, 325, 326, 327). The decreased amount or availability of isoleucine in general and valine in particular led to an inhibition of leukopoiesis, involution of lymphoid organs (especially the thymus), and a drop in blood lymphocyte levels (324, 328).

In 1977 Chevalier and Aschkenasy (324) reported an immunodepressive effect of excess leucine in rats on protein-poor diets (four percent casein). These effects were not seen, however, in well-nourished animals. Rats need only a very small intake of protein in order to preserve immunological potential, providing no imbalanced intake of BCAA is present (324).

Goldfarb et al. (329) have suggested that excess leucine may exert cardiodepressant activity. They demonstrated this by infusing leucine into endotoxin-shocked rats and measuring survival times and blood concentrations of leucine. Leucine infusion accelerated the development of shock (329); no explanation for the mechanism was offered.

An important point to remember concerning high intakes of leucine is the fact that leucine can serve as a significant precursor of serum cholesterol in man (330, 331, 332). High levels of serum cholesterol have been attributed to various cardiovascular disorders such as atherosclerosis, arteriosclerosis, high blood pressure, and stroke. Leucine is metabolized through several intermediary steps to beta-hydroxy-beta-methylglutaryl CoA (HMG-CoA) which is a direct precursor of cholesterol (330). Since HMG-CoA can also produce acetoacetate and acetyl CoA (11)

it is clear that leucine can serve as a precursor to several major classes of lipids in addition to cholesterol (331).

Hagenfeldt et al. (333) infused 70 micromoles per minute of leucine into splanchnic tissue of humans. This resulted in an increase in ketone body production by 120 micromoles per minute. Considering that one mole of leucine can be metabolized to three moles acetyl-CoA, it was assumed that extra leucine taken up by the liver is used mainly for ketogenesis. As mentioned earlier, it has been shown by others that excess leucine increases ketone levels in the blood (321).

To assess the consequences of elevated branched-chain amino acid levels on alanine, glutamine, and ammonia metabolism in muscle, Aoki et al. (334) performed bilateral forearm studies on six post-absorptive individuals following ingestion of a leucine meal (14.7 grams leucine) and light exercise. Excess nitrogen was routed out of muscle via glutamine formation and release with alanine and ammonia pathways playing minor roles. The ingestion of leucine by normal subjects, presumably in optimal nitrogen balance, resulted in a net loss of nitrogen from muscle. It was postulated that the leucine meal may have initiated proteolysis despite an increase in insulin levels (334). Further investigation is necessary before definite conclusions can be drawn.

As in other excess amino acid intakes, signs of toxicity have been seen in the form of decreased growth rate and food intake (320, 334, 335). This has been seen using various levels of protein and leucine intakes (10 percent protein plus five percent leucine, 10.5 percent protein plus 1.32 percent leucine). Leucine levels above 6.6 grams per day resulted in hypoglycemic symptoms in humans due to insulin stimulation

(176, 336). Administration of seven times minimum requirement to young rats lowered serotonin in the brain and caused poor maze performance.

As with many of the other amino acids being reviewed, more research is required before conclusive statements can be made regarding the safety and/or benefits of leucine supplementation.

ISOLEUCINE

Isoleucine is a nutritionally essential branched-chain amino acid which is both ketogenic via acetyl CoA, and glucogenic via propionyl CoA which enters the Krebs cycle as succinyl CoA (11). The other two branched-chain amino acids, valine and leucine, share similar catabolic pathways with isoleucine in that they all are oxidatively decarboxylated to yield a derivative of CoA. While isoleucine is nutritionally essential for humans, mammalian tissues do contain transaminases that catalyze interconversion of the three branched-chain amino acids with their corresponding alpha-keto acids (11).

Isoleucine can be formed endogenously through dehydration and deamination of threonine. Threonine deaminase, the enzyme that catalyzes the conversion to isoleucine is inhibited by isoleucine through biofeedback (20).

The branched-chain amino acids play a prominent role in muscle amino acid metabolism; they are oxidized principally in the liver (56). Isoleucine is also involved in fatty acid synthesis (337).

Isoleucine is available in the diet through animal proteins, legumes, seeds, and nuts. The RDA for isoleucine is 12 milligrams per kilogram body weight per day (1).

Although isoleucine is available OTC as a dietary supplement in 300 milligram tablets, no information was available on its purpose other than a recommended dosage of one tablet daily. The available scientific information on isoleucine specifically was sparse. The majority of

CHAPTER XIII

information regarding isoleucine was in connection with its properties

as a branched-chain amino acid. Isoleucine is a branched-chain amino acid which exists among these amino acids (338).

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All three branched-chain amino acids are competitive inhibitors of L-glutamate dehydrogenase

Although isoleucine is available OTC as a dietary supplement in 500 milligram tablets, no information was available on its purpose other than a recommended dosage of one tablet daily. The available scientific information on isoleucine specifically was sparse. The majority of information regarding isoleucine was in connection with its properties as a branched-chain amino acid and the antagonism which exists among these amino acids (338).

Potential Hazards of Isoleucine Excess

Excess isoleucine intake can produce several metabolic changes as a result of amino acid imbalance. For instance, excess isoleucine could be involved in alterations of neurotransmitter synthesis since it shares the same transport system across the BBB as the neurotransmitter precursors tryptophan and tyrosine (56).

In addition, excess isoleucine has been implicated in convulsions, respiratory disturbances, coma, mental retardation, and neurochemical disturbances in maple syrup urine disease which is characterized by elevated plasma and urinary levels of branched-chain amino acids and their corresponding alpha-keto and alpha-hydroxy acids. For this reason, maple syrup urine disease is also known as branched-chain ketonuria. The biochemical defect is the absence or reduction in activity of the alpha-keto acid decarboxylase which catalyzes conversion of all three branched-chain amino acids to carbon dioxide and CoA esters (11). The mechanisms of branched-chain amino acids toxicity could include the ability of excesses to impair transport of other amino acids or alter amino acid pool size thus impairing protein synthesis (11). All three branched-chain amino acids are competitive inhibitors of L-glutamate dehydrogenase

which suggests possible alterations in amino acid catabolism and biosynthesis (11).

In contrast to the above-mentioned alterations, Eriksson et al. (339) extravenously infused 150 micromoles per minute of L-isoleucine into man and found no significant changes in whole blood concentrations of phenylalanine, methionine, valine, or leucine. Arterial glucose fell slightly but not significantly and insulin level was unchanged.

It appears that in normal man, isoleucine is one of the least toxic amino acids. The lack of information being circulated by isoleucine promoters also suggests that isoleucine is not a popular amino acid supplement.

Cysteine serves as a precursor of pyruvate and thus plays a role in energy production; it is a cofactor of various enzymes that conjugates with bile acids forming one of the primary bile acids, taurochenodeoxycholic acid; it is a sulfur donor for the synthesis of glutathione and a precursor for the production of various amino acids for use in detoxification of phenols, cresols, etc. (37).

Methyl cysteine is a product of methionine catabolism and, like methionine, is one of the most toxic of the amino acids (6).

Cysteine is one of the more popular amino acid supplements. It is being promoted for many uses, most notably: (a) to slow down the aging process, (b) prevent cancer initiation and pollution,

(c) neutralize toxins, (d) promote hair growth, (e) detoxification of acetaldehyde, and (f) improve the immune system (32, 58, 60). Pearson and Shaw (12) list, in addition, use of cysteine supplementation for

CHAPTER XIV

CYSTEINE

Cysteine, like methionine, is a sulfur-containing amino acid.

While not itself nutritionally essential, it is formed from methionine (nutritionally essential) and serine (nutritionally non-essential). The sulfur of cysteine derives from methionine and the carbon skeleton from serine. Methionine is first converted to homocysteine via S-adenosyl-vitamin C should be used in amounts approximating two-methionine and S-adenosylhomocysteine. Through redox reactions, cystine and cysteine co-conversion occurs (11).

Cysteine serves various functions in the body: it is a precursor of pyruvate and thus acetyl CoA which is a key element in energy production; it is a precursor of the taurine that conjugates with bile acids forming one of the primary bile acids, taurocholic acid; it is a sulfur donor for use in production of mucopolysaccharide and a precursor for the production of sulfuric acids for use in detoxification of phenols, cresols, etc. (87).

