SOME NUTRITIONAL AND METABOLIC EFFECTS OF HISTIDINE TOXICITY IN THE RAT

by

Robert Darryl Steele

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

A. R. Kemmerer — 3 Aug 73

ARTHUR R. KEMMERER — Date

Professor of Agricultural Biochemistry

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES--Continued

LIST OF ILLUSTRATIONS

ABSTRACT

The effects of feeding a diet containing an excessive amount of histidine were studied in the white rat. When a 6% casein diet supplemented with 2% histidine was fed, growth and food intake were depressed substantially. Plasma and brain histidine concentrations were elevated on the first day and remained elevated throughout the 14 day experiment. When the protein content of the diet was increased to 12%, growth and food intake depression were less, as were the plasma and brain histidine concentrations. By the fourteenth day, tissue histidine concentrations returned toward control values. The addition of vitamin A to this diet had a beneficial effect on plasma and brain histidine concentrations. When the amount of protein and histidine were proportionately increased, similar patterns in growth and food intake were observed. Plasma and brain histidine concentrations remained elevated for 14 days. The concentration of glycine, threonine, phenylalanine, and tyrosine were depressed substantially.

Rats fed a diet containing excessive levels of histidine showed increased oxidation of labeled phenylalanine, threonine, and histidine to carbon dioxide and increased incorporation of these amino acids into liver

protein. Liver size in relation to body weight was also increased.

INTRODUCTION

When rats are fed a diet containing an excessive amount of an indispensable amino acid, depressions in growth and food intake occur (Sauberlich, 1961, and Peng et al., 1973). The degree of severity depends on the individual amino acid fed, its concentration in the diet, and the protein content of the diet (Harper, Benevenga, and Wohlhueter, 1970). Much of the work on amino acid toxicity has been done in an attempt to develop experimental models for the study of genetic defects of amino acid metabolism. Therefore, many of the amino acids have received little attention.

Harper, Becker, and Stucki (1966) found that when rats were fed an excessive amount of histidine in a low protein diet, growth and food intake were severely depressed. When the amount of protein in the diet was increased, the observed depressions were less severe. Likewise, when the amount of protein and histidine in the diet were increased proportionally, the depressions of growth and food intake were less severe.

Peng et al. (1973) recently reported that rats force-fed a diet high in histidine exhibited a 20-fold increase of histidine in the plasma and a 10-fold increase in the brain. The concentrations of isoleucine, leucine, tyrosine, and

phenylalanine decreased to about 50% of their control values in the brain.

Rats tend to adapt to a diet high in an individual amino acid after a few days (Daniel and Waisman, 1968). It has been speculated that adaptation to the diet occurs as a result of the animal becoming able to metabolize the amino acid in excess at a greater rate, thus allowing tissue concentrations to return to normal. However, as noted by Harper and associates (1970), little evidence has been presented that would support this theory.

The objectives of this experiment were to determine the effects of excessive levels of histidine in diets containing low or moderate amounts of protein on growth, food intake, and the plasma and brain amino acid patterns in rats over a 2 week period. It was also the author's intent to observe the effects of an elevated level of dietary histidine on the metabolism of selected essential amino acids, before and after adaptation to the diet, utilizing radioactive tracer techniques.

LITERATURE REVIEW

Histidine Metabolism

Histidine is an essential amino acid for most animals (rat [Rose, 1937], dog [Rose and Rice, 1939], pig [Rechcigl, et al., 1956], chick [Almquist and Grau, 1944]), and the human infant (Snyderman et al., 1963). Albanese et al. (1944); Rose, Haines, and Warner (1951); and Rose, Haines, Warner, and Johnson (1951) found that nitrogen balance could be maintained on a diet devoid of histidine and therefore concluded that histidine was not an essential amino acid for the adult human. In another experiment Weller, Calloway, and Morgen (1971) fed young men a mixture of amino acids based on Rose's requirements for 81 days and noted a negative nitrogen balance in all subjects. They suggested that an indispensible amino acid, most probably histidine, could have been missing from the diet. However, they fed 1-1.3 times the minimum required levels of the essential amino acids whereas Rose and Wixom (1955) fed twice the minimum required levels, thus making it possible that their diets did not contain adedquate amounts of one of the known essential amino acids. Irwin and Hegsted (1971) cited additional experiments that suggest histidine may be an essential amino acid for the adult as well as the infant. A final conclusion cannot be drawn since studies have not

been conducted to ascertain if the adult human can synthesize histidine. Thus far, pathways for the synthesis of histidine have been shown to occur only in microorganisms and plants (Meister, 1965).

The degenerative pathways of histidine are shown in Figure 1. The pathway yielding formiminoglutamic acid (FIGLU), also called the urocanic acid pathway, is the main degradative pathway of histidine. Histidine is first deaminated by the enzyme histidase or histidine deaminase to form urocanic acid (Mehler and Tabor, 1953). Urocanic acid is then converted by urocanase to hydroxyimidazolepropionate with spontaneous tautomerization to imidazoleproprionate (Kaeppli and Rétey, 1971). Imidazoloneproprionate hydrolase acts on the latter compound to convert it to FIGLU (Rao and Greenberg, 1961). In folic acid-deficient animals the presence of FIGLU was detected in the urine, which suggested that derivatives of folic acid might be necessary in the degradation of histidine. This was shown to be the case by Sagers et al. (1956), who showed that the formimino group is transferred to tetrahydrofolic acid (THF) to form formimino - THF, with glutamic acid being the other major product of this reaction. Thus the result of this major pathway of histidine catabolism is that one of the six carbon atoms of histidine enters the one-carbon pool and the other five carbon atoms are used for protein synthesis, gluconeogenesis, or oxidized to carbon dioxide by way of the Krebs cycle.

