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ADVERSE EFFECTS OF EXCESSIVE CONSUMPTION OF AMINO ACIDS

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INTRODUCTION

The purpose of this review is to survey the literature on amino acid toxicities published since the extensive review of Harper et al (52) in 1970. The approach taken in that earlier review is followed here, and where possible we emphasize the development of animal models as related to inherited disorders, except in the discussions of methionine and the D-amino acids, where we focus on the supplementation of low-protein diets with free amino acids. There has been considerable recent progress in the understanding of the metabolic implications of high cellular concentrations of a number of amino acids; hence we have chosen to emphasize those amino acids. Recent reviews are available on the dicarboxylic acids (35, 40), so they are not included in this review. The metabolism of branched-chain amino acids is reviewed in another chapter in this volume.

As described earlier (52), adverse effects of consumption of excessive levels of amino acids can vary from a slight suppression of food intake, followed by an adaptation and return to normal food intake, to marked food intake suppression, massive tissue damage, and death. In many cases, the level of the amino acid in the diet can be adjusted so that marked food intake suppression as well as tissue histological changes can be seen, but if the animal continues to receive the diet it will adapt and gain weight, and the tissue histology will return to normal. In this review, the term toxicity generally refers to a marked alteration in food intake and/or tissue histology. A detailed description of amino acid imbalance, antagonisms, and toxicities, and of the impact of the diet and age of the animal on these is presented by Harper et al (52). We do not review the relative toxicities of the amino acids because recent evidence does not alter that published earlier (52).

PHENYLALANINE TOXICITY

Interest in the toxicity of phenylalanine stems from a desire to understand the inherited disorder phenylketonuria (PKU). Individuals with this disease are profoundly retarded if they do not receive a low-phenylalanine-tyrosine diet soon after birth. The brains of young patients may show reduced lipid associated with myelin. Extensive reviews of the characteristics of this disease and a number of other hyperphenylalaninemias are available (64, 115, 116). Hyperphenylalaninemia can arise from a deficiency of phenylalanine hydroxylase, dihydropteridine reductase, or dihydrobiopterin synthetase. Classical PKU is due to a complete absence of phenylalanine hydroxylase. Successful treatment of these patients generally involves lifelong consumption of low-phenylalanine-tyrosine diets (121). Hyperphenylalaninemias due to a cofactor deficiency (biopterin) have recently been discovered and are thought to account for about 3% of those with hyperphenylalaninemia (116).

A number of approaches to the development of animal models of this disease have been made. A considerable number of the earlier studies utilized the rat and a low-protein diet supplemented with 3–7% of L-phenylalanine (52). Growth of animals fed this type of diet is not as greatly suppressed as that of animals fed similar levels of tyrosine or methionine (52, 85). In some cases, rats fed diets containing 7% of L-phenylalanine exhibited symptoms similar to those seen when excess tyrosine is fed (29). The observation that rats slowly adapt to lower levels of phenylalanine (52) raises questions about the utility of this model. Another problem with this model utilizing high dietary levels of phenylalanine to simulate PKU is that tyrosine levels increase in animals fed high levels of phenylalanine, whereas tyrosine levels are normal or slightly suppressed in individuals with PKU (67). However, abnormal brain development has been shown in 50-day-old rats that have been injected with 4 mg of phenylalanine/g of body weight from days 8 to 11 of life. By day 50, body and brain weights were 90 and 95% of control values whereas cerebellar DNA, RNA, and protein were three fourths or less that of control animals, suggesting that high levels of phenylalanine specifically affect the cerebellum (96).

The use of *p*-chlorophenylalanine, which inhibits liver phenylalanine hydroxylase (38, 70, 72) as well as tryptophan hydroxylase (68), has received considerable attention. At a dose of 300 mg/kg body weight (mg/kg BW), this inhibitor suppresses liver phenylalanine hydroxylase to 20% of control (64, 72) and increases the ratio of phenylalanine to tyrosine from a normal value of 1.2 to a value of 8–20 in animals injected four times per day with 50 mg of L-phenylalanine/kg BW. This compares with a phenylalanine/tyrosine ratio of 15–50 seen in patients with classical PKU (64, 72). In 21-day-old rats, injections of phenylalanine (300 mg/kg BW) twice daily in concert with *p*-chlorophenylalanine over 30 days resulted in an 8% decrease in brain weight and a 25% decline in brain myelin (106). The composition of lipids in myelin was altered significantly, as the sulfatide content was only 21% of that of the control group. The use of *p*-chlorophenylalanine as part of the development of a model system to investigate PKU has the risk of decreasing the concentrations of serotonin, dopamine, or norepinephrine; therefore, others have tried different inhibitors or combinations of inhibitors in addition to high levels of phenylalanine in efforts to develop a PKU model. A combination of 50 mg/kg BW of *p*-chlorophenylalanine, 0.00625 mg/kg BW of amethopterin (an inhibitor of dihydrobiopterin reductase), and 400–500 mg/kg BW/day of L-phenylalanine offered in four separate doses per day via stomach tube resulted in a 60-fold increase in plasma phenylalanine and a phenylalanine:tyrosine ratio in excess of 15 by the termination of the two-week-long (days 0–14 of age) experiment (74). The combination of the inhibitor and high phenylalanine depressed brain weight to 65% of control without an effect on the concentration of DNA, RNA, or protein. An alteration in the external proliferative layers of the cerebellum and a depressed number of layers of myelin lamellae in the

cerebellum were noted (73). The same level of phenylalanine without the inhibitor did not decrease growth, but did decrease brain size by 7%. The inhibitor alone decreased growth to 68% of control and decreased brain size to 89% of control. In addition to the decrease in body and brain weight and the altered brain histology in neonatal (0–21-day-old) rats treated with *p*-chlorophenylalanine and phenylalanine, Andersen et al (3, 4) found that the treated rats were slower to learn a new task and disregard a previously learned task.