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Cysteine is one of the more popular food-faddish amino acid supplements. It is being promoted for many uses, most notably: (a) to slow down the aging process, (b) protect against radiation and pollution, which acts as a "scavenger of free radicals" (59, 60). Pearson and Shaw

(c) neutralize toxins, (d) promote hair growth, (e) detoxification of acetaldehyde, and (f) improve the immune system (12, 58, 60). Pearson and Shaw (12) list, in addition, use of cysteine supplementation for treatment of arthritis; prevention of strokes, atherosclerosis, cancer, balding, and obesity; and reduction of detrimental effects of alcohol drinking and smoking; and for smoker's cough. Cysteine is marketed in 500 and 1000 milligram tablets with recommended label dosages of one tablet per day.

Several incongruencies were found in the literature supplied by the retailers. For example, one publication warned that in order to prevent cysteine from being converted into cystine, a supplemental dose of vitamin C should accompany cysteine intake in amounts approximating two-to-three times greater than cysteine (12, 58). Another publication stated that since vitamin C is converted in the body to a form that requires sulfur, those who are supplementing with vitamin C may be placing demands on their sulfur reserves and need to take extra amounts of sulfur amino acids (59).

Another source of inconsistency involved the references cited by several of these publications. Upon searching for these reference sources in order to evaluate the research, it was found that either the cited studies did not exist or they were misreferenced making it impossible to find them.

The claimed ability of cysteine supplements to slow aging, protect against radiation and pollution, improve the immune system, prevent arthritis, etc., stem from its being a sulfur-containing amino acid which acts as a "scavenger of free radicals" (59, 60). Pearson and Shaw

(12) stated: "the tiniest killer isn't a virus. It is a type of promiscuously reactive molecule or molecular fragment called a free radical." They further explained that "high energy radiation kills by generating free radicals in living organisms. Uncontrolled, these free radicals can damage proteins, fats, and nucleic acids." It is possible to prevent much of this damage by taking supplements of nutrients which provide protection from free radicals. Many of these have even extended the life span of experimental animals. These anti-free-radical nutrients (also called antioxidants) include vitamins A, C, E, B-1, B-5 (calcium pantothenate), B-6; cysteine, glutathione, and catecholic amino acids tyrosine, and LDOPA, zinc, selenium, bioflavonoids, BHT, and BHA" (12).

Pearson and Shaw (12) made a very disturbing comment concerning saturated versus unsaturated fats. "One unfortunate strategy for reducing serum cholesterol which has been widely recommended by the American Heart Association and the American Medical Association is to substitute polyunsaturated fats for saturated fats in the diet. Although this substitution can slightly reduce serum cholesterol, it does not lower the death rate from heart attacks, and in addition, there is an increased incidence of cancer." Pearson and Shaw (12) suggested that since polyunsaturated fats are more subject to "free-radical oxidative attack," one should substitute saturated fats in the diet instead of polyunsaturates. "The more polyunsaturated fats you eat, the more antioxidants your body requires to be healthy"(12).

While it is true that the double bonds in polyunsaturated fats are especially vulnerable to oxidation (11), this spontaneous auto-oxidation is markedly inhibited by the presence of natural antioxidants

(tocopherols) in the fat. In addition, the common practice of adding anti-oxidants such as BHA and BHT to vegetable oils also inhibit auto-oxidation (62).

Although the requirement for antioxidants is relatively uncontroversial, the possible need for antioxidants to promote improved function and decreased rate of aging is another matter. Here again, distinction must be made between normal dosages to maintain a healthy human versus megadoses from supplements for claimed therapeutic uses. The story of free radical pathologic conditions and the theory that antioxidants can inhibit such conditions has been reviewed by Goodhart and Shils (62). At best, it can be concluded that concrete evidence to support this theory has not yet been established.

Cysteine enters the proposed free-radical scavenger scheme as one of the amino acids in the tripeptide, glutathione (GSH). It is claimed that since cysteine is a part of GSH, which is a component of GSH peroxidase, an increased intake of cysteine will increase GSH production which will increase GSH peroxidase, thus increasing antioxidant capacity. GSH peroxidase may play a protective role as an antioxidant by destroying hydrogen peroxide (62); it must be remembered, however, that there is no evidence yet that dietary cysteine supplements will increase the production or utilization of GSH peroxidase.

Concerning cysteine's ability to neutralize toxins, it is true that the metabolism of sulfur amino acids within cells yields sulfuric acid. One of the important functions of sulfuric acid is the conjugation of phenols, cresols, and steroid sex hormones, thereby detoxifying compounds which would otherwise be harmful (87). The thiol group of

cysteine has three major roles in proteins and peptides: (a) act as metal ion ligand, (b) redox reactions and electron transport, and (c) as a component of intramolecular linkage in protein tertiary structure (340). Here again, the distinction must be made between cysteine's functions in normal recommended amounts versus cysteine in excess amounts.

At present, the available evidence indicates that excess cysteine either has no effect or actually decreases GSH. In 1966, high doses of cysteine, administered intravenously to rats, failed to increase liver GSH (341). More recently excess dietary levels (two-to-four times requirement) in chicks failed to increase hepatic GSH (342). By contrast, isolated rat hepatocytes were reported to undergo extensive loss of GSH during incubation in a cysteine-rich medium (343, 344, 345). In addition, intraperitoneal injections of large doses of cysteine into rats also promoted a depletion of liver and brain GSH (344). In 1983 Estrela et al (345) reported that cysteine administered to rats both intraperitoneally and orally resulted in a depletion of hepatic GSH.

The preponderance of evidence suggests that excess cysteine depleted, rather than increased GSH which contradicts the claims that cysteine supplementation is needed to increase GSH to protect against free radical damage. In fact, autoxidation of cysteine produces thiol and hydroxyl radicals which disrupt the integrity of hepatocytes causing depletion of GSH, ATP, and lactate dehydrogenase (LDH) (340, 345). Saez et al. (340) concluded that cysteine was of little value as a therapeutic antioxidant.

As was mentioned earlier, discrepancy exists among cysteine supplement promoters concerning the need for simultaneous vitamin C

supplementation. Some promoters believe vitamin C is necessary to prevent the rapid conversion of cysteine to cystine which in turn can lead to "cystine bladder or kidney stones" (12, 58). Other promoters warn that if vitamin C supplements were taken, cysteine supplementation should increase (59). Cystine, cysteine, and GSH have been shown to be equivalent as dietary sources of cysteine (340).

The use of cysteine to promote hair growth is based upon the fact that hair contains a high concentration of cystine. Exogenous and/or endogenous cysteine does act as a cystine precursor for hair production (346) but the ability of excess cysteine to enhance hair growth rate or promote growth in male pattern baldness is undocumented.

Cysteine is also being promoted as an acetaldehyde detoxifier (12, 58, 60). Presumably, acetaldehyde makes undesirable chemical bonds via free radical reactions with proteins and nucleic acids. When these "cross-links" form, the protein or nucleic acid becomes stiff and rigid "much the same as old rubber becomes brittle and stiff" (12). Examples are wrinkled, inelastic skin and inelastic, hardened arteries. "The combination of vitamin C and B-1 and the amino acid cysteine is effective in protecting experimental animals from toxic effects of acetaldehyde" (12). Supposedly, cysteine and the other mentioned nutrients slow the cross-linking caused by acetaldehyde; cross-linking of proteins is presumed to be a cause for aging, cancer, heart attacks, strokes, detrimental effects of smoking, alcohol ingestion, eating saturated fats, and smog (12).

Pearson and Shaw (12) present two disturbing chapters in their book entitled "Smoking; Making it Safer with Nutrients" and "Drinking;

Making it Safer with Nutrients" in which they state: "if you do not wish to alter your life style or your habits, this need not preclude your taking effective steps to extend your lifespan." They claim it is possible to reduce the damage of cigarette smoking and/or excessive alcohol intake by daily ingestion of one gram each of vitamin B-1 and cysteine plus five grams of vitamin C to detoxify the acetaldehyde produced from alcohol metabolism or cigarette smoking. No substantiating evidence was found to support the aldehyde-damaging theory.

The connection between cysteine and arthritis prevention is related to stimulation of the immune system by cysteine-mediated prevention of T-cell damage from free radicals. Cysteine supposedly works as an antioxidant preventing oxidation of fats, thus preventing free-radicals from forming and attacking T-cells (12).