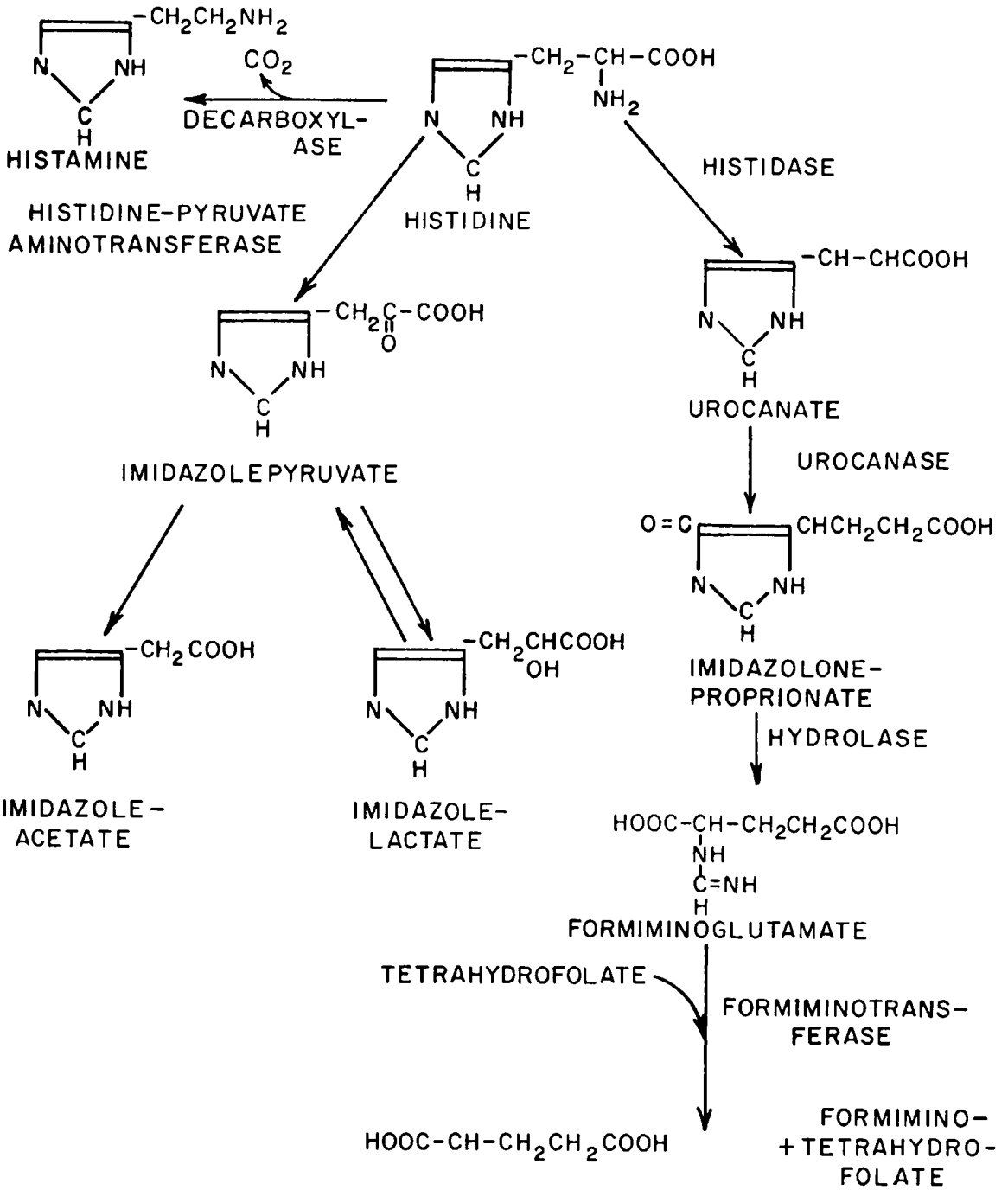

Figure 1. Major Metabolic Pathways of Histidine Degradation

Another pathway for the catabolism of histidine is by transamination with pyruvate to imidazolepyruvate which is then either reduced to imidazolelacetate or decarboxylated to imidazoleacetate There are two forms of the aminotransferase that catalyzes this reaction in the rat liver. One form is found in the mitochondria and the other is found in the cytosol (Spolter and Baldridge, 1964). Morris (1972) has found that the two forms of aminotransferase respond to most of the same dietary and hormonal treatments, but to varying degrees. The significance of this is not yet known. Imidazoleacetate is not known to be utilized any further and is excreted in the urine. Imidazolelactate and imidazolepyruvate can be reconverted to histidine and used somewhat less efficiently to support growth in rats (Cox and Rose, 1926). These compounds also have been observed in the urine.

Dietary and hormonal treatments have different effects on the enzymes of the two major pathways of histidine catabolism (Schirmer and Harper, 1970; Lee and Harper, 1971; Lee et al., 1972; and Morris, Lee, and Harper, 1972). Histidine-pyruvate aminotransferase is not induced in rats fed a diet containing a large amount of protein, but is induced to high activity when rats are injected with glucagon or glucagon plus cortisol. A high protein diet or injections of glucagon or glucagon plus cortisol will induce histadase activity as well in the rat. Morris et al. (1972)

have shown that dietary and hormonal treatment causing the enzyme inductions had a functional significance in helping to maintain a normal homeostatic environment after an oral load of histidine was given to rats.

Other pathways of histidine degradation are decarboxylation to histamine, and conjugation of histidine or 1-methyl histidine with β-alanine to form carnosine or anserine, respectively. Histamine is oxidized to imidazoleacetaldehyde, then to imidazoleacetic acid. The metabolism of histidine by these two pathways is discussed in detail by Stifel and Herman (1971).

Amino Acid Toxicity

When rats are fed an excessive amount of individual essential amino acids in a low or moderate protein diet, depressions in growth and food intake are generally observed (Sauberlich, 1961; Daniel and Waisman, 1968; and Peng et al., 1973). The severity of the observed depressions in growth and food intake depend on the specific amino acid fed and its relative concentration in the diet. Many of the studies on amino acid toxicity have been done under such a wide variety of dietary and experimental conditions that a clear comparison is difficult. Also, only a few amino acids have been studied in much detail. Therefore, in the review that follows, the amino acids are generally discussed individually.

Rats receiving diets containing approximately 2% cystine exhibited a reduced growth rate, but no mortality (Harper et al., 1970). However, reduced growth rate and a 50% mortality was observed in rats fed a 10% casein diet containing 2.42% cysteine (Benevenga, Harper, and Rogers, 1968). Lillie (1932) found damage to the liver and kidney when rats were fed a 4% casein diet containing 5, 10, or 20% of cystine.

Methionine consistently has been the most toxic amino acid in experiments devised to assess the relative toxicity of dietary amino acids (Russell, Taylor, and Hogan, 1952; Sauberlich, 1961, Daniel and Waisman, 1968; and Peng et al., 1973). The growth of rats was depressed 12% by the inclusion of only 0.3% of DL-methionine in diets containing amino acids in amounts to meet the minimum requirements of the rate (Russell et al., 1952). Additions of glycine and serine to high methionine diets alleviated the methionine toxicity (Benevenga and Harper, 1967), but only after the high methionine diets had been fed for about 3 days. This indicated that an adaptation period to the high methionine diet was required by the rats. Evidence for an adaptive period was supplied by Benevenga and Harper (1970) who observed that supplements of glycine and serine enhanced methionine oxidation, lowered plasma methionine levels, and increased stomach emptying rate, but only after the third day of the experiment. The effects of serine and glycine

were also paralleled by increased growth and food consumption.

Due to the large interest in devising experimental models for studying the genetic errors of metabolism of phenylalanine and tyrosine, the effects of ingestion of excesses of these amino acids have been studied more intensely.

When phenylalanine is fed in excess to rats there is a marked depression in growth and some mortality when the levels of this amino acid approached 7% in a low protein diet (Wang and Waisman, 1961). When the quantity of protein was increased in the diet the depression in growth was less (Harper et al., 1966). Plasma concentrations of phenylalanine and tyrosine are elevated when rats are fed diets high in phenylalanine (Sauberlich, 1961 and Peng et al., 1973). High concentrations of these amino acids have also been reported in the brains of rats (McKean, Boggs, and Peterson, 1968, and Peng et al., 1973). In a study by Godin and Dolan (1966), more radioactive carbon was excreted as respiratory carbon dioxide by rats receiving a high phenylalanine diet than in the controls when both groups were injected with DL-phenylalanine-3-^{14}C or DL-phenylalanine-1-^{14}C, They also observed less incorporation of radioactivity into liver protein in the high phenylalanine group. However, these results are questionable since the amount of radioactivity given was the same in both the control and the

high phenylalanine group. In effect, the radioactivity would be greatly diluted in the high phenylalanine group; thus the specific activity would be lower in the liver phenylalanine pool of rats fed the toxic diet. If a correction factor is introduced to correct the differences in specific activities, incorporation of radioactivity into liver proteins by rats fed a high phenylalanine diet is actually increased.

As is the case with most amino acids ingested in excess, animals on a diet high in phenylalanine gradually adapt to the diet and show improvement in growth and food intake (Daniel and Waisman, 1968).

Rats fed a diet containing increased amounts of tyrosine develop distinct swelling and redness of the paws and a dark discharge around the eyes (Sullivan, Hess, and Sebrell, 1932). Studies by Boctor and Harper (1968) have shown that most of the rats fed a diet containing 5% tyrosine died within 2 weeks. The rats that did not die showed no evidence of adaptation to the diets. By supplementing a diet containing 3% tyrosine with 1.25% threonine, the toxic symptoms were alleviated but cataracts developed. However a further supplementation with tryptophan prevented the cataracts (Alam et al., 1966). The possibility of increased threonine in the diet of rats having an effect on the tryptophan requirement has been discussed by these authors and by Harper et al. (1970). Alam and associates

(1967) showed that tyrosine concentrations in the plasma are decreased and liver tyrosine transaminase activities are increased in rats fed amino acid supplements to correct for tyrosine toxicity. These authors also reported that cortisol is effective in suppressing tyrosine toxicity.

An increased dietary intake of tryptophan causes depressed growth and food intake and an increased concentration of tryptophan in the blood (Sauberlich, 1961), and brain (Peng et al., 1973) of rats. The activities of several enzymes including tryptophan pyrrolase are elevated in rats fed or injected with high amounts of tryptophan, but the significance of these increases with respect to the toxic effects of increased tryptophan intake has not been assessed (Harper et al., 1970). Increasing the amount of protein in the diet of rats has been reported by Daniel and Waisman (1968) to decrease the toxic effects attributed to high levels of tryptophan.

Few studies have been conducted on the effects of feeding a diet high in threonine. Harper et al. (1966) reported a moderate depression in growth in rats fed a low protein diet containing 6% of DL-threonine. Peng and associates (1973) and Sauberlich (1961) reported similar observations in rats fed a low protein diet containing 5% DL-threonine, and also showed that an elevated level of threonine in the diet greatly increased plasma and brain threonine concentrations. Liver threonine dehydratase

activity is not increased when rats are fed a high threonine diet (Goldstein, Knox, and Berman, 1962).

Studies by Muramatsu and colleagues (1971) showed that rats fed either a 10% or 20% casein diet containing 5% L-histidine exhibited marked depressions in growth and food intake over a period of two weeks. Increased plasma and brain histidine concentrations were reported, by Peng and others (1973), three hours after force-feeding a low protein diet supplemented with 5% L-histidine. They also observed that the concentrations of essential amino acids other than threonine, lysine, and arginine were decreased in the brain. High concentrations of histidine in the plasma of rats fed diets high in histidine also have been reported by other workers (Sauberlich, 1961 and Daniel and Waisman, 1968). Martin (1946, 1947) observed that some rats died when fed a diet containing 10% L-histidine over a period of two months. If the diet was also deficient in riboflavin, the death rate was 100%. When the diet was only deficient in riboflavin the rats survived.

