July 30, 1970

To the Graduate Council:

I am submitting herewith a thesis written by Rose Mae Tindall entitled "The Effect of High Dietary Phenylalanine on the Concentration of Brain and Liver Serotonin and Nicotinamide Adenine Dinucleotide in Rats." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

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THE EFFECT OF HIGH DIETARY PHENYLALANINE ON THE CONCENTRATION OF BRAIN AND LIVER SEROTONIN AND NICOTINAMIDE ADENINE DINUCLEOTIDE IN RATS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Rose Mae Tindall

August 1970

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ABSTRACT

Fifty-four weanling Long-Evans-Sprague-Dawley rats were fed a niacin-free, 12 percent casein basal diet for one week. At the end of the pre-experimental adjustment period each rat was assigned to one of seven groups so that the average weight per group did not differ by more than one or two grams. Three of the seven groups ("A," "B" and "C") were fed the experimental diets (basal supplemented with L-phenylalanine to 3, 5 and 7 percent levels). One group of rats ("BC") was fed the basal diet (0.62 percent L-phenylalanine) ad libitum. Feed consumption of all rats fed the experimental diets was measured daily and rations of basal diet equal to the amounts eaten by experimental rats were given to the pair-fed control rats ("AA," "BB" and "CC"). At the end of the two-week experimental period the rats were sacrificed by decapitation. The right hemisphere of the brain was used for the analysis of NAD and the left hemisphere for serotonin assay. Liver (two separate lobes) samples were taken for serotonin and NAD analysis.

The effect of high levels of L-phenylalanine on feed consumption, weight gain, the concentration of serotonin in brain and liver, and the concentration of NAD in brain and liver were studied. The major purposes of this research were twofold: (1) To study the effect of excess amounts of

L-phenylalanine on the kynurenine pathway of tryptophan metabolism, and (2) To study the relationship between the two major metabolites of tryptophan metabolism, serotonin and NAD, in the same animal as affected by high levels of L-phenylalanine.

As the percentage of L-phenylalanine in the diet increased to 5 and 7 percent, the feed consumption and weight gain decreased respectively in relation to rats fed the basal control diet. The decrease in feed consumption was significant ($P<0.005$) for rats fed the 7 percent L-phenylalanine diet and approached significance for rats fed the diet containing 5 percent L-phenylalanine. Decreased feed consumption and growth depression might be attributed to the excess amounts of L-phenylalanine.

The concentration of both brain and liver serotonin decreased in relation to the values obtained for "BC" and pair-fed basal ("AA," "BB" and "CC") rats as the level of L-phenylalanine in the diet increased from 3 to 7 percent. The greatest differences were found at the 5 and 7 percent levels of L-phenylalanine.

The only significant difference ($P<0.005$) in the concentration of brain NAD of the experimental rats as compared to rats fed the basal control diet was the decrease demonstrated by rats ("A") fed the 3 percent level of L-phenylalanine. It might appear that L-phenylalanine fed at the

3 percent level either caused more tryptophan to enter the serotonin pathway than the kynurenine pathway in the brain or that the low concentration of NAD found in "A" rats might be attributed to failure of NAD already formed in the liver to pass from the blood stream to the brain.

The decreased concentration of liver NAD in rats fed the diet containing 7 percent L-phenylalanine might be attributed to decreased feed intake as a result of an excess of the amino acid in the diet. It appears that the failure of the concentration of liver NAD of rats in group "B" to decrease as expected due to decreased tryptophan intake might be a result of the 5 percent level of L-phenylalanine causing more tryptophan to enter the NAD pathway of tryptophan metabolism than entered the serotonin pathway.

The mean total brain weight and serotonin content of brain decreased significantly in rats fed diets containing 5 and 7 percent levels of L-phenylalanine. These reductions might not be attributed to decreased feed consumption alone but also to the level of L-phenylalanine in the experimental diets. A tendency for the NAD content to increase from the significantly low content found in "A" rats toward values comparable to that found in rats fed the basal control diet was demonstrated.

It appears that higher levels of L-phenylalanine (5 and 7 percent) decreased feed consumption, weight gain, brain size and serotonin content while NAD was decreased at the 3 percent level. Thus it seems that the higher levels of L-phenylalanine (5 and 7 percent) might have caused more of the tryptophan eaten to enter the kynurenine pathway of tryptophan metabolism with a correspondingly smaller amount entering the serotonin pathway. The opposite relationship was indicated for the 3 percent level of L-phenylalanine.