The claimed use for cysteine in cancer treatment is supposedly as a preventative nutrient mainly in connection with cancer resulting from drinking alcohol and smoking cigarettes (12). There is accumulating evidence that some forms of cancer are directly related to alcohol and/or tobacco use (347, 348, 349, 350). Hamster lung cultures exposed one-to-eight weeks to fresh smoke from either tobacco or marihuana displayed cytotoxic alterations, loss of lysosomes, marked abnormalities of cell division and of chromosomal DNA content. However, when L-cysteine was added to the culture, lysosomes increased and significantly less abnormalities in cell division and chromosomal DNA content were seen (347). This suggested that L-cysteine possibly protects against or reverses abnormal growth and malignant transformation after carcinogenesis from smoke. More specifically, it was suggested that L-cysteine

could inhibit mutagenesis by acting as a nucleophile competing with nucleophilic sites on DNA for the electrophilic mutagen (350). In 1983 Kedryna et al. (349) suggested that L-cysteine is one of the negative signal molecules which, in addition to other effectors, might switch off the glycolytic pathway of energy production in acites tumor cells.

Research pertaining to arthritic treatment with cysteine was not found in the scientific literature. The claimed connection between cysteine and detoxification of acetaldehyde to prevent free radicals from "stiffening the joints" (12) appears to be merely speculative.

Pearson and Shaw (12) speak of cysteine's use for people who become fat after breaking the smoking habit. They attribute the health problems associated with the post-smoker's weight gain to abnormal, uncontrolled fat oxidation. Thus, free radicals are formed causing damage to DNA, carcinogenesis, and abnormal cell function. "The oxidized fats inhibit certain white blood cells (macrophages) which normally attack and eat bacteria, viruses, cancer cells, and other undesirables in the body. But in fat people, who are full of oxidized fats, white blood cells are deterred from attacking these enemies. The result is that fat people get sick more often than people of normal weight." Therefore, cysteine is recommended by them as an antioxidant to prevent harmful free-radical attack (12).

Pearson and Shaw's theory (12) on obesity and health risks was inconsistent with many who believe that in order to lower health risks associated with obesity, weight loss is imperative (116). Consequently, weight loss is achieved through catabolism of fats which involves oxidation (11). Therefore it would seem rather presumptuous to suggest that

fat people suffer health risks due to excess fat oxidation, when this fat oxidation would be contributory to weight loss itself and a lessening of health risks. In addition, it is known that accelerated fat oxidation occurs when the dietary intake of carbohydrate and protein is insufficient which occurs in normal and even underweight subjects postprandially or during vigorous exercise or even during sleep.

investigators have suggested that cysteine was involved in the inactivation of various hepatic enzymes (364, 365). The inactivation of

Potential Hazards of Excess Cysteine Intake

Cysteine toxicity studies have been performed on a number of different microorganisms, fungi, and animals (351, 352, 353). Toxic L-cysteine has been investigated in several laboratories (365). A effects were attributed to different mechanisms of inhibition. For modification of the thiol group of the enzyme protein has been proposed, example, cysteine toxicity for E. coli was shown to be due to a however, the exact mechanism is unknown (365).

cysteine-specific inhibition of branched-chain amino acid synthesis

In 1984 Murakami et al. (364) reported that ornithine (351, 354, 355). It was suggested that hydrogen peroxide formation decarboxylase (ODC) was inactivated in vitro in the presence of L-cysteine. The inactivation correlated with H₂S production and formicacy seen with Salmonella typhimurium (353) and E. coli (352); hydrogen peroxide caused DNA strand scissions and degradation (353, 356, therefore, it was suggested that H₂S produced from cysteine converted (357). In fungi, the growth inhibiting effect of cysteine was correlated with cysteine's ability to chelate metal ions essential for growth inactivated ODC. Since H₂S production did not occur in vitamin-B6-deficient rats, it has been suggested that H₂S production from cysteine urinary zinc excretion in animals increased with cysteine supplementation, tissue levels of cysteine-fed rats were not depleted (359, 360,

Cysteine was shown to be neurotoxic (366, 367, 368, 369). Judging from the data for growth, organ weights and hematology, Pedersen and Karlson (366) subcutaneously injected four-day-old rats supplemental cysteine at three percent had no adverse effects (359). with a single dose of L-cysteine (1.2 milligrams per gram body weight) Additionally, Wong and Fresco (362) showed that long-term five percent which produced a 50 percent mortality rate. Approximately 10 percent cysteine intake was not toxic to mice. Cysteine supplementation was of the survivors developed severe brain damage with almost complete

shown to induce increased absorption of non-heme iron but had no effect on tissue levels of iron or fecal losses of iron (363).

Cysteine supplementation has also been implicated in liver cellular damage (340, 364, 365). In addition to the previously reported study by Saez (340) on the free-radical formation of auto-oxidizing cysteine with resulting disruption of hepatocyte integrity, several investigators have suggested that cysteine was involved in the inactivation of various hepatic enzymes (364, 365). The inactivation of tyrosine aminotransferase in the cytoplasmic membrane in the presence of L-cysteine has been investigated in several laboratories (365). A modification of the thiol group of the enzyme protein has been proposed, however, the exact mechanism is unknown (365).

In 1984 Murakami et al. (364) reported that ornithine decarboxylase (ODC) was inactivated in vitro in the presence of L-cysteine. The inactivation correlated with H_2S production and formation of iron loosely bound to cytosolic and microsomal molecules. therefore, it was suggested that H_2S produced from cysteine converted cytosolic and microsomal iron into a reactive loosely bound form that inactivated ODC. Since H_2S production did not occur in vitamin-B6-deficient rats, it has been suggested that H_2S production from cysteine is due to some vitamin B6-dependent enzyme (366, 367, 368, 369, 370).

Cysteine was shown to be neurotoxic (366, 367, 368, 369). Pedersen and Karlsen (366) subcutaneously injected four-day-old rats with a single dose of L-cysteine (1.2 milligrams per gram body weight) which produced a 50 percent mortality rate. Approximately 10 percent of the survivors developed severe brain damage with almost complete

disappearance of the cortex. The remaining survivors developed more moderate cortical atrophy with permanent retinal dystrophy. The basic mechanism for the neurotoxicity was unknown but postulated to be due to the formation of the acidic metabolites cysteic acid or cysteine sulfinic acid or even cysteine itself (366, 371).

Smith et al. (372) in 1983 demonstrated in rats that supplementation of a 15 percent casein diet with 0.505 grams cysteine per 100 grams body weight produced changes in the utilization and storage of energy. They found decreased activity of malic enzyme and lipogenesis with a concomitant increase in gluconeogenesis. A decrease in lipogenesis and decreased malic enzyme activity would conserve three carbon fragments for gluconeogenesis as well as channel energy into glucose synthesis. What effect this would ultimately have on blood glucose levels in humans regularly ingesting cysteine supplements warrants investigation.

a normal, well-balanced diet is slim. Methionine supplements are available OTC in 500 milligram tablets with a recommended dosage of one tablet daily with meals.

Literature obtained from various retail outlets claim several "useful" purposes for methionine supplements. These include: removal of poisonous waste from the liver and kidney and liver regeneration, stimulation of hair follicles and hair growth, treatment of baldness (12) and as an antioxidant (60). Other uses include: supplier of sulfur to prevent disorders of the skin and nails, free-radical deactivator, neutralizer of toxins which "help slow the aging process," and chelation of heavy metals such as lead, mercury, or cadmium which helps

to eliminate the build-up of toxic minerals in the body. Since methionine is a methyl donor substance, it is touted as an "anti-fatigue" agent (59). Most of these claims were undocumented in the faddist literature.

CHAPTER XV

The following proposed therapeutic uses for methionine supplements were documented: methionine's ability to remove poison-

ous waste. Methionine is an essential, sulfur-containing amino acid; it is a precursor for the non-essential amino acid cysteine. In the form of S-adenosylmethionine, it is the principal methyl group donor in the body which may be utilized directly, condensed with glycine to form serine, or oxidized (11).

The 1980 RDA for methionine in the reference adult is 0.70 grams per day (1). The estimated U.S. adult intake is 2.1 grams per day. The likelihood of a deficiency of methionine in the average American who consumes a normal, well-balanced diet is slim. Methionine supplements are available OTC in 500 milligram tablets with a recommended dosage of one tablet daily with meals.