In a study by Harper et al. (1966) rats were fed a diet containing 6% casein and 2% L-histidine. Growth depression was severe but if the protein content of the diet was increased to 12%, the degree of growth depression was less. This indicates that the rat can better tolerate an excess of histidine if the protein content of the diet is increased. When the amounts of protein and histidine

were proportionately increased from 6:2 to 12:4 (casein: histidine), the depression of growth was decreased also.

In chicks fed a diet containing 3% histidine, the depressions in growth were reduced by the addition of glycine to the diet (Snetsinger and Scott, 1961). The addition of 2% histidine to the diets of zinc-deficient chicks was found by Nielson, Sunde, and Hoekstra (1967) to prevent the characteristic leg disorder in zinc deficiency in chicks, but had no effect on the reduced growth observed. Of the other metabolites of histidine tested, only the addition of 0.2% histamine was effective in preventing the leg disorder.

In experiments with infant monkeys, Kerr, Wolf, and Waisman (1965) found that hyperlipemia occurred in animals fed a high histidine diet for four months. The liver appeared to be essentially normal. The relationship between lipid metabolism and excesses of dietary amino acids is presently unknown.

The genetic defect of histidine metabolism, histidinemia, was first discovered by Ghadimi, Partington, and Hunter (1961). In this disease histidase is lacking from the liver and skin (LaDu et al., 1962 and Baldridge and Auerbach, 1964). Thus histidinemic persons apparently are not able to convert histidine to urocanic acid in the liver and skin. Ghadimi, Partington, and Hunter (1961, 1962) noted hyperalaninemia in some patients. In patients

remaining untreated, blood serotinin levels are reduced but return to normal when plasma histidine concentrations are lowered by treatment (Corner and associates, 1968).

Less attention has been given to the effects of feeding large amounts of individual non-essential amino acids in animals. Generally no toxic effects have been observed other than small depressions in growth and food intake in animals fed large excesses of a dispensible amino acid. For more detail on this subject and information on the separate area of amino acid antagonisms observed between the branched chain amino acids and the lysine-arginine antagonism, the reader is referred to the excellent review article by Harper et al. (1970).

METHODS

Animals and Diets

Male rats of the Holtzman strain were used in all experiments. They were individually housed in screen bottomed cages with water offered ad libitum. The animal room was maintained at a temperature of 76 $\pm$ 2°F. The room was kept under a controlled light-dark cycle of 12 hours. The rats were weighed daily and fed as described below. All diets were fed as agar gels, unless otherwise stated, by mixing the dry diet with an equal volume of boiling 3% agar solution. The agar diets were then poured into plastic containers and allowed to cool. After cooling at room temperature, the diets were stored under refrigeration.

Growth and Food Intake

Rats weighing 55-60g were divided into 2 groups and fed ad libitum a 6 or 12% casein diet until the group receiving the 6% casein diet reached an average weight of approximately 100g. The composition of each control diet is given in Table 1. When the desired weight was attained, the rats receiving the 6% casein diet were divided into 2 groups of 7 rats each and fed ad libitum a diet containing either 6% casein or 6% casein plus 2% L-histidine. The rats receiving the 12% casein diet were divided into 4 groups of

Table 1. Composition of Diets

Constituents	6% Casein[a]	12% Casein[b]
Casein	6	12
L-Methionine	0.2	0.2
DL-Threonine	0.55	0.55
Corn oil	5	5
Salt mixture[c]	5	5
Vitamin mixture[d]	0.5	0.5
Choline bitartrate	0.36	0.36
Glucose monohydrate	41.2	38.2
Cornstarch	41.2	38.2

[a]The diet containing 6% casein plus 2% L-histidine was prepared by adding 2% of L-histidine to the 6% casein diet at the equal expense of glucose monohydrate and cornstarch.

[b]The diets containing 12% casein plus 2% L-histidine and 12% casein plus 4% L-histidine were prepared by adding either 2% of L-histidine or 4% of L-histidine to the 12% casein diet at the equal expense of glucose monohydrate and cornstarch.

[c]Rogers-Harper salt mix, General Biochemicals (Rogers and Harper, 1965).

[d]0.5% of the vitamin mixture in the diet provided the rats with the following vitamins in mg/kg diet: thiamine·HCL, 5; riboflavin, 5; niacinamide, 25.0; Ca-D-pantothenate, 20; pyridoxine·HCL, 5; folic acid, 0.5; menadione, 0.5; d-biotin, 0.2; vitamin B_{12} (0.1% in mannitol), 30; ascorbic acid, 50; vitamin E acetate, 400; vitamin A acetate and vitamin D_2 (500,000 IU A, 50,000 IU D_2/g), 8.

7 rats each and were fed ad libitum a diet containing either 12% casein, 12% casein plus 2% L-histidine, 12% casein plus 4% L-histidine, or 12% casein plus 2% L-histidine plus 100,000 IU of vitamin A per 100g diet. Body weight and food consumption were measured daily. Food intake was measured on a dry-weight basis.

In another experiment, rats weighing 85-90g were fed a 12% casein diet for 10 days. On day 11 the rats were divided into 4 groups of 6 rats per group. Each group was fed ad libitum a diet containing either 12% casein, 12% casein plus 33mg of folic acid per kg of diet, 12% casein plus 4$ L-histidine, or 12% casein plus 4% L-histidine plus 33mg of folic acid per kg of diet. Body weight and food consumption were measured daily.

Plasma and Brain Amino Acid Concentrations

Rats weighing 55-60g were divided into 2 groups and fed ad libitum either a 6% casein or 12% casein diet (Table 1) until the group receiving the 6% casein diet reached an average weight of about 100g. The rats were then divided into 6 groups of 14 rats each and fed the various diets described above except that in the group receiving the diet containing additional vitamin A, the vitamin A concentration was reduced to 3,300 IU of vitamin A per 100g of diet. Three rats were sacrificed from each group after having been fed their respective diets for 0, 1, 3, 8, and 14 days. The

rats were fasted for 8 hours from midnight the night before to 8 a.m. of the day of sampling. They were then force-fed a single meal (2g per 100g of body weight) of their respective diets in liquid form (1g dry diet plus 1g distilled water) by stomach intubation. Five hours later the rats were anesthetized with ether and killed. Blood was drawn with a heparinized syringe by exposed heart puncture and the head was removed within 4 minutes and frozen in liquid nitrogen before storage in the freezer.

The plasma was obtained from the heparinized blood by centrifugation at 12,100 x g for 15 minutes in a refrigerated centrifuge. Equal volumes of the plasma from the three blood samples from each group were pooled and subsequently deproteinized by adding 0.25ml of 15% sulfosalicylic acid (SSA) per ml of pooled plasma. The precipitated proteins were removed by centrifugation at 12,100 x g for 15 minutes. The supernates were used for free amino acid analysis with a Beckman Model 121 amino acid analyzer.

The brain samples were prepared by homogenizing the cerebrum with 3 volumes of distilled water. The cerebral homogenate was deproteinized with 15% SSA (1ml homogenate plus 0.25ml SSA) and centrifuged at 12,100 x g for 15 minutes. Equal volumes of the supernates from each group were pooled and analyzed for free amino acids on the amino acid analyzer.

Labeled Diets

Uniformly labeled L-threonine, L-phenylalanine, L-lysine, or L-histidine was used in making the radioactive diets.[1] Six grams of the dry diet were weighed into a large disposable syringe which had the end cut off so that the agar gel diets could easily be expelled. In mixing the radioactive diets, 1ml of the isotope solution and 6ml of boiling 3% agar solution were added to the diet in the syringe and thoroughly mixed with a glass rod. The diets were allowed to cool at room temperature and subsequently were stored in the refrigerator. Except for diets containing labeled histidine, each diet contained 3.0μc of a labeled amino acid. In the labeled histidine diets, 0.8μc of labeled histidine was incorporated into the control (12% casein) diet and 10.0μc were incorporated into the high histidine (12% casein plus 4% histidine) diet. This was done so that constant dietary specific activity would be maintained since there is 12.5 times as much histidine in the high histidine diet as in the control diet. If no corrections were made, the radioactivity would be greatly diluted in the high histidine diet due to the high content of histidine in the diet and the increased histidine pool size in the body of the rat.

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1. The labeled amino acids were purchased from New England Nuclear Corporation, Boston, Massachusetts, 02118.

Metabolism Experiments

During a 2 week period rats were trained to eat a control diet (12% casein) within 2 hours by gradually decreasing the amount of time the diet was offered. They were then divided into 2 groups of the same average weight. One group was fed a 12% casein diet (control), and the other group was fed a diet containing 12% casein plus 4% histidine (high histidine). After being fed the diets for 2 days, some rats were placed in glass metabolism cages on the morning of the third day and fed the control or high histidine diet containing either uniformly labeled lysine, phenylalanine, threonine, or histidine. The amount of labeled amino acid added to the diets was not in sufficient quantity to affect the protein quality of the diets.