It is not known whether alterations in the concentrations of phenylalanine or its metabolites in the brain have any effect on learning, but young (15-day-old) rats that were injected with *p*-chlorophenylalanine and phenylalanine for 10 days showed a 12-fold increase in brain phenylalanine, a 25-fold increase in phenylacetic acid, and a 7-fold increase in phenylpyruvic acid six hours after receiving an injection of 400 mg of L-phenylalanine/kg BW (75). Rowe et al (100) compared the urine metabolites of rats treated with *p*-chlorophenylalanine plus phenylalanine obtained after phenylalanine loading of 1 or 4 g of phenylalanine/kg BW. They concluded that the PKU-like behavioral and pathological changes are due to phenylalanine itself, because the behavioral effects were seen at lower levels of phenylalanine loading when no buildup in metabolites was apparent.

Developing the α-Methylphenylalanine Model

The possibility that the neonatal rat model developed with *p*-chlorophenylalanine might be compromised by the action of the drug on other hydroxylating systems (tryptophan and tyrosine) gave impetus to the development of a more specific inhibitor. Many of the observations described with respect to use of *p*-chlorophenylalanine are also seen when α-methylphenylalanine is used (28). Injections of 6-day-old rats with 24 μmol of α-methylphenylalanine/10 g BW resulted in a decrease in liver phenylalanine hydroxylase to 25% of control within 24 hours. This level of inhibition did not cause a decrease in body weight, brain weight, or the concentrations of DNA or protein in brain. One of the problems encountered with this model is the return of circulating phenylalanine to normal levels by day 21 even when the treatment is continued. Weaned rats fed a diet containing 0.5% of α-methylphenylalanine and 3% of L-phenylalanine had a liver phenylalanine hydroxylase activity that was 25% of control and circulating phenylalanine levels that were elevated 16-fold within 4 days of initiating the treatment. The combination of the injection of the inhibitor and phenylalanine from day 3 through day 21 with the feeding of the inhibitor in the diet from day 21 through day 33 resulted in slower learning as evidenced by significant reductions in the running wheel and maze tests. Thus it seems that useful information on PKU may become available via this model. Lane et al (70) found that the use of

α-methylphenylalanine and phenylalanine in suckling rat pups at levels suggested by Delvalle et al (28) resulted in characteristics that were similar to those seen in PKU. Slight suppression of brain weights were observed without the alterations in the concentrations of serotonin, dopamine, or norepinephrine that were seen when *p*-chlorophenylalanine was used. Increases in the excretion of phenylalanine and its metabolites were observed. A signficant (40–50%) increase in the time required to learn a task was also noted. Interestingly, a 20–30% decrease in myelin proteins was observed in rats that had received the inhibitor and phenylalanine from day 3 through day 30. An alteration in the cellular architecture of the cerebellum was noted. Probably the most important observation was the marked (50%) reduction in the brain concentration of aliphatic and aromatic amino acids seen from 4 to 20 hours after the last injection of inhibitor along with phenylalanine. These observations support the earlier reports on the depression of brain protein synthesis in neonatal rats injected with phenylalanine (52).

Use of the α-Methylphenylalanine Model in Brain Protein Synthesis

A direct test of the effect of high phenylalanine levels on brain cell protein synthesis has been made. Inclusion of phenylalanine at 100-fold its normal plasma level in an in vitro system with brain cells (58) resulted in a 40% decrease in protein synthesis over a one-hour incubation when labeled leucine, isoleucine, or methionine was used. A 25 or 5% reduction was observed when labeled alanine or lysine was used. These results and the others that show altered concentrations of free amino acids in brain cells incubated with or without 14 mM phenylalanine show that high levels of phenylalanine suppress the uptake of the large neutral amino acids. The magnitude of the reduction in the amino acid concentration itself may not be sufficient to predict the depression in protein synthesis. Direct measurement of the saturation of the aminoacyl-tRNA with individual amino acids revealed an 11% suppression in leucine and isoleucine tRNA and a slight (0–5%) change in alanine and lysine tRNA. When the changes in aminoacyl-tRNAs were taken into account, brain cell protein synthesis was depressed 20% by 14 mM phenylalanine. These observations raised the question of whether the decrease in brain protein synthesis caused by high levels of phenylalanine could be counteracted by increasing the concentration of the amino acids shown to be competitive with phenylalanine in their transport into brain. The rate of lysine incorporation into brain protein increased from a low of 65% of control in the α-methylphenylalanine- and phenylalanine-treated rats to 93% of control in those animals receiving in addition to phenylalanine supplemental methionine, isoleucine, leucine, or valine (12). Attempts to prevent the detrimental effects of high levels of maternal phenylalanine on the developing brain of the fetus have utilized

dietary supplements of large neutral amino acids to counteract the high maternal concentrations of phenylalanine generated by diets containing 0.12% of *p*-chlorophenylalanine plus 3% of phenylalanine. Of the branched-chain amino acids used, the only one that was effective was isoleucine, incorporated into the diet at a level of 3% (16). These data and those of Lane et al (70) support the suggestion of Kaufman (64) that the culprit in PKU is a high level of phenylalanine. He suggests that high blood levels of phenylalanine present in individuals with PKU prevent uptake of some amino acids in the brain and that this affects protein synthesis and thus the development of the brain.

TYROSINE TOXICITY

As with phenylalanine, interest in the effects of excessive intakes of tyrosine on experimental animals has as its basis the need to understand the changes in tissue and in metabolism of tyrosine in individuals with the inherited disorder of tyrosine metabolism, tyrosinemia II. Tyrosinemia II is inherited as an autosomal recessive trait and involves a complete lack of the liver enzyme tyrosine aminotransferase (48). Individuals with this disease have corneal lesions and palm and sole erosions in the early months of life. Mental retardation is not always seen.

A description of attempts to develop animal models that mimic this disease was published some time ago (52). While detailed accounts of the case histories of individuals with this disease have been quite instructive, major progress in understanding the disease has come from observation of laboratory rats fed low-protein diets containing high levels of tyrosine and more recently, from studies of mink that have an inherited disorder of tyrosine metabolism.