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The following proposed therapeutic uses for methionine supplements were documented: methionine's ability to remove poisonous wastes from the liver, to regenerate kidneys and liver, treatment of baldness, anti-stress factor, neutralize toxins, anti-fatigue factor, and as an antioxidant to "deactivate" free radicals.

Regeneration of liver and kidney cells occurs, as in every body cell, with the use of many amino acids. While it is true that methionine is essential for protein synthesis, the implication that methionine alone performs this feat is invalid. Furthermore, there is no evidence that excess methionine enhances the cellular regeneration process. Indeed, the very opposite, i.e., cellular degeneration, may occur.

A remote connection can be made between methionine and some of the faddists' claims by examining the biochemical role of glutathione (GSH). GSH is a tripeptide, gamma glutamyl cysteinyl glycine, that is present in all life forms and implicated in the following important physiological functions: protection against radiation (373), lipid peroxidation (374), and infections (375); detoxification of ethanol (376), drugs (377), and heavy metals (378); regulation of insulin degradation (379); maintenance of membrane stability (380); and a possible role in amino acid transport (381).

Seligson and Rotruck (382) suggested that maximization of tissue GSH might be desirable. Since methionine serves as the sulfur donor in GSH synthesis via the intermediate cystathionine (383), it is conceivable that methionine supplement promoters have used this fact to "sell" their idea. It is well established that dietary sulfur-containing amino acid content affects tissue GSH (384, 385); however, much remains to be learned about the physiochemistry of the process.

The present faddist claims for methionine use appear to be premature. The unverified promotion of supplemental methionine could present dangers to humans since it is one of the most toxic of the naturally occurring amino acids (386). Indeed, it has been reported that the range between the amount of methionine which is beneficial and that which is toxic is relatively small (387).

Potential Hazards of Excess Methionine Intake

Methionine excess has been implicated in several physiological changes in animals. These include antagonistic effects upon glycine (387), histidine (388), cysteine (389), choline (390), and folic acid (391), liver enzymes (392, 393), energy utilization (372, 394, 395, 396), as well as histological alterations in various organs (397, 398).

When chicks were fed a diet containing 13 percent protein supplemented with 0.84 percent methionine, the choline requirement for maximum growth rate and the prevention of perosis was 800 milligrams per kilogram of diet compared to 300 milligrams per kilogram of diet without methionine supplements (390). Thus, the choline requirement of chicks was elevated 266 percent by increased dietary levels of

methionine which may be due to increased choline degradation as a result of increased liver choline oxidase activity.

Excess dietary methionine increased the need for serine or glycine in rats (387). Similarly, a feeding of 1.25 percent excess methionine to chicks produced an antagonism between methionine and glycine as well as a 20 percent depression in threonine utilization which lead to threonine deficiency (387).

In 1978 Connor et al. (391) found that feeding human subjects eight grams of methionine for four days resulted in a significant decrease in serum folate concentration. Three days after the end of methionine supplementation, serum folate concentration had not completely returned to normal. It was suggested that methionine loading increased the proportion of methyltetrahydrofolic acid and other reduced folates in the serum. This could cause a redistribution of folate from serum to cells and thereby cause a relative lack of folate in some tissues. The validity of this explanation remains to be proven.

More recently, it was shown that high dietary methionine enhanced histidine catabolism by stimulating one-carbon metabolism (388). This effect was not immediate since the urinary excretion of formiminoglutamic acid (FIGLU), a metabolite of histidine, was not increased until five days following test initiation. The metabolism of histidine is folic acid-dependent in that FIGLU requires tetrahydrofolate (THF) for catabolism. It could be postulated that the fall in serum folate concentration with increased methionine intake seen in Conner's study could be due to methionine increasing the

catabolism of histidine therefore increasing the need of THF. Without pyruvate transaminase (GPT) was seen in liver and muscle, and hepatic folic acid supplementation during a high methionine intake, folate glutamate dehydrogenase (GHDH) was increased. The elevations and reductions of these transaminases may have adverse effects on protein biosynthesis because of their role in non-essential amino acid synthesis and indirect energy production. The relationship between the activities of deoxygenase, cysteine desulfhydrase, and cystathionase, three enzymes involved in cysteine catabolism. Therefore, it appears that excess methionine intake could have an antagonistic effect upon cysteine by accelerating its catabolism.

Stipanuk (389) found that three percent methionine fed to rats on a ten percent casein diet increased the activities of cysteine indirect energy production. The relationship between the activities of deoxygenase, cysteine desulfhydrase, and cystathionase, three enzymes involved in cysteine catabolism. Therefore, it appears that excess methionine intake could have an antagonistic effect upon cysteine by accelerating its catabolism.

In several of the above studies, experimental animals either gained less weight than controls or actually lost weight during the first five-to-ten-days of excess methionine ingestion. Thereafter, the methionine-fed animals gained weight similar to controls. The initial weight disturbance was attributed to decreased food intake by the ad-libitum fed animals.; thereafter, adaptation to high levels of methionine occurred (387, 388, 389, 390).

Hepatic enzyme function, particularly those enzymes involved in protein metabolism, may be disturbed by excess methionine intake (392, 393). In 1982 Balogun (398) reported results of excessive intake of DL-methionine on liver enzymes. The DL form was chosen for this study because it is the most widely used form in dietary supplements. L-methionine, the natural form, has been established as the most toxic while the D form is considerably less.

Excessive methionine intake increased glutamate oxaloacetate transaminase (GOT) activity in the brain and liver of the rats tested and decreased GOT activity in muscle. In addition, decreased glutamate catabolizing the excess methionine (401).

pyruvate transaminase (GPT) was seen in liver and muscle, and hepatic glutamate dehydrogenase (GLDH) was increased. The elevations and reductions of these transaminases may have adverse effects on protein biosynthesis because of their role in non-essential amino acid synthesis and indirect energy production. The relationship between the activities of these enzymes, growth, and other indirect protein utilization measurements resulting from excess methionine intake is being investigated (399).

In 1979 Yokota et al. (400) reported nutritional anemia induced in rats by two-and-one-half percent excess methionine. The anemia was attributed to decreased biosynthesis of globin as a result of excess methionine(amino acid imbalance) and not to alterations in the biosynthesis of heme. The nitrogen efficiency ration was markedly reduced in the excess methionine group and iron deposition in the spleen increased. It was speculated that the deposited iron had not been utilized due to decreased hemoglobin synthesis and thus collected in the spleen. Judging from the immediate return of nitrogen efficiency to normal after excess dietary methionine was discontinued, it was deduced that nitrogen efficiency in rats was greatly affected by addition of excess methionine.

Excess methionine has been implicated in alterations of energy and fat metabolism. Fau et al. (396) showed that one month of feeding a two percent methionine diet to rats reduced their body lipids while maintaining dietary protein efficiency. There was also an increased energy need during increased methionine intake; if the rats were offered a protein-free diet ad libitum during excess methionine ingestion, they tended to overconsume to acquire extra energy, possibly for use in catabolizing the excess methionine (401).

Providing the animal was well fed, methionine excess increased lipogenesis and decreased gluconeogenesis. Methionine supplementation stimulated the animal to store energy as fat as opposed to glycogen storage. A 0.62 percent methionine intake caused decreased activity of phosphoenolpyruvate carboxykinase and increased activity of citric cleavage enzyme, malic enzyme, and glucose-6-phosphate dehydrogenase, thereby decreasing gluconeogenesis (372).

More specifically, excess methionine has been shown to cause an increase in cholesterol and triglyceride concentrations in liver, aorta, and heart in rats. Methionine added at the rate of 1.08 grams per 100 grams control diet decreased serum high density lipoprotein (HDL) and increased low density lipoprotein (LDL) and very low density lipoprotein (VLDL). The fecal bile acids, cholic and deoxycholic acids, were also decreased. Liver malic enzyme activity increased while glucose-6-phosphate dehydrogenase decreased. Lipoprotein lipase and triglycerides lipase activity in the tissues decreased. The observed increase in cholesterol and triglycerides in the aorta, the increase in LDL and VLDL cholesterol in the serum, and the decrease of HDL cholesterol associated with excess methionine intake suggest possible implication of methionine excess in atherogenesis (395).