Expired carbon dioxide was collected at hourly intervals for 6 hours by trapping it in a 15ml solution of methyl-cellosolve and ethanolamine (1:1 V/V). A 3ml aliquot of these samples was taken and, using the scintillation fluid detailed by Jeffay and Alvarez (1961), the radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. The automatic external standard technique was used to estimate the counting efficiency of each sample.

At the end of the 6 hour time period the rats were removed from the metabolism cages and anesthetized with ether. The liver, head, stomach, and small intestine of

each rat was removed and frozen in dry ice before being transported to the freezer until analysis could be done.

To determine the amount of radioactivity that was absorbed during the 6 hour period, the stomach and small intestine were thoroughly washed out with saline. Any particles of food that were left in the cages were combined with the washings. They were then brought to constant volume and mixed in a Waring blender. A 1ml aliquot was counted for radioactivity using the fluid as described by Bruno and Christian (1961). After correcting for the dilution, the amount of radioactivity found was subtracted from the amount added to the diet, thus giving the amount absorbed. Counting efficiency was estimated by the external standard technique.

The livers were thawed and washed with saline, then blotted on filter paper. They were then weighed and homogenized with distilled water (1g liver plus 4ml water). Total protein content was determined by the method of Lowry et al. (1951). A portion of the liver homogenate was deproteinized by adding 15% SSA and analyzed for free amino acids using the same procedure described above for brain homogenates. Measurement of the incorporation of radioactivity into liver protein was done by using the filter paper disc method of Mans and Novelli (1961). In some liver homogenates the amount of radioactivity present in the acid-soluble fraction was measured. Two ml of 10% tricloroacetic

were added to 2ml of the homogenate and centrifuged at 12,100 x g for 15 minutes. One ml aliquots of the resulting supernates were counted for radioactivity as described by Bruno and Christian (1961).

In some animals the cerebrum was homogenized with distilled water to give a 25% homogenate. The homogenates were then analyzed for total protein and incorporation of radioactivity into brain protein by the same methods described above for liver homogenates.

After consuming either a control or high histidine diet for 7 days, rats were placed in the glass metabolism cages on day 8, and the respective control or high histidine diet containing uniformly labeled histidine or threonine fed. The same procedures described above for collection and preparation of samples on day 3 were used on day 8 except that brain samples were not analyzed.

Statistical Treatment of Data

Student's t-test was used on all tests of significance between the means of two samples.

RESULTS

As shown in Figure 2, the growth of rats fed diets containing an excess of histidine was depressed. Rats fed the diet containing 6% casein plus 2% histidine showed the least total gain in weight over the 2 week period. Rats fed 2% histidine in their diets exhibited less growth depression when the protein content of the diet was 12% as compared to the 6% casein diet. When 100,000 IU vitamin A per 100g diet was included in the diet growth depression was increased. On day 10 the vitamin A content of the diet was reduced to 3,300 IU per 100g of diet. After reducing the vitamin A content of the diet, the growth rate appreciably increased. When the amounts of protein and histidine in the diet were proportionally increased greater total weight gain was observed, but when compared with respective controls, the growth depressions were essentially the same. In all groups receiving an excess of histidine, an adaptation to the diet appeared to occur after the third day as indicated by general increases in the slopes of the growth curves.

Figure 3 depicts the average daily food intakes of the rats described in Figure 2. The food intake curves are directly related to the growth curves discussed above. The groups showing the greatest growth depressions (12% casein plus 4% histidine, and 6% casein plus 2% histidine) also

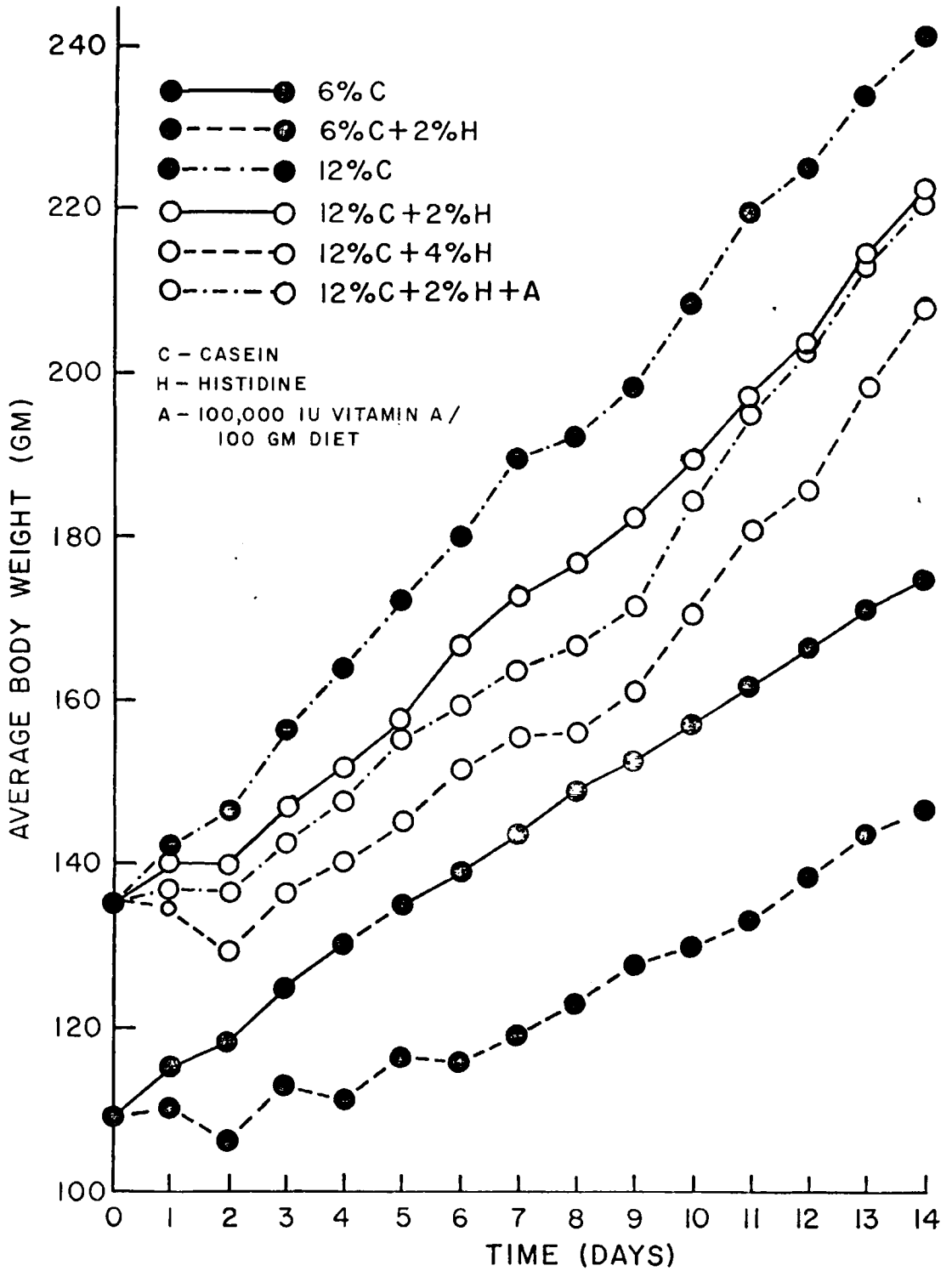

Figure 2. Average Daily Body Weight of Rats Fed Diets with and without Excessive Levels of Histidine