General Nutritional Observations

Work on the development of a rat model system to study tyrosine toxicity involved the use of purified diets containing 6–10% casein and 3–5% L-tyrosine (52). Higher levels of tyrosine were used in later experiments because growth depression and lesion severity lessened over the two-week durations of the experiments, which suggested that the animals could adapt to the diet (1, 14). Over the course of a two-week experiment, the growth depression of rats fed a 6% casein diet with 3% of L-tyrosine was depressed to 20–30% of control values, whereas weight loss was observed in rats fed a 10% casein diet supplemented with 5% of L-tyrosine (13, 14, 59, 84–86). The formation of cataracts and lesions on the bottom of the paws (46) were noticeable in rats fed either of the high-tyrosine diets mentioned above. Generally these lesions were observed within five to eight days after initiation of consumption of the high-tyrosine diet.

A number of modifications to the diet have been made in an attempt to alleviate the growth depression and tissue-destroying effects of high dietary levels of tyrosine. Increasing the protein content of the diet from 6 to 15 or 24% (59) or from 10 to 25 or 50% (86) resulted in little or no growth suppression and prevented the development of lesions despite high levels of tyrosine (85, 86). Specific amino acid additions to the low-protein diet have been effective in improving growth and suppressing the severity of lesions in the eyes and paws. Inclusion of 0.5–1.25% of L-threonine (1, 27, 43), 0.66% of L-methionine plus 0.9% of L-threonine (84, 122), or 1.25% of L-threonine plus 0.2% of L-tryptophan (1) improved growth to near-control levels; addition of 0.08% of L-cystine (84) prevented only weight loss. In most cases the incidence of cataract formation was eliminated or markedly reduced. While these amino acids were effective when casein was used as the protein source, lysine or lysine and threonine were required to prevent growth depression and eye and paw lesions when the source of dietary protein was 20% of wheat gluten (123). When 10% of wheat gluten was the sole source of dietary protein, methionine was required in addition to lysine and threonine (123). Taken in total, these studies strongly suggest that first or first and second limiting amino acids are protective against the toxicity of tyrosine. In each case, a substantial increase in growth was observed; thus, a substantial reduction in the circulating level of tyrosine and urinary excretion of phenols would be expected.

Factors Affecting Tissue Concentrations of Tyrosine

The plasma concentration of tyrosine in animals fed low or moderate levels of a high-quality protein is 0.04 mM (2, 27, 123), while it is 1.5–3.0 mM in animals fed low-protein diets supplemented with 3 or 5% of L-tyrosine. Tissue concentrations may approximate or be higher than those seen in the plasma (2, 99, 123). The concentration of tyrosine in the whole rat eye is 4.7–6.0 mM (2), whereas that in the aqueous humor varies from 2.8 to 5.0 mM (99). The high concentration of tyrosine in the eye may be responsible for the lesions, because the concentrations observed exceed the solubility of tyrosine in water at 35°, which is 3.5 mM.

In general, the dietary supplements that increase the growth of rats consuming low-protein diets supplemented with high levels of tyrosine decrease the blood and tissue levels of tyrosine from levels that are 10–70 times control to levels that are approximately 2–3 times control levels (2, 27, 59, 122). Most of the effective agents in the work reviewed have been amino acids or proteins added to the diet; however, hormone administration that results in an increase in the activity of tyrosine aminotransferase (EC 2.6.1.5), the rate-limiting enzyme of tyrosine degradation, have improved growth, presumably by lowering the tyrosine concentration in tissues (19, 104). However, the use of thyroxine (14), which causes a threefold increase in the activity of liver tyrosine

aminotransferase, resulted in a twofold increase in circulating tyrosine and a depression in growth, while glucagon administration (59) resulted in a two- to threefold increase in liver tyrosine aminotransferase, a decrease in plasma tyrosine to 30% of that seen in the tyrosine-supplemented rats, and no improvement in weight gain. In other experiments (104), improvement in weight gain and food intake and a depression in the severity of lesions have been noted with the use of steroids, which do not increase the activity of tyrosine aminotransferase. It is clear that there is no obvious, direct link between enhanced tyrosine aminotransferase activity and a depression in plasma tyrosine concentration and/or animal well-being. There is a good correlation, however, between enhanced tyrosine aminotransferase activity in animals fed high-protein diets (with or without supplemental tyrosine) and the conversion of the carboxyl carbon of tyrosine to carbon dioxide in intact rats (59). Interestingly, the three- to fourfold increase in tyrosine aminotransferase activity over a two to four hour period in response to a tyrosine load was not reflected in a concomitant reduction in plasma or liver tyrosine concentration. The beneficial effect of treatments that enhance the activity of tyrosine aminotransferase may be obvious, but the explanation for these effects may not rest on enhanced catabolism of tyrosine, although a reduction in tyrosine concentration is clearly helpful. This and the observation by Boctor & Harper (13) that rats fed diets containing 5% of *p*-hydroxyphenylpyruvic acid did not develop eye and paw lesions suggest that intermediates derived from the main pathway of tyrosine degradation are probably not responsible for the toxicity of tyrosine.

Probable Cause of Tyrosine Toxicity

The idea that the high concentration of plasma and tissue tyrosine causes eye and paw lesions has good support. Since the solubility of tyrosine in water at 35° is 3.5 mM, concentrations above this level should give rise to a precipitate or crystal formation. Within 24 hours of the initiation of feeding diets containing 5% of tyrosine to laboratory rats, alterations appear in the cornea, which are described as dots that progress into "snow flake" opacity (7). The cellular architecture becomes distorted, the integrity of the tissue lessens, and the tissue is invaded by polymorphonuclear leukocytes. The cornea thickens and becomes opaque. If the diet is continued for an additional three to four weeks, the cornea appears nearly normal. Recovery from the effects of consumption of high-tyrosine diets was described by Alam et al (1). The positive identification of crystals within the cells of the cornea of rats fed low-protein diets containing 5% of L-tyrosine has been made (42). The crystals were only seen in areas where lesions occurred. The needle-like crystals (0.5–1.1 by 10–25 μm) were seen to pass from one cell into another, often disrupting membranes and nuclei. The crystals in the tissues are similar to those of L-tyrosine, but positive identification of the crystals in the tissue as tyrosine was prevented by their

small width (0.5–1.1 μm). Factors that prevent tyrosine toxicity in rats also prevent crystal formation and corneal lesions (19).