Excess methionine can also damage various organs. Klavins et al. (397) observed the following effects on the body organs of rats fed a two or four percent methionine-supplemental basal diet:

Liver - No damage to liver cells.

Pancreas - Distortion of acinar cells. Increased nuclei.

GI tract - Atrophy of esophageal mucosa and forestomach.

Glands distorted and shrunken.

Decreased secretory activity in pyloric and Brunner's glands.

Decreased thickness of mucosa of colon and rectum.

Salivary glands - Shrinking and distortion of acini in parotid and submaxillary glands.

Increased nuclei of acinar cells.

Genitourinary system - Dilation of renal tubules and increase in glomerular size.

Increased weight of kidneys.

Spleen - Decreased number of lymphocytes.

Sinusoids were dilated and filled with blood.

Reticulum cells were prominent and contained large amounts of iron.

Thymus - No change seen.

Thyroid - Decrease in number of large follicles.

Adrenals - Decreased cytoplasmic vacuolization.

Other glands - No changes were seen in the pituitary, parathyroid, or islets of Langerhans.

Bones - No change with two percent methionine.

With four percent methionine, there was shortening and blunting of the bone trabeculae with decreased thickness of epiphyseal plate.

Weight - Weight loss was seen with two percent and four percent methionine supplementation.

In 1983 Balogun (398) conducted a similar study using 1.6 or 3.6 percent DL-methionine fed to rats. On an absolute basis, excess DL-methionine intake caused a significant decrease in weight of all tissues investigated as well as a decrease in water, DNA and RNA contents, and nitrogen. Other metabolic alterations included: (a) accumulation of calcium in all tissues, especially in muscles; (b) iron accumulation in spleen, liver, and muscles; (c) depression of zinc in the liver, kidneys, brain, lungs, and muscles; (d) excessive zinc accumulation in spleen (398). This study suggested that the overall growth depression was due to a collective reduction of protein synthesis in each of the tissues.

The increased level of tissue calcium suggested a withdrawal of calcium from the blood and bones. Balogun (398) suggested that the only possible advantage of excess DL-methionine was the availability of surplus calcium in the tissues for membrane transport and excitation, muscle contraction, and cell motility. The depression of zinc in other tissues and accumulation in the spleen could lead to metabolic disturbances in tissue functioning which remains to be investigated.

Excess methionine may be advantageous in the clinical management of malignant tumors. Methionine administration not only inhibited progression of ethionine-induced hepatic tumors, but also may have achieved a full remission of the neoplastic process in some rats (402).

Most of the therapeutic claims for methionine supplementation were undocumented in the scientific literature. Considering the toxic potential of excess methionine ingestion, it would be advisable to abstain from its use until further investigations prove it to be safe and effective.

L-threonine is available OTC in 500 milligram tablets with recommended dosages of one tablet per day. Since threonine is widely distributed in plant and animal proteins, it is unclear why this amino acid is being promoted. Garrison (59) stated that threonine is an important constituent of collagen, elastin, and tooth enamel protein. He added that when dietary choline was deficient, threonine assumes the role of a lipotropic agent to prevent fatty buildup in the liver. Lee

(60) simply states, "Threonine helps the digestive and intestinal tracts function more smoothly. It also assists metabolism and assimilation."

Neither Garrison nor Lee gave dosage information or an explanation for the need to supplement one's diet with threonine. Pearson and Shaw (12)

CHAPTER XVI

did not include threonine in their book.

THREONINE

When rats were injected intraperitoneally with 0 to 400

milligrams per kilogram body weight of L-threonine, a dose-dependent rise

Threonine is an essential, neutral amino acid. Unlike other amino acids, threonine cannot be replaced in the diet by an alpha-keto acid analogue (56). Threonine, along with lysine, constitutes 80 to 90 percent of the total indispensable amino acid concentration in muscle (56). It is glycogenic and ketogenic. The catabolism of threonine proceeds along three pathways: (a) in the presence of threonine aldolase, threonine is cleaved into acetaldehyde which is transformed into acetyl CoA, and glycine, which forms serine and

In 1982 Barbeau, Roy and Chouza (404) used 500 milligrams of threonine per day in six patients suffering from spasticity. After 12 months of treatment, all patients displayed improved motor performance by dehydrogenation and decarboxylation of threonine yields amino-acetone (20).

L-threonine is available OTC in 500 milligram tablets with also stated that long term use of low doses of threonine appeared to be recommended dosages of one tablet per day. Since threonine is widely distributed in plant and animal proteins, it is unclear why this amino acid is being promoted.

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When rats were injected intraperitoneally with 0 to 400 milligrams per kilogram body weight of L-threonine, a dose-dependent rise in spinal cord and brain threonine levels were seen (403). In addition, glycine concentration in the spinal cord increased. These findings suggested that plasma threonine concentration might affect glycine production by the central nervous system (CNS) neurons. The authors suggested that exogenous threonine might be useful as a drug for stimulating neuronal glycine synthesis and hence modifying glycinergic transmission (403).

In 1982 Barbeau, Roy and Chouza (404) used 500 milligrams of threonine per day in six patients suffering from spasticity. After 12 months of treatment, all patients displayed improved motor performance and reduced spasticity, particularly in the lower limbs; painful muscular spasms and hyperactive knee jerks were also reduced. The investigators also stated that long term use of low doses of threonine appeared to be without serious clinical or biochemical side-effects.

Potential Hazards of Excess Threonine

In 1978 Reddi (405) reported a case of threoninemia in an eight-month-old boy. The serum concentration of threonine was elevated as was the urinary excretion of threonine. The boy was anemic and

suffered periodic episodes of minor motor seizures with occasional fever. His feeding was irregular and at times he was anorectic. It was proposed that threoninemia resulted from inadequate catabolism of threonine due to a defective enzyme system, a low rate of incorporation of threonine into protein, or both.

Threonine supplements ranging from six-to-eight percent produced growth depressions in laboratory animals maintained on an adequate protein diet (10, 236, 406, 407). Excess threonine has also induced cataracts in rats. The severity of the cataracts was increased by tyrosine supplementation (407, 408).

An increased plasma concentration of threonine as a result of excessive threonine intake during pregnancy resulted in decreased birth weight and decreased cranial volume of the fetus (241).

Obviously, threonine has not received the same amount of attention as other amino acids have by either reputable scientific investigations or faddists. However, laboratory animal studies of one human metabolic error and one excess ingestion during pregnancy have recorded adverse effects. In view of these facts, it would appear that supplemental threonine is not without risk and should be avoided pending clarification of its safety and potential therapeutic usefulness.

would healing, and weight control (12, 60).

The rationale behind dietary ornithine supplementation involves its use as an antioxidant and a growth hormone stimulator. (Refer to the chapter on arginine for current review of this rationale.) Ornithine was equated to arginine in its therapeutic value with only dosage

CHAPTER XVII

ORNITHINE

Ornithine is nutritionally non-essential; is not usually found in proteins and is synthesized in the body primarily from arginine.

Ornithine is an intermediate in the biosynthesis of urea, functioning as a carrier of carbon and nitrogen atoms (11). Within the urea cycle,

ammonia from amino acid catabolism combines with carbon dioxide and adenosine triphosphate (ATP) to form carbamyl phosphate which combines

with ornithine to initiate the urea cycle. Aspartic acid contributes a second molecule of ammonia. Along with the enzyme arginase and magnesium,

arginine yields one urea molecule and one ornithine molecule. Thus, the cycle regenerates itself (87).

Ornithine is available OTC in 500 milligram tablets with a recommended dosage of one tablet per day. It is promoted for the same therapeutic uses as arginine. These include prevention and/or treatment of arthritis, immune system stimulant, cancer prevention, enhanced wound healing, and weight control (12, 60).

The rationale behind dietary ornithine supplementation involves its use as an antioxidant and a growth hormone stimulator. (Refer to the chapter on arginine for current review of this rationale.) Ornithine was equated to arginine in its therapeutic value with only dosage

recommendations differing. It was recommended that ornithine be taken at one-half the dosage of arginine "since it is one of the intermediates in the synthesis of arginine" (33). Pearson and Shaw (12) recommended that two-and-one-half to five grams ornithine per day taken on an empty stomach can cause growth hormone release which presumably is involved in the burning of fat and building of muscle tissue (12, 60). Lee (60) stated, "Even if you don't cut down on the amount of food you eat, you will still find that extra fat will frequently diminish and muscle tone will improve." In addition, KAL Series #5 (33) states, "These two supplements" (arginine and ornithine) "when used properly may hold the potential to dramatically alter the health status of our nation's population. What a welcome turn of events that would be." While it is true that these amino acids may "alter" our nation's health status, it remains questionable whether this alteration will be for better or worse.