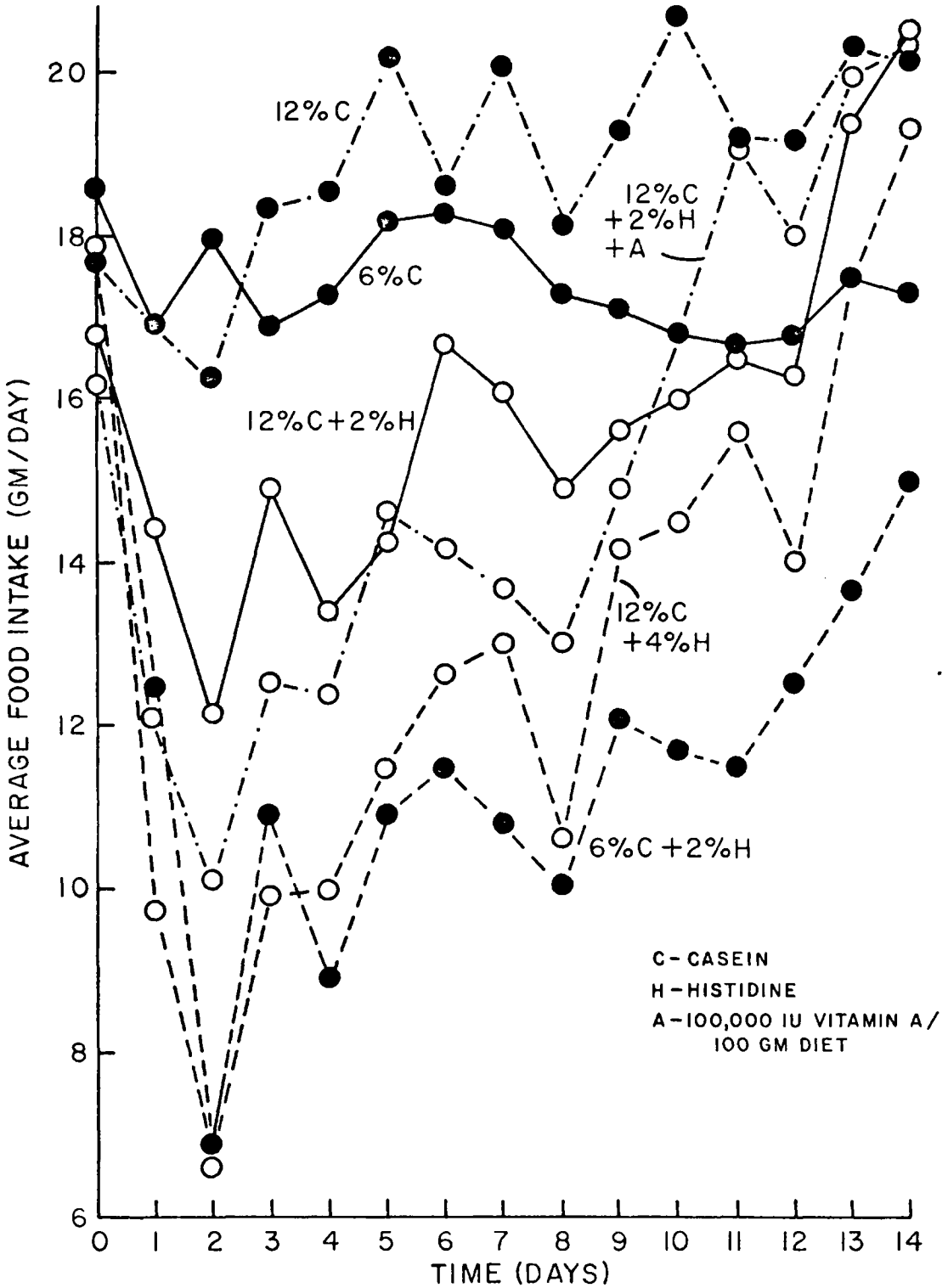

Figure 3. Average Daily Food Intake of Rats Fed Diets with and without Excessive Levels of Histidine

showed the greatest depressions in food intake. That an adaptive response to excess histidine seems to occur on the third day is even more evident from the food intake data.

The results of including 33mg of folic acid per kg of diet in the diets of the control (12% casein) and high histidine (12% casein plus 4% histidine) rats on growth and food intake are shown in Figure 4 and 5. Initially folic acid depressed growth in both the control and high histidine groups, but stimulated the growth of rats receiving the high histidine diet after the third day. However the stimulation observed was not statistically significant. Folic acid had no significant effect on food intake in either the control or high histidine groups.

Tables 2 through 5 show the relative concentrations of plasma amino acids, expressed as a percentage of their respective control values (6% casein or 12% casein), 5 hours after force-feeding the diet on the days indicated.

When a diet containing 6% casein plus 2% histidine was force-fed to rats (Table 2), the plasma level of histidine was increased 55-fold on day 1 and remained elevated on days 3, 8, and 14. The concentrations of the other indispensible amino acids were generally decreased and the concentrations of the dispensible amino acids, with the exception of glycine, generally were increased throughout the course of the experiment. By the third day the concentrations of threonine and phenylalanine were depressed

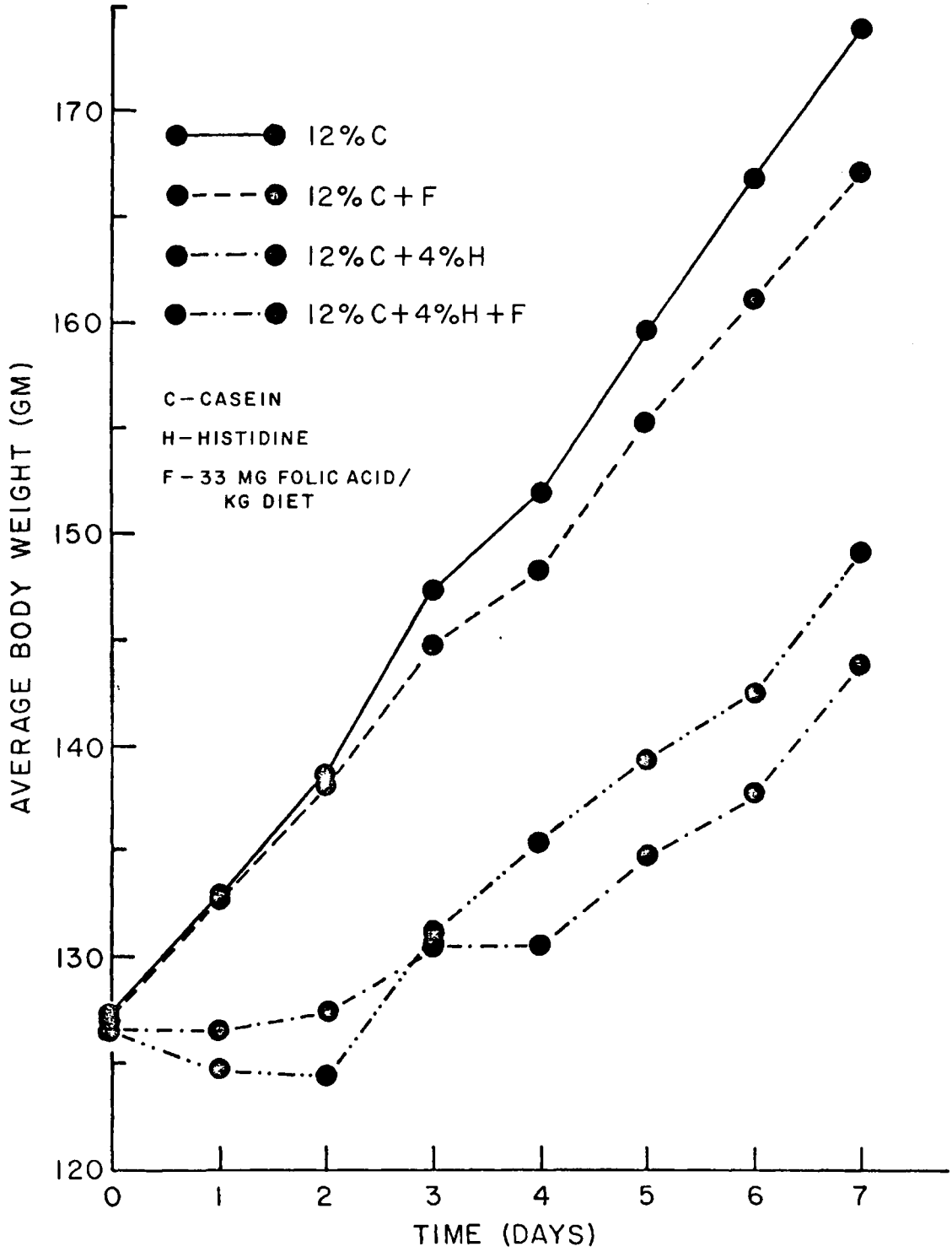

Figure 4. The Effect of Folic Acid Supplementation on Average Body Weight of Rats Fed a Diet with and without an Excessive Level of Histidine