Goldsmith (44, 45) has studied interactions of human erythrocytes and rat liver lysosomes with L-tyrosine crystals. It appeared that crystal size was important, as was direct crystal contact in the lysis of these particles. The mechanism by which the interaction of the tyrosine crystal and the membrane results in hydrolysis is not known, but hydrogen ion donation from the phenolic groups in the tyrosine is strongly considered.

A case history of a human infant with tyrosinemia II recently reported by Goldsmith & Reed (49) is startling in the similarity of the symptoms described to those described in laboratory rats fed low-protein diets supplemented with high levels of L-tyrosine. The patient developed corneal lesions similar to those described in the rat. Erosions on the fingertips and palms of the hands were noted. The concentration of tyrosine in the serum of this patient prior to consumption of a low-tyrosine, low-phenylalanine diet was approximately 2.5 mM. Within six weeks after the introduction of the low-tyrosine, low-phenylalanine diet, the corneas were clearing and the eruptions on the palms of the hands and fingers had regressed (47, 49). Plasma tyrosine fell from 2.5 mM to approximately 0.4 mM by the fourth day of feeding the low-tyrosine, low-phenylalanine diet.

A New Animal Model for Human Tyrosinemia II

Tyrosinemia is inherited as an autosomal recessive trait in mink (24, 50). Lesions of the eyes and paws have been noted. Plasma tyrosine concentrations varied from 2.2 to 2.8 mM in 50% of the affected kits. The level in control kits was 0.3 mM (24). The capacity for the transamination of tyrosine in the liver of tyrosinemic kits was 20% of that of unaffected controls (50). While antibodies against rat liver tyrosine aminotransferase would inactivate the tyrosine aminotransferase in the livers of control mink, it would not suppress the activity in livers of tyrosinemic mink (50). The residual tyrosine aminotransferase activity was thought to be due to an enhanced capacity of mitochondrial aspartate aminotransferase. The disease is observed in animals approximately six weeks of age under farm conditions as a result of a dietary change to a high-protein diet to promote pelt production. Fed this diet, kits usually die within a week (24).

TRYPTOPHAN TOXICITY

General Nutritional Observations

The results of both long-term and short-term studies indicate that tryptophan is generally considered to be one of the more toxic of the amino acids based upon growth and food intake measurements of rats fed low-protein diets supplemented with high levels (usually 5% or more) of amino acids (52, 85, 87,

90). However, Peng et al (90) have pointed out in their studies that while tryptophan and methionine appeared to be equally toxic when fed to rats for four days as 5% supplements to a 6% casein diet, the amount of tryptophan in the diet was 33-fold the requirement for growth compared to the methionine-supplemented diet, where the methionine level was only 8-fold the requirement for growth. Therefore, relative to the requirement, tryptophan may indeed be less toxic to animals than is commonly believed.

The growth-depressing effects of excess tryptophan are also markedly affected by dietary protein content (85, 87). After three weeks, rats fed a 6% casein diet supplemented with 5% of L-tryptophan exhibited a 78% depression in weight gain compared to controls; however, when the dietary protein content was increased to 25% casein, the addition of 5% tryptophan to the diet did not result in any growth depression compared to controls after three weeks. Similar results have been reported in a study designed to assess the carcinogenicity of L-tryptophan (117). Tryptophan was added at levels of 2.5 or 5% to a commercial laboratory ration and fed to groups of male and female rats and mice for one year. No significant differences were found in body weights due to tryptophan feeding in the rats; however, mice of both sexes that were fed these levels of tryptophan had lower body weights. The reason for this difference between rats and mice is not apparent. No evidence of carcinogenicity due to tryptophan administration was found in either species. Thus, based upon growth and food intake data, it may be concluded that tryptophan is not toxic under conditions of moderate protein intake.

Factors Affecting Tryptophan Toxicity

Although excess tryptophan may be tolerated well by normal growing rats given an adequate protein intake, recent evidence suggests that this is not the case in rats with compromised liver function due to the construction of a portacaval shunt (PCS) (17, 18). Following two months of receiving 200 mg of tryptophan/kg BW/day given in the drinking water, tryptophan-supplemented PCS rats lost 25% of their body weight; nonsupplemented PCS rats gained 54% of their initial body weight; and tryptophan-supplemented, sham-operated controls gained 65% of their initial body weight. As expected, PCS rats had liver weights approximately 15–20% smaller than sham-operated controls, but liver weights in the tryptophan-supplemented PCS rats were decreased by 35–40% and distinct liver atrophy was present, which the authors diagnosed as toxic hepatitis. Considerable alterations in brain histology were also evident in the tryptophan-supplemented PCS rats, which the authors suggested was due to higher brain tryptophan concentrations as a result of delayed metabolism of tryptophan in the liver by the kynurenine pathway. However, no metabolite measurements were made in these experiments.

Badawy & Evans (5) have speculated that the toxicity of tryptophan among

animal species is in part a function of the presence of the apoenzyme of tryptophan 2,3-dioxygenase. Animals lacking the apoenzyme (cat, frog, gerbil, guinea pig, hamster, sheep, ox, and rabbit) lack the hormonal mechanism of induction of tryptophan 2,3-dioxygenase, the initial step in the kynurenine pathway for tryptophan catabolism. Based upon preliminary studies, Badawy & Evans (5) proposed that tryptophan toxicity in animals lacking the potential for induction of this pathway results from an accumulation of toxic intermediate(s) from another pathway, such as the indoleamine pathway. While the amount of tryptophan metabolized by the kynurenine pathway does appear to be proportional to tryptophan intake under physiological conditions in the rat (89), it is not yet clear whether increased catabolism of tryptophan by another pathway is responsible for any of the adverse effects produced by excessive tryptophan consumption.