As with arginine supplements, Pearson and Shaw (12) provided cautionary statements regarding inappropriate use of GH releasers. These precautions were: (a) they should not be used by persons who have not completed their long bone growth unless they have been advised to do so by their physician; (b) excess GH will cause skin to grow so rapidly that it becomes noticeably coarser and thicker which is reversible when GH or GH releasers are withdrawn; (c) excessive GH can cause irreversible joint enlargement ("which may be unsightly, but not dangerous"); (d) lowering of voice pitch due to larynx growth; (e) schizophrenics should use GH releasers with caution since they may increase the severity of their symptoms, possible due to effects of methyl donation by polyamines made from these amino acids in the body (12).

The statement was made that there "will be a GH-release scandal in the body-building business and in the Olympics. Although officials may bar the use of L-DOPA, they can't do anything about the use of tryptophan, arginine, ornithine, phenylalanine, tyrosine, and other nutrients to boost GH release and stimulate muscle growth" (12). Even though research concerning ornithine is still in progress, it appears that some who promote its use also question its safety.

Potential Hazards of Excess Ornithine

Excess ornithine has produced decreased food intake and decreased growth in rats. This has been correlated with an increased tissue ornithine concentration and a decreased brain lysine pool as a result of competitive transport inhibition of lysine into the brain (409). Ornithine is still a relatively unstudied amino acid with potentially serious consequences associated with large doses; its use for the unsubstantiated treatments and cures of various ailments should await further research.

Hyperornithinemia has been associated with gyrate atrophy (GA), a progressive chorioretinal degeneration characterized by the onset of night blindness and diminished peripheral vision (410, 411). In 1973 Simell and Takki (412) found 10-to-20 fold increases in ornithine concentration in the body fluids in patients with GA. The exact pathophysiological mechanisms responsible for chorioretinal degeneration in GA is unknown.

The hyperornithinemia seen in GA patients has been attributed to a deficiency of ornithine transaminase (OTA) (413, 414, 415, 416). While people with normal amounts of OTA would be able to catalyze the conversion of ornithine to glutamate semialdehyde, it is not unreasonable to imagine hyperornithinemia resulting from excessive intakes of exogenous ornithine to exceed OTA's ability to catabolize it.

However, ornithine, per se, is probably not the cause of GA since ornithine concentration was also increased in other abnormalities without any eye or muscular changes (411). It was suggested that excess ornithine inhibited arginine-glycine amidinotransferase, the rate-limiting enzyme in creatine production. CHAPTER XVIII
Simell and Takki (412) concluded that in gyrate atrophy patients, formation of guanidinoacetate, creatine, and possibly phosphocreatine was inhibited at the transamidination step by high concentrations of ornithine. Deficiency of high energy phosphates may, therefore, underlie the pathogenesis of eye and muscle atrophies.

Ornithine is still a relatively unstudied amino acid with potentially serious consequences associated with large doses; its use for the unsubstantiated treatments and cures of various ailments should await further research.

OTC amino acid supplements, as well as vitamins and/or minerals, are technically classified as "foods" by the Food and Drug Administration (FDA). As "foods," these nutrients may be freely sold OTC as long as they meet the FDA-established standards of identity, purity and labelling and neither make nor imply any therapeutic claims (417). Furthermore, the FDA is prohibited by law from placing limits upon the consumption of "foods" unless it can be clearly demonstrated that excessive consumption represents a real hazard to human health (417). In other words, the manufacturer or producer of a "food" (in this instance an amino acid, vitamin or mineral) does not have to demonstrate its safety; the consumer or some other interested individual would have to demonstrate a real hazard before the FDA could even begin

to consider placing a limitation or restriction on the sale and/or distribution of the "food" in question. Needless to say, food faddists in general and dietary supplement producers in particular have learned how to exploit the idiosyncrasies of the FDA regulations to their own

CHAPTER XVIII

advantage via false or misleading advertising.

Although there are a

DISCUSSION

supplement cases, the "vitamin D story" can be cited as an analogy to

In the interest of intelligibility, the discussion of the illustrate how the FDA regulations can be changed by an individual who purported benefits versus risks of each of the readily available over-the-counter (OTC) amino acid supplements surveyed in this study has can demonstrate that excessive consumption of a "food" represents a distinct human health hazard. Prior to 1966 one could purchase vitamin D singly or in combination with other vitamins OTC in which the been included in the review of each amino acid (Chapters IV through XVII). Therefore, this discussion will concentrate upon a general overview of the amino acid supplement problem and, by analogy, its similarities to the more widely recognized vitamin toxicities.

OTC amino acid supplements, as well as vitamins and/or minerals, farina, corn meal, corn grits, rice, macaroni, noodles, breads, milk are technically classified as "foods" by the Food and Drug Administration (FDA). As "foods," these nutrients may be freely sold OTC as long as they meet the FDA-established standards of identity, purity and

labelling and neither make nor imply any therapeutic claims (417).

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In other words, the manufacturer or producer of a "food" (in this instance an amino acid, vitamin or mineral) does not have to demonstrate its safety; the consumer or some other interested individual would have to demonstrate a real hazard before the FDA could even begin

to consider placing a limitation or restriction on the sale and/or distribution of the "food" in question. Needless to say, food faddists in general and dietary supplement producers in particular have learned how to exploit the idiosyncrasies of the FDA regulations to their own advantage via false or misleading advertising.

Although there are at present no comparable amino acid supplement cases, the "vitamin D story" can be cited as an analogy to illustrate how the FDA regulations can be changed by an individual who can demonstrate that excessive consumption of a "food" represents a distinct human health hazard. Prior to 1966 one could purchase vitamin D singly or in combination with other vitamins OTC in which the single dose far exceeded the recommended dietary allowance (RDA) for adults of 400 International Units (IU) per day. In addition, vitamin D was permitted as a deliberate food additive in margarine, flour, polfarina, corn meal, corn grits, rice, macaroni, noodles, breads, milk (fluid, dry whole, evaporated, concentrated) and infant feeding formulations used in place of milk (418).

The toxicity of excess vitamin D was first reported in 1930 (419); the Council on Pharmacy and Chemistry of the American Medical Association established the lethal dose as 2000 times the RDA in 1949 (420) making vitamin D the most toxic vitamin known.

In 1965 Dr. Robert E. Cooke, chief of pediatrics at Johns Hopkins Hospital, unequivocally established vitamin D toxicity in the neonates that was acquired in utero from mothers who were consuming slightly more than 1200 IU's of vitamin D per day from prenatal supplements and vitamin-D enriched foods (418, 421). Cooke's revelation

was supported by the American Academy of Pediatrics and the American Medical Association who collectively petitioned the FDA to limit OTC vitamin D to 400 IU's per dose and to ban the enrichment of all foods with vitamin D except for milk at a level of 400 IU's per quart. The FDA approved the petition and supporting evidence and published its proposed rule changes governing vitamin D in the Federal Register of August 28, 1965 (418); the new rules went into effect 60 days after publication. "overdose" appears to be part of human nature; indeed,

"overdose" The status of unrestricted OTC amino acid supplements today is somewhat comparable to the pre-1965 status of vitamin D. Although vitamin D was known to be the most toxic vitamin as early as 1949, it was readily available OTC in single dose capsules containing 50,000 IU's (125 times the RDA); in addition, massive doses (50,000 to 1,000,000 IU's daily) were used indiscriminately in futile attempts to treat pulmonary tuberculosis, various allergic states and rheumatoid arthritis (419). Many of the fourteen amino acids researched for this thesis also have known, well-established toxicities and are being advocated for the treatment of human illnesses which make as much sense as the archaic uses of vitamin D cited above. Americans of all ages (excluding preg-

nant and Unlike pre-1965 OTC vitamin D supplements which were readily available in single dosages representing as much as 125 times the RDA, most of the fourteen OTC amino acid supplements surveyed for this study are marketed as 500 milligram tablets with labeled directions for the consumer to "take one or two tablets daily." A daily intake of 500 to 1000 milligrams of an essential amino acid is within the general minimum requirement range of a young, healthy adult male provided adequate RDA

quantities of all other amino acids of nutritive importance are also included in the diet (1). Thus, the manufacturers or distributors of OTC amino acid supplements are able to exploit the idiosyncratic FDA regulations by supplying a "food" rather than a "drug" simply by limiting the amount of an amino acid in a single dose! However, nobody can prevent the consumer from ingesting the entire contents (typically one-hundred 500 milligram tablets) within a matter of minutes. The potential to "overdose" appears to be part of human nature; indeed, "overdosing" is recommended and encouraged by food-faddists reviewed in this thesis (12, 60).