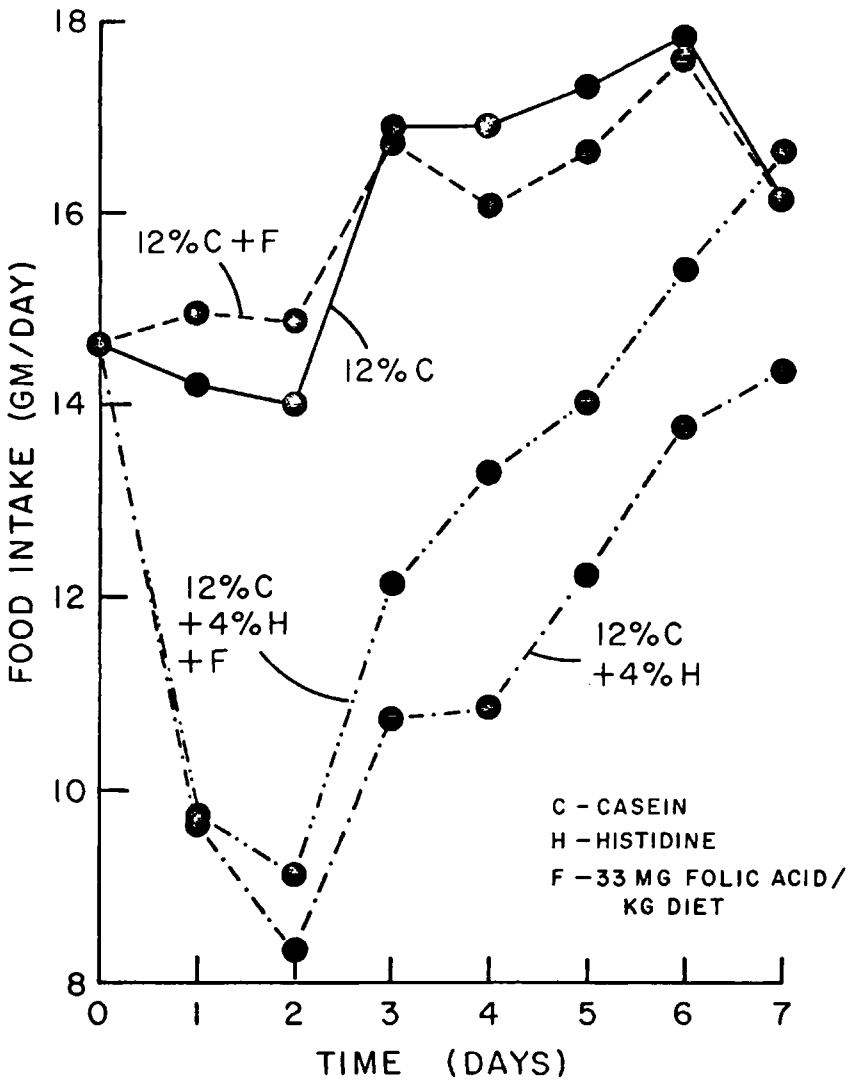

Figure 5. The Effect of Folic Acid Supplementation on Average Daily Food Intake of Rats Fed a Diet with and without an Excessive Level of Histidine

Table 2. Relative Concentrations of Plasma Amino Acids 5 Hours After Force-Feeding Rats a 6% Casein Plus 2% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	95	123	89	77
Histidine	5498	6441	6491	5799
Arginine	122	107	136	127
Aspartic Acid	109	131	133	119
Threonine	159	44	53	46
Serine	142	220	182	174
Glutamic Acid	115	171	147	151
Proline	127	148	125	127
Glycine	141	135	126	100
Alanine	174	223	220	168
Cystine	134	93	114	101
Valine	81	97	110	92
Methionine	101	91	58	103
Isoleucine	90	96	73	99
Tyrosine	42	101	65	79
Phenylalanine	53	61	46	65

[a]Average of 3 rats/group.

[b]The control group was force-fed a 6% casein diet on the days indicated.

Table 3. Relative Concentrations of Plasma Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 4% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	135	115	111	65
Histidine	5584	5554	7131	4515
Arginine	129	81	66	74
Aspartic Acid	83	84	93	100
Threonine	158	73	49	32
Serine	152	129	168	118
Glutamic Acid	141	75	76	102
Proline	181	103	105	89
Glycine	123	129	103	84
Alanine	181	93	94	112
Cystine	130	100	74	95
Valine	106	80	103	74
Methionine	207	102	156	86
Isoleucine	98	86	111	71
Tyrosine	126	59	29	33
Phenylalanine	70	69	58	52

[a]Average of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

Table 4. Relative Concentrations of Plasma Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 2% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	119	112	92	81
Histidine	812	934	667	672
Arginine	106	85	98	101
Aspartic Acid	95	107	82	92
Threonine	126	73	61	56
Serine	119	121	119	112
Glutamic Acid	126	91	68	114
Proline	104	95	103	87
Glycine	101	90	61	51
Alanine	117	108	91	98
Cystine	110	93	76	93
Valine	91	82	93	88
Methionine	180	99	111	101
Isoleucine	92	91	89	97
Leucine	79	81	82	89
Tyrosine	74	60	47	60
Phenylalanine	69	61	72	78

[a]Average of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

Table 5. Relative Concentrations of Plasma Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 2% Histidine Diet Plus 3,300 IU Vitamin A/100 g Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	146	112	102	80
Histidine	905	736	688	191
Arginine	140	94	97	92
Aspartic Acid	101	99	97	88
Threonine	153	79	73	83
Serine	120	129	107	89
Glutamic Acid	154	126	96	94
Proline	170	100	103	99
Glycine	100	76	55	54
Alanine	146	106	97	103
Cystine	116	117	96	100
Valine	103	85	98	89
Methionine	216	94	118	82
Isoleucine	99	94	90	78
Leucine	97	90	82	79
Tyrosine	115	62	60	79
Phenylalanine	89	62	71	86

[a]Average of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

substantially and remained depressed throughout the experiment.

In rats force-fed a 12% casein plus 4% histidine diet (Table 3), the plasma level of histidine was greatly elevated through the eighth day and then decreased somewhat on day 14. The concentrations of most amino acids were more balanced on the first day than was observed for rats fed a low protein toxic diet (Table 2). However, by the fourteenth day the essential amino acid concentrations were again appreciably decreased. Threonine, phenylalanine, and tyrosine showed a drastic decrease in relative concentrations throughout the experiment. Glycine, a dispensible amino acid for rats, exhibited a gradual decline in the plasma.

In the plasma of rats force-fed a diet containing 12% casein plus 2% histidine (Table 4) the relative concentrations of histidine are increased only 8.1, 9.3, 6.7, and 6.7 times on days 1, 3, 8, and 14, respectively. As in the other groups, essential amino acids and glycine showed a steady decrease in concentration throughout the time period studied.

When vitamin A was added to the moderately toxic diet (Table 5), the relative plasma concentrations of most amino acids had generally decreased to varying degrees by day 14. This is evident in the concentration of histidine on day 1 compared to day 14. Though still above control

concentration, the decline in plasma histidine during the 2 week period was greater in this group than in other groups studied. As in the other groups studied, glycine and most of the essential amino acids showed the lowest relative concentrations by the fourteenth day.

The relative concentrations of brain free amino acids 5 hours after force feeding rats various high histidine diets are shown in Tables 6 through 9. The concentrations are expressed as a percentage of the respective control values obtained from force-feeding rats the control diets (6% casein and 12% casein).

In rats force-fed a diet containing 6% casein plus 2% histidine (Table 6), the histidine was increased 19-fold on the first day. Between the third and eighth day the concentration apparently reached a maximum, then decreased to a 27-fold increase over the control by day 14. The amino acid pattern for histidine appears to be similar to the plasma (Table 2) but less marked. The relative concentrations of all amino acids were gradually decreased throughout the experiment. Most notable of these were the essential amino acids.

When a 12% casein plus 4% histidine diet was force-fed (Table 7), the histidine concentration in the brain reached a maximum on day 8. By day 14 the concentration was less than 50% of the value obtained on day 8 but was still 40% higher than the histidine concentration observed on

Table 6. Relative Concentrations of Brain Amino Acids 5 Hours After Force-Feeding Rats a 6% Casein Plus 2% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	130	137	120	103
Histidine	1926	5635	5489	2679
Arginine	130	98	94	126
Aspartic Acid	91	140	88	88
Threonine	99	56	58	60
Serine	102	87	102	99
Glutamic Acid	103	126	91	80
Proline	100	114	96	92
Glycine	93	116	105	97
Alanine	107	105	104	97
Cystine	88	89	75	70
Valine	71	73	69	67
Methionine	74	67	54	65
Isoleucine	79	97	81	59
Leucine	80	82	80	69
Tyrosine	92	108	112	84
Phenylalanine	69	66	114	69

[a]Average of 3 rats/group.

[b]The control group was force-fed a 6% casein diet on the days indicated.

Table 7. Relative Concentrations of Brain Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 4% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	118	156	146	131
Histidine	1802	5614	5999	2581
Arginine	68	112	100	140
Aspartic Acid	146	168	100	124
Threonine	92	71	54	72
Serine	75	99	139	122
Glutamic Acid	174	269	149	321
Proline	89	125	117	153
Glycine	102	122	115	143
Alanine	92	91	105	140
Cystine	89	105	81	127
Valine	79	78	85	119
Methionine	141	141	197	167
Isoleucine	64	91	91	91
Leucine	70	99	90	110
Tyrosine	113	79	40	73
Phenylalanine	75	88	78	125

[a]Pooled samples of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

Table 8. Relative Concentrations of Brain Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 2% Histidine Diet[a]

	Per Cent of Control[b]			
Amino Acid	Day 1	Day 3	Day 8	Day 14
Lysine	108	123	114	136
Histidine	743	965	740	687
Arginine	66	97	107	142
Aspartic Acid	106	127	100	125
Threonine	106	84	77	107
Serine	89	101	116	119
Glutamic Acid	117	163	117	279
Proline	88	105	116	160
Glycine	94	110	105	131
Alanine	100	102	109	137
Cystine	88	110	115	132
Valine	92	100	100	143
Methionine	206	147	171	179
Isoleucine	72	94	87	105
Leucine	70	98	91	128
Tyrosine	89	79	62	102
Phenylalanine	82	84	96	146

[a]Pooled samples of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

Table 9. Relative Concentrations of Brain Amino Acids 5 Hours After Force-Feeding Rats a 12% Casein Plus 2% Histidine Diet Plus 3,300 IU Vitamin A/100 g Diet[a]

Amino Acid	Per Cent of Control[b]			
	Day 1	Day 3	Day 8	Day 14
Lysine	113	109	120	127
Histidine	656	685	747	232
Arginine	80	85	115	126
Aspartic Acid	175	100	102	119
Threonine	108	77	92	125
Serine	68	75	110	114
Glutamic Acid	211	92	134	229
Proline	96	103	120	159
Glycine	117	98	108	128
Alanine	89	87	116	123
Cystine	95	118	117	146
Valine	92	80	109	122
Methionine	211	99	134	145
Isoleucine	74	95	90	81
Leucine	82	95	103	105
Tyrosine	114	73	81	120
Phenylalanine	91	76	101	138

[a]Pooled samples of 3 rats/group.