In recent years, considerable effort has been directed toward establishing the role of tryptophan and its metabolites of both the indoleamine pathway and the kynurenine pathway in the etiology and treatment of a number of neurological disorders (26, 39, 55, 71). However, whether any or all of these observations may be attributed to toxic effects of L-tryptophan awaits further investigation.

Tryptophan and Ruminant Interstitial Pulmonary Emphysema and Edema

Substantial progress has been made during the past 15 years in the understanding of the role of tryptophan in the development of interstitial pulmonary emphysema and edema in ruminants. Cattle develop this syndrome within a few days after an intraruminal or oral dose of tryptophan (20), presumably as a result of production of 3-methylindole (skatole) from tryptophan by rumen fermentation (21). The 3-methylindole is activated by a microsomal mixed function oxidase to a reactive free radical intermediate. Recent evidence has indicated that cysteine and glutathione may alleviate this syndrome both in vitro (80) and in vivo (81). Tissue covalent binding of a reactive metabolite of 3-methylindole was significantly higher in the lung than in the liver of goats, but the opposite was noted in rats. Glutathione or cysteine was effective in decreasing the level of metabolite binding in both tissues of each species (80). In in vivo studies, measures that were effective in raising tissue glutathione concentrations resulted in an alleviation of the lung lesions induced by 3-methylindole. Conversely, the lowering of tissue glutathione concentrations as a result of diethyl maleate treatment exacerbated the development of lung lesions (81). In goats fed a high-protein diet, an increase in 3-methylindole-induced toxicity was noted, as was an increase in cytochrome P-450 content of goat tissue (81). These results appear to indicate that feeding a high-protein diet either resulted in an enhanced capability for production of a reactive metabolite, or less likely, in a decreased ability for detoxification of the reactive metabolite.

HISTIDINE TOXICITY

Harper et al (52) have summarized the earlier studies that demonstrated the marked growth-depressing effects of diets containing high levels of histidine on numerous mammalian and avian species. More recent studies with rats have been directed toward determining the mechanism of the growth and food intake depressions, the characterization of other specific symptoms of histidine toxicity, and the alleviation of histidine toxicity.

General Nutritional Observations

In a comparative study of amino acid toxicity in rats, Peng et al (90) found that rats fed a low-protein diet supplemented with 5% of L-histidine lost approximately 15% of their initial body weight within four days. Of the amino acids studied (glu, thr, lys, phe, leu, his, trp, and met), a more severe weight loss was observed only in the rats fed diets supplemented with 5% of tryptophan or methionine; the rats fed the basal 6% casein diet increased their body weight by about 12% during the four-day experimental period. These results are qualitatively similar to studies of longer duration (three to four weeks) that assessed the relative toxicities of amino acids under a variety of dietary and experimental conditions (85, 87, 101).

While histidine is clearly one of the more toxic of the amino acids based on growth-depressing effects, the degree of growth depression caused by supplements of histidine is a function of protein quantity and quality. Muramatsu et al (85) found that rats fed a 10% casein diet supplemented with 5% of L-histidine for three weeks exhibited a growth depression of 77% compared to rats fed an unsupplemented 10% casein control diet. However in a subsequent experiment, rats fed a 25% casein diet supplemented with 5% of L-histidine for three weeks demonstrated only a 23% growth depression compared to the unsupplemented control rats. These findings are consistent with the observation that the activities of the histidine-catabolizing enzymes are higher in rats fed moderate- and high-protein diets compared to low-protein diets and that this results in an enhanced ability to degrade excess histidine with a concomitant decrease in histidine toxicity (102). Muramatsu et al (87) have also demonstrated that rats fed higher-quality proteins seem better able to tolerate excess histidine, although tolerance did not correlate with plasma histidine concentrations. It was not determined whether rats fed a lower-quality protein such as wheat gluten had a decreased ability to oxidize histidine due to lower levels of the histidine-catabolizing enzymes.

In addition to depressions in growth and food intake, a number of other metabolic effects have been documented in animals fed high levels of histidine. As expected, concentrations of histidine in plasma and tissues are markedly elevated in rats fed histidine (52, 90). Three hours after rats were force-fed a

6% casein diet supplemented with 5% of L-histidine, their plasma histidine increased 20-fold and brain histidine increased 10-fold compared to rats force-fed the control diet. However, the concentration of other large neutral amino acids (leu, ile, phe, and tyr) in brain were reduced by 50%, while serine, valine, methionine, and threonine concentrations were 10–20% less than in control rats. The amino acid depression in response to force-feeding of high levels of a single neutral amino acid appears to be the consequence of a marked competition at the blood-brain barrier for uptake of amino acids that utilize the same carrier (88). The effect of the resultant changes in the amino acid spectrum in brain on the control of food intake has been of considerable interest for a number of years (52, 90, 94).

Metabolic Aspects

Other metabolites of histidine catabolism are elevated in tissues and urine of rats fed high-histidine diets (33). The urinary excretion of formiminoglutamic acid (Figlu) by rats fed 10% casein diets supplemented with 1% of L-histidine was demonstrated within a few days. After 11 days, histidine-fed rats excreted about 5 mg of Figlu/mg creatinine, while urinary Figlu was barely detectable in control rats. Thus it appears that the capacity of the folic acid–dependent one-carbon pool to catabolize the carbon from the ring portion of the histidine molecule is exceeded in rats fed moderately elevated levels of dietary histidine. These data suggested that the rate-limiting step in the metabolism of large loads of histidine in rats fed 10% casein diets is due not to a limitation in enzyme activity, but to a sufficient availability of polyglutamyl tetrahydrofolic acid.