In 1972 the FDA published the results of a comprehensive survey of American's health practices and opinions (422). One of the thirteen major topics in the survey examined vitamin pill and other nutritional supplement usage. Although amino acid supplement usage was not documented (the survey was actually completed several years before amino acids were readily available in retail markets) the survey findings identified some characteristics of vitamin supplement users which probably apply, at least in part, to amino acid supplement users. Approximately 40 percent of all Americans of all ages (excluding pregnant and lactating women) were consuming one or more supplements often at five to ten times the RDA in the belief that "if a little is good, a lot will be better."

The "more is better" belief seems to permeate Americans' thinking and has given rise to the term "megadose." According to Herbert and Barrett (423) five times the RDA of a fat-soluble vitamin, or ten times the RDA of a water-soluble vitamin or three times the RDA

of a mineral constitutes a "megadose." By analogy, one might consider essential amino acid "megadosing" to occur somewhere within the range of three-to-ten times the RDA adjusted for age, gender and health status. Unfortunately, this assumptive analogy does not consider the fact that L-methionine (the natural form) is the most toxic of all the essential amino acids and that toxicities have been demonstrated in animals receiving two times the RDA (7, 387). Furthermore, non-essential amino acids are omitted from this analogy although some of them have toxicities which are documented in this thesis and elsewhere (7).

Methionine is of interest for another important reason. Of the fourteen amino acids surveyed in this thesis, methionine is the only amino acid withan FDA-approved therapeutic usage. DL-methionine as "Pedameth"^R or "Uracid"^R has been approved as an urinary acidifer to control diaper rash or urine odor, dermatitis and ulcerations caused by ammoniacal urine in the incontinent adult patient (424). The recommended adult dosage is three-to-four 200 milligram capsules per day (600 to 800 milligrams). OTC L-methionine labels as well as accompanying informational literature carry no warnings or precautionary messages concerning methione toxicity. As a matter of fact, the informational brochures appear to reinforce the "more is better" belief.

By contrast, pharmaceutical DL-methionine obtained by prescription clearly states that it should not be used in patients with a history of liver disease since methionine exacerbates the toxemia of the disease; furthermore, loss of weight is noted as a distinct possibility (424). Finally, as noted in Chapter XV, L-methionine (the natural form) is more toxic than D-methionine. Since OTC methionine is the

"L" form, it is potentially more toxic than the pharmaceutical "DL" form on a milligram for milligram basis.

Even physiological dosages, i.e., normal dietary intakes, of some amino acids can have serious consequences under special circumstances. For example, physiological dosages of phenylalanine leads to brain damage, mental retardation and behavioral abnormalities in infants with phenylketonuria (PKU) discussed in Chapter IX. In PKU, liver phenylalanine hydroxylase, the enzyme responsible for converting excess phenylalanine to tyrosine, is deficient as a result of an autosomal recessive gene. Since excess phenylalanine cannot be converted to tyrosine, an alternate metabolic pathway produces phenylpyruvic, phenyllactic and phenylacetic acids (the phenylketones) which circulate in the blood and are believed to be responsible for the adverse effects of the condition (125).

Since phenylalanine is essential for protein synthesis, the "trick" to feeding a PKU infant in order to minimize the adverse effects of the disease is to supply just enough dietary phenylalanine to meet the essential needs without providing an excess which would form the phenylketones. While an exact dietary prescription for a PKU infant exceeds the scope of this thesis, the PKU infant's diet is normally restricted to 25 milligrams of phenylalanine per kilogram per day whereas a normal, non-PKU infant safely consumes 47 to 90 milligrams/kilogram/day (425). Thus, PKU is but one of 29 known aminoacidurias or inborn errors of metabolism (426) in which physiological dosages of amino acids elicit, evoke or exacerbate adverse health effects.

A very real problem could arise with physiological dosages of phenylalanine if a PKU female becomes pregnant. Available evidence indicates that during pregnancy, the PKU female must restrict her phenylalanine intake in order to prevent central nervous system (CNS) damage to the fetus. For maximal fetal CNS protection, the prospective mother's dietary phenylalanine restriction should be initiated before conception (427); in reality, this dietary modification is likely to be rare and some irreversible fetal CNS damage could occur before the PKU female realized she was pregnant.

By analogy, it is possible that a non-PKU pregnant female taking excessive amounts of OTC phenylalanine for "weight control" (12) could unwittingly induce CNS fetal damage or some other as yet unidentified birth defect. After all, excess histidine, glutamine, and threonine have each been associated retrospectively with fetal abnormalities (241).

None of the fourteen OTC amino acid supplements surveyed for this study carried any precautionary messages concerning use during pregnancy. The effects of supplemental phenylalanine or any other amino acid on the physical development and ultimate mental abilities of the fetus are simply not known at this time.

In PKU individuals, physiological dosages of phenylalanine in excess of the body's immediate protein synthetic requirements are converted via liver phenylalanine hydroxylase to tyrosine. In a minor metabolic pathway, tyrosine can be decarboxylated to tyramine, a potent vasoconstrictor which is rapidly inactivated by monoamine oxidase; the resulting parahydroxyphenylacetic acid is rapidly eliminated from the body via the urine (428).

Tyramine occurs in a variety of foods either naturally (avocado, banana, pineapple, etc.) or as the result of fermentation as a means of food preparation (beer, cheese, wine, etc.) Tyramine can also be formed in the intestinal tract as a result of bacterial decarboxylation of tyrosine (429). Under ordinary circumstances, tyramine, irrespective of its origin, is of little concern because of its rapid inactivation and excretion. However, there are special circumstances which can render an otherwise innocuous dietary component like tyramine into a potentially life-threatening situation and that is exactly what happens with a class of antidepressant drugs known as monoamine oxidase inhibitors (MAOI), examples of which are: "Marplan"^R (isocarboxazid); "Niamid"^R (nialamide); "nardil"^R (phenelzine sulfate) and "Parmate"^R (tranylcypromine sulfate) (430). Drugs under consideration might also exert an inhibitory effect on

As little as six milligrams of tyramine has invoked hypertensive crises in MAOI antidepressant-treated patients (431). Symptoms included "apprehension, restlessness, muscle twitching, dizziness, pallor, sweating, nausea, tachycardia, photophobia" along with diastolic and systolic hypertension producing severe headache "which may lead to intracranial hemorrhage and death" (430, 431). None of the

Although Pearson and Shaw (12) cautioned their readers about the dangers of phenylalanine and tyrosine with respect to MAOIs, none of the OTC supplements or accompanying literature warned of this potential life-threatening food-drug interaction. The synthetic sweetener aspartame

Decarboxylation of tryptophan produces tryptamine which is also a vasoconstrictor but apparently not as potent as tyramine. Normally, tryptamine is oxidatively deaminated to indoleacetic acid and excreted

in the urine (428). Interestingly enough, there are two reports in the literature (207, 208) describing behavioral and neurological symptoms in patients on MAOI therapy (phenelzine) after consumption of two-to-six grams of tryptophan. Whether the effects were the result of tryptamine or some other metabolite was not ascertained.

Decarboxylation of histidine produces histamine which is a potent vasodilator leading to hypotension and possible vascular collapse; indeed, excess histamine release has been related to traumatic shock. Histamine is inactivated by histaminase (a diamine oxidase) and ultimately converted to the urinary excretion product, beta-imidazoleacetic acid (432). Although monoamine oxidase is not involved in the inactivation of histamine, there is no evidence at this time that the antidepressant drugs under consideration might also exert an inhibitory effect on diamine oxidase thereby precipitating a hypotensive crisis.