[b]The control group was force-fed a 12% casein diet on the days indicated.

day 1. The concentrations of threonine, valine, isoleucine, leucine, tyrosine, and phenylalanine were generally less than their respective control values throughout the experiment. With the exception of histidine, the concentrations of all amino acids appeared to be relatively higher on day 14 than was observed in the rats fed the diet containing 6% casein plus 2% histidine.

Brain histidine levels were 7.4, 9.6, 7.4, and 6.9 times their control values on days 1, 3, 8, and 14, respectively, in rats force-fed a 12% casein plus 2% histidine diet (Table 8). The levels of threonine, valine, isoleucine, leucine, tyrosine, and phenylalanine were below their control values through day 8, but returned to the control values or higher by the fourteenth day. The concentration of methionine was increased to a moderate degree throughout the study.

The effects of an increased amount of vitamin A in a high histidine diet on the concentrations of brain amino acids are shown in Table 9. The brain concentrations of histidine were consistently lower in this group than in the other groups studied, as was also the case with plasma histidine levels in this group. By the third day, concentrations of threonine, serine, tyrosine, and phenylalanine were noticeably depressed, but by day 14 they all exhibited brain concentrations above their respective control values.

The total production of radioactive carbon dioxide from rats fed either a control (12% casein) or high histidine (12% casein plus 4% histidine) diet containing uniformly labeled phenylalanine, lysine, threonine, or histidine is shown in Table 10. The production of radioactive carbon dioxide was higher in rats fed the high histidine diet for three days in all cases. However the results were significantly higher only when labeled threonine or histidine was present in the diets. On the eighth day, rats consuming the high histidine diet oxidized more labeled threonine and histidine than the control animals. However the results were not significantly different. The small number of animals in each group and the relatively large standard error obtained might be an explanation for not detecting any significance between the two means.

Table 11 shows the relative concentrations of liver amino acids in rats 6 hours after being fed a high histidine meal. The liver concentration of histidine was increased 15-fold over its control value on day 3 and 16-fold after being fed the high histidine diet for eight days. With the exception of lysine, serine, glutamic acid, and alanine, all other amino acids showed a decrease in concentration on day 3. A moderate recovery in concentration of amino acids was exhibited on the eighth day. As observed in plasma and brain concentrations, threonine concentration was the most depressed of any amino acid in the liver.

Table 10. Total Production of Radioactive CO_2 from Rats Six Hours After Feeding the Labeled Diets[a]

Group	Isotope	Per Cent of Absorbed Dose Recovered as $^{14}CO_2$[b]	
		Day 3	Day 8
Control[c]	L-Phe-U-^{14}C	4.18 ± 0.29[e]	--
High histidine[c]	L-Phe-U-^{14}C	5.1	--
Control[c]	L-Lys-U-^{14}C	7.3	--
High histidine[c]	L-Lys-U-^{14}C	8.5	--
Control[d]	L-Thr-U-^{14}C	7.33 ± 0.48	10.53 ± 0.84
High histidine[d]	L-Thr-U-^{14}C	10.74 ± 1.28*	14.28 ± 1.34
Control[d]	L-His-U-^{14}C	4.96 ± 0.45	10.22 ± 2.53
High histidine[d]	L-His-U-^{14}C	15.50 ± 2.43**	20.82 ± 4.96

[a]The rats were trained to consume their diet within 2 hours.

[b]% absorbed =

$$\frac{\text{radioactivity ingested - radioactivity in GI contents}}{\text{radioactivity ingested}} \times 100$$

[c]Average of 3 rats/group.

[d]Average of 4 rats/group.

[e]Mean ± SE M.

*$P < 0.05$.

**$P < 0.01$.

Table 11. Relative Concentrations of Liver Amino Acids in Rats 6 Hours After Feeding a 12% Casein Plus 4% Histidine Meal[a]

	Per Cent of Control[b]	
Amino Acid	Day 3	Day 8
Lysine	107	133
Histidine	1520	1640
Aspartic Acid	73	79
Threonine	55	41
Serine	110	105
Glutamic Acid	141	128
Glycine	56	52
Alanine	99	100
Valine	53	75
Methionine	75	93
Isoleucine	61	87
Leucine	62	85
Tyrosine	53	67
Phenylalanine	60	73

[a]The rats were trained to consume their diet within 2 hours. Pooled samples of 8 rats.

[b]Control rats were fed a 12% casein diet on the days indicated.

Liver protein concentrations of rats fed either a control or high histidine radioactive diet are shown in Table 12. There was no significant difference in liver protein content when rats were fed a high histidine diet as compared to rats fed a control diet. The liver size of high histidine rats was increased over control rats which would increase total liver protein in rats fed a high histidine diet.

Rats fed a diet high in histidine for 3 days appeared to incorporate more phenylalanine, lysine, threonine, and histidine into liver protein as illustrated in Table 13. When fed the diets for 8 days, more labeled threonine and histidine were incorporated into liver protein in rats fed a high histidine diet. Statistical treatment showed that only histidine incorporation was significantly increased on day 3 and day 8. The amount of radioactivity from labeled threonine and its metabolites in the acid-soluble fraction was nearly the same in the control and high histidine groups. Recalling that the level of threonine in the liver was depressed by about 50% in rats fed a high histidine diet raises a question concerning the specific activity of labeled threonine in the high histidine group. Since the concentration of threonine in the liver was decreased in rats fed a high histidine diet, the specific activity of the labeled threonine may be greater in this group than in the control group. Unfortunately, the true specific

Table 12. Liver Protein of Rats 6 Hours After Feeding[a]

Group	Isotope	Body Weight g	Liver Weight g	Protein mg/g liver
		Day 3		
Control[b]	L-Phe-U-^{14}C	179±0.7[d]	7.5±0.08	209± 2.1
High histidine[b]	L-Phe-U-^{14}C	173±9.5	7.4±1.2	198±11.0
Control[b]	L-Lys-U-^{14}C	185±3.5	8.1±0.35	204± 2.9
High histidine[b]	L-Lys-U-^{14}C	175±3.9	8.5±1.3	206± 4.6
Control[c]	L-Thr-U-^{14}C	163±3.9	6.9±0.1	187± 1.7
High histidine[c]	L-Thr-U-^{14}C	159±4.3	7.5±0.1	189± 2.3
Control[c]	L-His-U-^{14}C	167±1.7	7.4±0.1	187± 1.3
High histidine[c]	L-His-U-^{14}C	164±2.6	8.2±0.2	191± 1.5
		Day 8		
Control[c]	L-Thr-U-^{14}C	180±1.2	7.3±0.2	193± 2.2
High histidine[c]	L-Thr-U-^{14}C	174±6.3	7.9±0.2	191± 2.4
Control[c]	L-His-U-^{14}C	174±2.5	6.9±0.2	194± 1.3
High histidine[c]	L-His-U-^{14}C	163±3.0	6.8±0.1	193± 2.3

[a]The rats were trained to consume their diet within 2 hours.

[b]Average of 3 rats/group.

[c]Average of 4 rats/group.

[d]Mean ± SEM.

Table 13. Incorporation of Radioactivity Into Liver Protein by Rats 6 Hours After Feeding[a]

Group	Isotope	CPM/mg liver protein	Acid-soluble Fraction DPM/100 mg liver
		Day 3	
Control[b]	L-Phe-U-^{14}C	159 ± 16[d]	--
High histidine[b]	L-Phe-U-^{14}C	199 ± 6	--
Control[b]	L-Lys-U-^{14}C	126 ± 10	--
High histidine[b]	L-Lys-U-^{14}C	153 ± 8	--
Control[c]	L-Thr-U-^{14}C	144 ± 17	1520 ± 60
High histidine[c]	L-Thr-U-^{14}C	186 ± 27	1532 ± 96
Control[c]	L-His-U-^{14}C	51 ± 3	523 ± 26
High histidine[c]	L-His-U-^{14}C	221 ± 53*	19607 ± 2328
		Day 8	
Control[c]	L-Thr-U-^{14}C	146 ± 9	1410 ± 113
High histidine[c]	L-Thr-U-^{14}C	165 ± 10	1019 ± 81
Control[c]	L-His-U-^{14}C	66 ± 5	542 ± 70
High histidine[c]	L-His-U-^{14}C	247 ± 47**	19204 ± 2568

[a]The rats were trained to consume their diet within 2 hours.