In 1965, it was demonstrated in Waisman's laboratory (65) that infant monkeys fed excessive levels of histidine (3 g/kg BW) exhibited a marked hyperlipidemia that was not found when infant monkeys were fed similar levels of other amino acids (trp, gly, phe, leu, ile, and val). Hyperlipidemia has not been reported in humans with histidinemia that is due to a genetic error resulting in a lack of histidase activity (69). Subsequent studies reported from Waisman's laboratory showed that rabbits (41) and rats (109) fed diets containing 5% or 8% of L-histidine exhibited elevated plasma cholesterol (40–50%) and phospholipid (20–50%) levels, although these effects were not as severe as those found in histidine-fed monkeys. In the rat, the marked hepatomegaly due to histidine or urocanic acid feeding was accompanied by a 20% decrease in the ratio of DNA to protein, indicating an increase in liver cell size rather than an increase in cell number. Within a week of removal of the excess histidine from the rats' diet, plasma cholesterol returned to normal.

Liver slices taken from rats fed diets supplemented with 5% of L-histidine for 4 days incorporated 100% more radioactivity into cholesterol from [2-^{14}C] acetate or [1-^{14}C] octanoate than slices from control rats, while incorporation of label into triglycerides was reduced by 38% compared to controls (110). In

longer-term studies (98) using liver homogenates from rats fed diets supplemented with 5% of L-histidine for 18 days, incorporation of [2–^{14}C] acetate and [2–^{14}C] mevalonate into the nonsaponifiable fraction were elevated ninefold and sevenfold respectively, while a sevenfold to eightfold increase in incorporation of label into the digitonin-precipitable fraction was observed with either radioactive substrate. Subsequent studies by some of these investigators (30) led to the proposal that the hypercholesterolemia due to histidine feeding was a consequence of an inhibition of lipogenic enzyme activity resulting in increased availability of acetyl-CoA for cholesterol biosynthesis. Compared to similarly treated controls, rats fed high levels of histidine had markedly decreased liver activities of fatty acid synthetase (25–50%) and acetyl-CoA carboxylase (52%) after fasting and refeeding.

An astute observation was made by Harvey et al (54) when they noted the similarity between the hypercholesterolemia produced by high levels of dietary histidine and that produced as a result of feeding rats diets deficient in copper. This, coupled with the well-known function of histidine as a zinc and copper ligand in mammalian blood (56), led them to examine the copper status of rats fed diets containing 8% of L-histidine for about eight weeks. As expected, histidine feeding produced depressed growth and hepatomegaly and more than doubled plasma cholesterol, but in addition, significant reductions in concentrations of plasma zinc, plasma copper, and liver copper were found. Feeding additional zinc supplements elevated plasma zinc levels above control values but did not affect the histidine-induced hypercholesterolemia. On the other hand, supplementation to the 8% histidine diet with 56.6 μg Cu/g lessened the hepatomegaly, elevated the tissue copper levels, and completely alleviated the hypercholesterolemia. While these studies and those discussed above were not designed and performed in an identical manner, it seems likely that many of the observed alterations in lipid metabolism due to excess histidine feeding may be attributed to the secondary production of copper deficiency due to increased binding of copper by histidine and subsequent excretion in the urine. Although zinc supplementation was not effective in alleviating histidine-induced hypercholesterolemia, it has been reported to alleviate anorexia and taste and smell dysfunction and to improve the mental states of patients undergoing oral histidine treatment for progressive systemic sclerosis (57).

Alleviation of Histidine Toxicity

Many of the metabolic effects of feeding excess histidine may be alleviated by pharmacological supplements of methionine (32) or retinol (33). Rats fed 10% casein diets with 1% of L-histidine and an additional 0.5% of L-methionine had plasma histidine concentrations approximately 50% less and about 80% less excretion of urinary Figlu than rats fed the histidine-supplemented diet without additional methionine for ten days. Histidase and urocanase activities in liver were not significantly different; however, a greater percentage of a histidine

load was oxidized to carbon dioxide and less of the dose was excreted as urinary Figlu in rats fed the high-histidine diet supplemented with 1% methionine. These results suggest that the alleviation of the effects of histidine loading by methionine are the result of an enhancement of histidine degradation by stimulation of one-carbon metabolism. Similar results were found with histidine-fed rats that were fed an additional 1,000 IU of retinol/g diet. Retinol-supplemented, histidine-fed (1%) rats excreted 50–70% less urinary Figlu and their plasma histidine was reduced by 30–70% compared to rats fed histidine without supplemental retinol over a 16-day period. Hepatic activities of histidase, urocanase, and formiminotransferase were not affected by retinol supplementation, but oxidation of histidine to carbon dioxide was enhanced significantly. Therefore, these data suggest that both retinol and methionine supplementation of a high-histidine diet result in alleviation of histidine toxicity by stimulating the oxidation of histidine and thus lowering plasma and tissue histidine concentrations.

A number of studies have clearly shown that rats will adapt to a diet containing a moderate excess of histidine within one to two weeks (33, 52). This adaptation did not involve an increase in the activities of the histidine catabolic enzymes, histidase and urocanase (33, 52, 102), although after three weeks growth and food intake were significantly increased and plasma histidine concentrations and urinary Figlu excretion were significantly decreased in rats that consumed diets containing 1% of L-histidine compared to values obtained during the first ten days of feeding. This is in contrast to recent studies (103) with chicks which show that addition of 5–6% histidine to a basal diet resulted in a doubling of histidase and histidine-pyruvate aminotransferase activities in liver. Thus, the metabolic adaptation in rats appears to be the result of factors that stimulate one-carbon metabolism, resulting in an enhanced catabolism of excess histidine. The biochemical mechanism of this adaptation is not yet known.

METHIONINE TOXICITY

Interest in methionine toxicity in general is not related to a need to know more about an inherited disorder, as is the case with the interest in phenylalanine and tryosine. Although hypermethioninemia has been reported (83), interest in the toxicity of methionine has been stimulated by the growth suppression seen in animals consuming low-protein diets supplemented with small successive increments of methionine (52).