Physiological dosages of either tryptophan or histidine have the potential to form vasoactive amines; the normal inactivation of these amines could be inhibited by drugs, such as the antidepressants, leading to hypertensive or hypotensive crises. Under similar circumstances, OTC tryptophan or histidine supplements could be disastrous. None of the OTC supplements examined in this study indicated the possibility of food-drug interactions. Only one has been reviewed here, but others exist.

The potential for amino acid toxicity at physiological dosages became a key issue in the approval of the synthetic sweetener aspartame (aspartyl phenylalanyl methyl ester). Roak-Foltz and Leveille (433), using a daily menu approach, were able to establish the fact that 750 milligrams of aspartame would be required to replace all of the

sucrose, corn sweetener and other deliberately added sugars and sugar alcohols in the average American's diet. In essence, a daily intake of 750 milligrams of aspartame would increase the average American's daily aspartate intake by four percent. This dietary projection was critical since Appelbaum et al. (434) had induced hypothalamic neuronal necrosis in neonatal mice with aspartic acid and had calculated that the threshold for damage was in the range of 500 to 650 milligrams per kilogram.

Using the lower (500 milligram) figure as the threshold for damage, the adult reference male of 70 kilograms (1) would have to inject 35 grams of aspartame daily to receive a comparable dosage of aspartic acid (16 grams). Thus it became apparent that the projected 750 milligrams of aspartame would provide 338 milligrams of aspartic acid which was only two percent of the toxic dose established for mice.

Aspartic acid, which is a non-essential amino acid, is probably one of the least toxic amino acids by virtue of its relatively simple and straightforward intermediary metabolism. Excess aspartic acid rapidly undergoes oxidative deamination producing oxaloacetic acid, a member of the citric acid cycle which can be completely catabolized to CO_2 , H_2O and generation of energy as adenosine triphosphate (ATP) or, it can give rise to glucose and glycogen (428, 432). In other words, aspartic acid, unlike many of the essential amino acids, does not have highly individualized or specialized functions producing a number of metabolically important intermediates such as those mentioned earlier for tyrosine, tryptophan or histidine.

The projected maximal daily ingestion of 750 milligrams of aspartame would also supply approximately 340 milligrams of phenylalanine

which by itself would supply all of the phenylalanine that could be safely utilized by a 13.6 kilogram PKU child. Since the PKU infant is usually limited to 25 milligrams of phenylalanine per kilogram per day from all dietary sources, it was apparent that aspartame could contribute significantly to the total dietary load of phenylalanine. Therefore, aspartame or any products containing aspartame must be labeled with a precautionary statement specifically directed to PKU individuals (433).

In the case of aspartame, valid concerns were expressed over projected maximal daily ingestions of aspartic acid and phenylalanine in the 350 milligram range. By contrast, as reviewed in this thesis, food faddists and some health professionals are advocating megadoses of some amino acids which are known to be toxic and/or have the potential for altering the production, via inhibition or potentiation, of metabolically important intermediates. As examples, Pearson and Shaw (12) advocated five-to-ten grams of arginine daily to release growth hormone or to delay immune system aging; KAL Series #6 (58) prescribed four grams of lysine daily for herpes outbreaks; Benedict et al. (97) advocated seven-and-one-half grams of tyrosine daily to increase brain catecholaminergic activity; Beitman and Dunner (159) suggested two-to-twelve grams daily of tryptophan as an antidepressant and, finally, Lee (60) and KAL Series #7 (138) recommended one-to-ten grams of glutamine daily to improve memory and intelligence.

None of the purported uses for amino acid megadosing reviewed in this thesis could be documented with certainty although there was ample evidence that the potential for toxicity exists. It is highly unlikely that controlled, long-term human experiments with amino acid

megadoses would ever be authorized in an approved health-care or clinical research setting. Therefore, the dangers inherent in amino acid megadosing will have to surface on an individual basis as happened in the cases reported by Moghissi et al. (241) who attributed self- or iatrogenically-administered histidine or glutamine or threonine during pregnancy to birth defects and retarded mental development.

Once again, a vitamin analogy will serve to illustrate the type of problem that is likely to emerge as a result of amino acid megadosing. In 1983 Schaumberg et al. (435) reported seven human cases of sensory neuropathy from megadoses of pyridoxine (vitamin B-6). In all seven cases the subjects developed unsteady gait, numbness of the hands and feet and some loss of motor ability after daily ingestion of two-to-six grams of pyridoxine (1,000 to 3,000 times the RDA) for periods of two to 40 months. Cessation of the pyridoxine megadoses was accompanied by dramatic improvement in the symptoms of all patients. However, nerve biopsies on two of the patients indicated widespread axonal degeneration which, it is believed, will be a permanent condition. All seven patients exhibited varying degrees of residual neuropathy six months after withdrawal from the pyridoxine megadoses. It is not known at this time whether or not the neuropathies will be permanent or reversible.

In all seven cases of pyridoxine toxicity, the subjects were encouraged by faddist publications or, in some instances by licensed "holistic" physicians (435), to consume pyridoxine megadoses in order to "cure" premenstrual tension and/or depression. Since pyridoxine is

water soluble, it was assumed that excesses would be rapidly excreted in the urine.

Americans in general and some health professionals in particular (who should know better) assume that megadoses of water soluble vitamins may be safely consumed since excesses are presumably rapidly excreted. Nothing could be further from the truth. In a recent review, Alhadeff et al. (436) have documented human toxicities as the result of megadoses of the water soluble vitamins C, thiamin, riboflavin, niacin, pantothenate, pyridoxine, cobalamin, folate and biotin.

As a result of this research on the physiological consequences of excess amino acids, this writer is convinced that some amino acids likely produce disease and possibly death. The amino acids reviewed in this thesis are no exception to the rule. One can only speculate at this particular point in time as to the number of Americans with aches, pains, mental disturbances or demonstrable biochemical abnormalities which are the direct result of self-administered or iatrogenic (437) amino acid supplementation. Typically, if such individuals were to seek medical help, they would probably be undiagnosed as to the true cause of their condition and treated symptomatically. This writer is also convinced that "detective work," comparable to that of Schaumberg et al. (435) will eventually reveal the accuracy of this writer's predictions.

Those which contain an aromatic or heterocyclic nucleus (phenylalanine, tyrosine, tryptophan, and histidine) or some specialized functional group such as the methyl-thio group of methionine, the sulfhydryl group of cysteine, the guanido group of arginine, etc.

Amino acids are the building blocks of proteins and Americans seem to know that proteins are essential for life. However, Americans

seem to be obsessed with the idea that if "a little is good, a lot is better." Therefore, it is not surprising that 40 percent of Americans use some form of dietary supplement (vitamins and/or minerals), often at five-to-ten times the RDA (422).

CHAPTER XIX

Amino acid supplementation is a relatively new activity for the American consumer; no data exists on the prevalence of the

CONCLUSIONS

amino acid supplement practice but there is a general belief that amino

There is an unwritten rule in nutrition which is universally applicable to all living things, including man. Deficiency of an essential nutrient will produce disease and ultimately death. Excessive

amounts of that same essential nutrient in the same species will most likely produce disease and possibly death. The amino acids reviewed in this thesis are no exception to the rule.

There is ample and, in some instances, overwhelming scientifically documented evidence that all eight essential, the two semi-essential and most, if not all of the twelve non-essential amino acids are potentially toxic for humans. Amino acid toxicity is most likely to occur with amino acids that have specialized chemical structures and a multiplicity of metabolic functions other than protein synthesis per se. The most likely amino acids to be toxic then are those which contain an aromatic or heterocyclic nucleus (phenylalanine, tyrosine, tryptophan, and histidine) or some specialized functional group such as the methyl-thio group of methionine, the sulfhydryl group of cysteine, the guanido group of arginine, etc.

Amino acids are the building blocks of proteins and Americans seem to know that proteins are essential for life. However, Americans

seem to be obsessed with the idea that if "a little is good, a lot is better." Therefore, it is not surprising that 40 percent of Americans use some form of dietary supplement (vitamins and/or minerals), often at five-to-ten times the RDA (422).

Amino acid supplementation is a relatively new activity for the American consumer; no data currently exists on the prevalence of the amino acid supplement practice but there is a general belief that amino acid supplements are "safe." This thesis offers evidence to the contrary. Indeed, the FDA removed all amino acids from its GRAS (Generally Regarded As Safe) list in 1974 (438).

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