[b]Average of 3 rats/group.

[c]Average of 4 rats/group.

[d]Mean ± SEM.

*$P < 0.02$.

**$P < 0.01$.

activity of the labeled threonine present in the livers of the control and the high histidine groups cannot be deduced from the data shown. The amount of radioactivity present in the acid-soluble fraction of livers from the rats fed a high histidine diet was about 33 times the control values on day 3 and day 8.

After consuming a diet high in histidine for 2 days, rats fed labeled diets on the third day did not exhibit increased incorporation of labeled phenylalanine or lysine into brain protein when compared with their respective controls (Table 14).

Table 14. Incorporation of Radioactivity Into Brain Protein by Rats 6 Hours After Feeding[a]

Group	Isotope	mg protein per g brain	CPM/mg brain protein
		Day 3	
Control[b]	L-Phe-U-^{14}C	120 ± 2.6[c]	33 ± 2
High histidine[b]	L-Phe-U-^{14}C	123 ± 1.7	34 ± 5
Control[b]	L-Lys-U-^{14}C	117 ± 3.3	34 ± 5
High histidine[b]	L-Lys-U-^{14}C	119 ± 2.7	32 ± 3

[a]The rats were trained to consume their diets within 2 hours.

[b]Average 3 rats/group.

[c]Mean ± SEM.

DISCUSSION

Harper et al. (1966) studied the effects of protein content of diet on histidine toxicity. They observed that when rats were fed a low protein diet, severe depressions in growth and food intake occurred when 2% histidine was added to the diet. When the amount of protein was doubled and 2% histidine was added to the diet, the depressions observed were less severe. If the protein and histidine content of the diet were proportionally increased, the growth depressions were also less severe. The results reported here generally agree with their observations. However, when expressed as a percentage of their respective controls, growth and food intake depressions were found to be essentially the same in diets containing proportional increases of protein and histidine.

An elevated level of dietary histidine produced elevated levels of histidine in the brain, plasma, and liver of rats. Elevations in brain histidine concentration were generally less than observed in the plasma of rats fed increased dietary concentrations of histidine. That this is an indication of a blood-brain barrier has been discussed most recently by Peng et al. (1973). When the amount of protein was increased and the amount of histidine was kept constant in the diet, elevations of histidine concentration

occurred in both the blood and brain but to a lesser extent. The growth and food intake of these rats was also depressed less. These results indicate that the rat is better able to tolerate a dietary load of histidine when the protein content of the diet is increased. The results also indicate that the degree of histidine toxicity depends on the degree of elevation of histidine in the blood and brain.

When vitamin A was added to the diet, the concentration of histidine in the plasma and brain was elevated less and gradually declined toward control values during the 14-day experimental time period. The amino acid patterns of this group appeared to be the most balanced of any group studied by the end of the experiment (day 14). The association of vitamin A with histidine metabolism is not known, but appears to warrant further investigation.

After the third day of feeding, rats seem to begin to adapt to a high dietary level of histidine as evidenced by a gradual increase in food intake and growth. This adaptation occurred even though the concentration of histidine remained elevated in the plasma and brain, especially when a large amount of histidine was included in the diet. However, though the plasma and brain levels of histidine do not appreciably decrease during adaptation, more histidine is being consumed due to increased food intake. Thus more histidine is apparently being metabolized. Evidence to support this assumption was shown in Table 10, indicating

that more labeled histidine was being oxidized to carbon dioxide after 8 days on the high histidine diet than after 3 days. Nevertheless, it seems that a great decrease in tissue concentrations of histidine is not necessary for adaptation to a diet high in histidine to occur in rats. The possibility of an overriding adaptive mechanism coming into play is an interesting one, but evidence of this has not been shown.

As the animals were adapting to a diet containing high levels of histidine, the relative concentrations of glycine and most of the essential amino acids decreased in the plasma, brain, and liver. A comparison of Tables 3 and 4 shows that if the histidine content of the diet is increased, the relative depressions in concentration of the above-mentioned amino acids are greater. This comparison shows that the dietary level of histidine had a direct effect on the depression in concentrations of other amino acids. The tissue concentrations of threonine, tyrosine, and phenylalanine show the greatest depression. Lee et al. (1972) and others cited by him have noted that repeated injections of glucagon induced the activities of threonine dehydratase, tyrosine transaminase, and phenylalanine-pyruvate aminotransferase. The results reported here showed an increased oxidation of threonine and phenylalanine to carbon dioxide in rats fed a diet high in histidine, thus indicating that a hormonal response might have been evoked

under the stressful conditions produced by an elevated level of histidine. However the animals would be expected to be in the most stressed condition when the effects of the toxicity were the greatest. On the basis of growth and food intake this was shown to occur during the first three days of ingestion of a diet high in histidine. The concentrations of threonine, tyrosine, and phenylalanine did not begin to decrease substantially until after the third day, with the greatest depression occurring at 2 weeks. By this time the rats were fairly well adapted to the diet. Thus it appears that the possible release of glucagon under stress is not a fully satisfactory explanation for the decrease in concentration of many amino acids. Whether the concentrations of these amino acids would continue to decrease over a long period of time is not known.

When labeled lysine was included in the control and high histidine diets, no increased oxidation to carbon dioxide or increased incorporation into liver protein was observed. The tissue concentrations of lysine also remained relatively stable throughout the experiment. These results, together with the observations that the concentrations of methionine and other amino acids did not increase in the plasma or brain of rats fed high in histidine, make it unlikely that a general effect on amino acid metabolism is exerted by increased levels of histidine.

The observations by Alam et al. (1966) on the alleviation of tyrosine toxicity by threonine, and by Snetsinger and Scott (1961) on the alleviation of histidine toxicity in the chick by glycine supplementation makes the possibility of alleviation of histidine toxicity in the rat by amino acid supplementation worthy of investigation, especially since glycine and tyrosine, plus other amino acids, have been shown by this investigation to decrease substantially in concentration in the tissues of rats fed diets high in histidine. However, as noted before, growth and food intake depressions occur before the concentrations of these amino acids decrease, thus indicating that the observed depressions in amino acid concentration are secondary effects of histidine toxicity. The primary mechanism of histidine toxicity remains undetected.

Although the effects of elevated levels of histidine on incorporation of histidine into liver protein have not been reported, the results obtained in this study are in general agreement with work done in vitro (Hanking and Roberts, 1964a, 1964b) and in vivo (Godin and Dolan, 1966) with elevated levels of other amino acids. These results indicate that protein synthesis and degradation were stimulated in the presence of increased levels of an amino acid. By inspection of the data for total liver protein (Table 12), it is shown that if liver protein concentration remained constant and amino acid incorporation increased,

not only was protein synthesis increased by protein degradation must also have been increased for liver protein concentrations to have remained stable. Therefore, an increased level of dietary histidine was shown to enhance both protein synthesis and degradation in the liver.

Jefferson and Korner (1969) found that maximal incorporation of a labeled amino acid into liver protein, and the ability of ribosome preparations to incorporate amino acids into liver protein were dependent on 11 amino acids. These amino acids are arginine, asparagine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, and valine. It is interesting to note that many of these amino acids were found to be decreased substantially in the tissues of rats fed diets containing an elevated amount of histidine. Although this work has not been demonstrated in the intact animal, it may provide a possible mechanism which would explain the increased liver size, increased incorporation of amino acids into liver protein, and decreased concentrations in the tissues of many of the above-mentioned amino acids when a rat ingests an excessive amount of dietary histidine.

Summary

When rats were fed a low protein diet containing an excessive amount of histidine, severe depression in growth and food intake were observed. When the protein content of

the diet was increased the depressions were less severe, indicating that the rat can better tolerate an excess of histidine when the protein content of the diet is increased. When the protein and histidine content of the diet were increased proportionally, there was no difference in severity of growth and food intake depression. The inclusion of 100,000 IU of vitamin A to a high histidine diet caused greater depressions in growth and food intake.than when vitamin A was present in normal concentration.

Relative concentrations of histidine were elevated in the plasma, brain, and liver of rats fed an excessive level of histidine for 14 days. The concentrations of glycine and most of the indispensible amino acids generally were decreased after the third day and remained depressed throughout the experiment. The total plasma and brain amino acid pattern depended on the level of protein and histidine in the diet which, along with growth and food intake, indicated that the amino acid pattern is a measure of the rat's ability to tolerate an excessive mount of histidine in the diet.

Rats fed an excessive amount of histidine oxidized more labeled phenylalanine, threonine, and histidine to carbon dioxide and incorporated more of these amino acids into liver protein than rats fed a control diet.

These results indicate that an excessive amount of dietary histidine involves not only the metabolism of histidine, but the metabolism of other amino acids as well.

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