General Nutritional Observations

Of the amino acids required for protein synthesis, methionine is clearly the most toxic, whether expressed on the basis of the percent of the diet, molality, or increase over the requirement (8). The first adverse effects of consumption

of excessive levels of methionine in animals fed low-protein diets (10% casein) with methionine levels three to four times the estimated requirement (0.5–0.6% of the diet) are marked suppression in voluntary food intake and near-cessation of growth. When the level of methionine is maintained at four to six times the requirement, food intake and growth remain suppressed. It is clear that some metabolic change or adaptation occurs because supplemental glycine or serine enhances growth after a few days of feeding the high-methionine diet, and a marked increase in the ability to oxidize methionine is seen (8, 52).

Tissue Damage

Alterations in tissue due to consumption of excess methionine have been reviewed (52). The most noticeable effect in the rat is that the spleen is enlarged twofold, darkened, and has twice the iron concentration; there is a 40% increase in kidney weight; and liver weight increases by 30% (11, 93). The increase in splenic iron is likely due to an increase in red cell turnover and destruction (25, 66, 79, 124).

Protective Effect of Glycine, Serine, or Retinol

In the rat, partial alleviation of the growth suppression and tissue damage due to excessive consumption of methionine may be obtained with supplemental glycine or serine (8, 52). The level of glycine or serine required is two times the molar quantity of methionine when methionine is incorporated into a low-protein diet at three or five times its requirement (10, 93).

A recent series of papers by Peng (91–93) has shown that pretreatment of rats with a diet containing 1000 IU of retinol/g diet for eight days prior to feeding a high-methionine diet restored body weight gain to control values when 2% of methionine was used but did not improve gain when rats were fed a 10% casein diet containing 3% of L-methionine. The effect of retinol in stimulation of growth seemed independent of the beneficial effect on growth seen with supplemental glycine or serine. The effect of retinol pretreatment on growth was fivefold greater when low levels of glycine or serine (0.5 or 0.7%) were used than when high levels (1.5 or 2.1%) were used (93).

Retinol treatment resulted in a slight (5%) improvement in the ability of rats to convert [U–^{14}C] or [methyl–^{14}C] methionine to carbon dioxide, and it decreased plasma methionine and splenic iron by one-half. The adrenal gland apparently plays a role because adrenalectomized rats were not protected from excess methionine by the retinol pretreatment. Interestingly, daily injection of corticosterone (1.5 mg/100 g BW/day) for two weeks did not protect rats from the adverse effects of consumption of excessive methionine and actually caused a significant decrease in gain compared to carrier-injected controls. The mechanism by which the retinol pretreatment alleviates the toxicity of methionine is not known, but the significant 11% increase in the ability of rats

to oxidize formate to carbon dioxide after the retinol treatment (34) may in part explain the enhanced methionine oxidation and the decrease in plasma methionine concentration. In short-term (12-hour) experiments with intact rats, Case & Benevenga (22) showed that more than one-half of the oxidation of the methionine methyl carbon involves formate production. However, the small increase in methionine oxidation due to retinol treatment would not seem to account totally for the beneficial effects of retinol pretreatment, and other explanations for the protective effect of retinol are needed.

Chemical Characteristics Related to the Toxicity of Methionine

Comparison of the adverse effects of methionine and homocysteine on the rat revealed that consumption of excessive levels of methionine caused a greater suppression in growth and more tissue damage than homocyst(e)ine when the two were compared on a molar or percent-of-diet basis (52). This, in part, was used as the basis for the suggestion that the metabolism of the methyl group of methionine was somehow involved in the toxicity of methionine (8). It is now becoming clear that it is the metabolism of the methylthio group that is related to the toxicity of methionine, because methionine, *S*-methyl-L-cysteine, 3-methylthiopropionate, and dimethylthetin all result in markedly suppressed food intake and growth when incorporated into low-protein diets at comparable levels, but also result in enlarged, darkened spleens (11, 52, 112). Interestingly, when choline, betaine, or sarcosine are incorporated into low-protein diets at methyl loads that are greater than or equal to those of methionine used above, they do not result in depression in growth or splenic abnormalities, even though it is clear that the conversion of their methyl groups to carbon dioxide is far greater than that reported for methionine (23). Support for a relationship between the production of methanethiol and the toxicity of methionine comes from the work of Steele & Benevenga (113), who showed that two very toxic substances, methanethiol and hydrogen sulfide, are produced in the metabolism of 3-methylthiopropionate by a rat liver homogenate system. Methylthiopropionate is thought to be an intermediate in an alternative route of degradation of methionine (9).

Damaging Effects of Methanethiol

There is good evidence that methanethiol can affect the activity of enzymes. Oxygen consumption of liver mitochondria was suppressed 10% by 1 μM methanethiol, but a 50% decrease was observed when 70 μM methanethiol was used (119). Oxygen consumption of brain mitochondria was suppressed to one-third of control by 170 μM methanethiol when glutamate plus malate or succinate were used as substrates (118). A direct effect of methanethiol on cytochrome c oxidase has been reported by Wilms et al (120). The K_i for methanethiol is 4.5 μM and that for hydrogen sulfide is 0.1 μM. Methanethiol

(100 μM) also has been shown to inhibit brain (Na^+,K^+)-ATPase by 50% (97). Modification of red cell membrane sulfhydryl groups with reagents such as *p*-mercuribenzoate results in greater susceptibility to hypotonic hemolysis (60), whereas sulfhydryl reagents such as *n*-ethylmaleimide, an alkylating agent, stablize the membrane at concentrations of 10 mM and accelerate hemolysis at 30 mM. Treatment of red cells with *n*-ethylmaleimide markedly lowers their deformability (36). An agent that alters the deformability of red cells would be expected to result in an increased uptake and destruction of modified cells by the spleen (112). This and the observed inhibition of the activity of rabbit muscle creatine kinase to 28% of control by modification of a cystinyl residue by methanethiol and complete recovery of activity upon treatment with 2-mercaptoethanol (76) suggest that the detrimental effects of consumption of high levels of methionine may be due to production of protein-bound methanethiol-cysteine mixed disulfides and possibly to a direct action of methanethiol on enzyme-bound metals (120).

Methionine Toxicity in Chickens

Considerable work by Baker's group (53, 62, 63) on the utilization of sulfur amino acids has shown that in chicks, as in rats, increasing the dietary methionine concentration to 1.5–2.0% of the diet suppresses growth and causes enlarged, darkened spleens that show a fourfold increase in iron concentration. As expected, feeding 1.35% of 3-methylthiopropionate resulted in a growth decrease similar to that seen with methionine but resulted in a splenic iron concentration that was three times that seen with methionine, suggesting that 3-methylthiopropionate is more damaging than methionine. Baker suggests that the toxicity of methionine in chickens is due to the production of homocysteine (6) because growth depression due to methionine and homocysteine are comparable; however, recent work with chickens (51) suggests that the decrease in growth due to homocystine may be half that of methionine. The responses of quail to high dietary levels of methionine appear to be similar to those of chickens (105).

Ethionine Toxicity

It is generally accepted that consumption of low levels of ethionine (i.e. 1% of the diet or less) results in marked growth suppression and tissue damage. One of the more accepted hypotheses for the toxicity of ethionine has been that ethionine, via its rapid conversion to S-adenosylethionine coupled with the slow rate of transethylation, results in a marked increase in the cellular concentration of S-adenosylethionine and a concommitant decrease in cellular ATP to 50 or 25% of normal (107, 108). This hypothesis is quite attractive because the integrity of subcellular organelles and the cytosol show moderate structural and functional alterations when the cellular concentration of ATP is reduced by

50%. However, cells fully recover within 24 hours even in cases where cellular concentration of ATP has fallen to 20% of control values for periods of in excess of 48 hours (31). Consumption of diets that result in an intake of 50 mg or more of ethionine per day would not be expected to decrease the cellular concentration of ATP to 50% of control values (108). Thus, if a lowering of the cellular concentration of ATP to 25% of normal is required for tissue alterations to be seen, the suppressed ATP concentrations discussed above could not account for the cytotoxic action of ethionine.

Observations on the relative toxicity of D- and L-ethionine raise additional questions in regard to the explanation of ethionine toxicity based on lowered cellular ATP. The LD_{50} for D-ethionine in Swiss mice is 185 mg/kg BW, whereas even 2500 mg/kg BW of L-ethionine do not cause death (37). D-ethionine would not be expected to be converted as efficiently to S-adenosylethionine as L-ethionine (15). Hence, a suppressed ATP concentration would be unlikely to account for the toxicity of D-ethionine. Recent observations on alternative routes of ethionine degradation (114) involving the production of ethylthiopropionate and ethanethiol may aid in a fuller understanding of ethionine toxicity. Comparison of the toxicity of ethionine and ethylthiopropionate revealed that both suppressed growth, but ethylthiopropionate caused considerably more tissue damage (111). Metabolism of ethionine by a transaminative route should receive serious consideration with respect to the toxicity of ethionine.

D-AMINO ACIDS

It was previously pointed out (52) that with the exceptions of aspartate and serine, the naturally occurring L-form of an amino acid is generally equal to or more toxic than the D-form. Since that time, a few studies have been published demonstrating the increased toxicity of some D isomers of dispensible amino acids.

Studies by Peterson & Carone (95) documented the marked nephrotoxicity of an intraperitoneal injection of D-serine (80 mg/100 g BW) and the acute tubular necrosis that occurred at the proximal tubule. Within 48 hours, regeneration of the kidney was observed, and re-epithelialization was complete by 16 days after injection. Subsequent studies (61) were directed at determining the structural requirements for the nephrotoxic action of the D isomer of serine. Eighteen compounds were tested, and necrosis of the proximal tubules of rat kidneys did not occur with analogs resulting in elimination or blocking of the amino group or hydroxyl group, lengthening of the carbon backbone (e.g. D-threonine), or substitution of a methyl group to the carbon at which the amino group was attached. The only compound tested that consistently produced tubular necrosis was D-2,3-diaminopropionic acid. The L isomer of both

compounds was without effect. In addition, the nephrotoxic action of the D isomer was apparently confined to the rat; the authors pointed out that they were unable to produce the lesions in dogs, hamsters, and gerbils, and that others had not been able to produce them in guinea pigs, rabbits, or mice (82). The mechanism of action of D-serine nephrotoxicity is unknown, but it appears that a rather stringent molecular configuration is required.

Marayama et al (77, 78) reported that high levels of D isomers of alanine, aspartate, and glutamate depress growth in chicks to a much greater extent than the respective L isomers. The addition of 2% D-alanine to the chick diet depressed growth after two weeks by 45–55%, depending on the dietary protein composition. No growth depression was observed with 2% L-alanine supplementation. Similarly, 2% of D-aspartate depressed growth by about 25%, while L-aspartate addition up to 6% of the diet did not depress growth in chicks over the 2-week experimental period. Plasma aspartate levels were increased 25-fold over controls within a few days of adding 3% D-aspartate to the diets. Replacement of D-aspartate with L-aspartate after one week resulted in an immediate decline in plasma aspartate to control levels and a rapid return of growth rate toward control values. While high-protein diets tend to decrease the toxicity of most L-amino acids (52), D-aspartate toxicity was not alleviated by increasing the protein to 50%.

Similar results were obtained with D-glutamic acid. While chicks tolerated up to 15% of L-glutamate in diets, the addition of 5% of D-glutamate resulted in a 38% depression in growth compared to control chicks after two weeks. Unlike addition of aspartate in the studies cited above, the addition of D-glutamate did not result in a significant elevation of plasma glutamate; however, 86% of the plasma glutamate was present in the D form. Therefore, the addition of D-glutamate to chick diets results in a marked reduction in the ability of the chick to synthesize L-glutamate. The mechanisms of the marked growth-depressing effects of excess D-alanine, D-aspartate, or D-glutamate are not yet known.

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