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CHAPTER I

INTRODUCTION

Biochemical studies of patients with phenylketonuria have led to the discovery of high levels of plasma phenylalanine in these patients as a result of the inability to metabolize this particular amino acid (1, 2). In rats made phenylketonuric, the concentration of brain serotonin was found to be significantly decreased while the plasma level of phenylalanine was significantly elevated (3). This work suggests a relationship between the concentration of phenylalanine present and the metabolism of the amino acid tryptophan.

Tryptophan is required in the diet for growth and for maintenance of nitrogen equilibrium. Since tryptophan is both a precursor of the niacin coenzymes which are important to cellular respiration, and also of serotonin, a constituent of brain and other tissues that produces marked effects on the central nervous system, any disturbance in the pathways of metabolism of this amino acid might result in adverse effects.

The present study was undertaken to determine the effects of an excess amount of the amino acid L-phenylalanine on tryptophan metabolism, in particular, the concentration of serotonin and nicotinamide adenine dinucleotide (NAD) in brain and liver tissue of rats. Through this work a relationship

might be established between serotonin and NAD content in the same animal as affected by the presence of an excess amount of the amino acid L-phenylalanine.

CHAPTER II

REVIEW OF THE LITERATURE

Tryptophan Metabolism

The indispensable amino acid, L-tryptophan, has been investigated with regard to its conversion to various tryptophan metabolites. One major pathway by which tryptophan may be metabolized leads to the formation of serotonin through the steps in Figure 1 (4, 5).

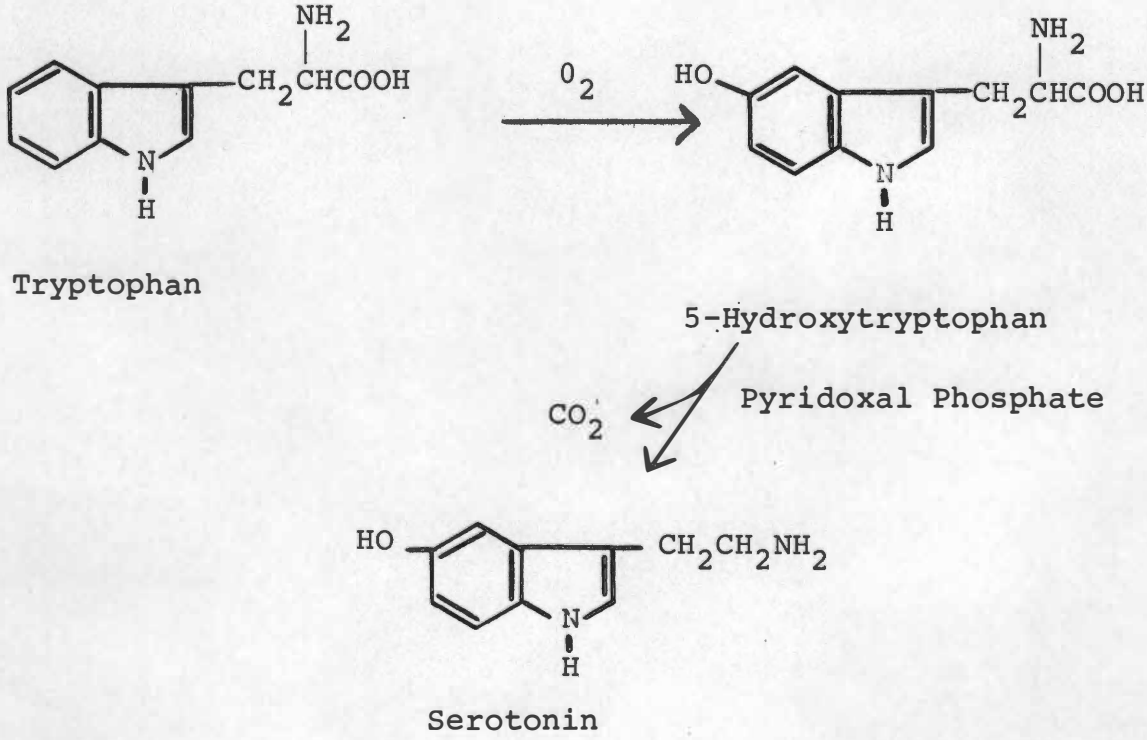
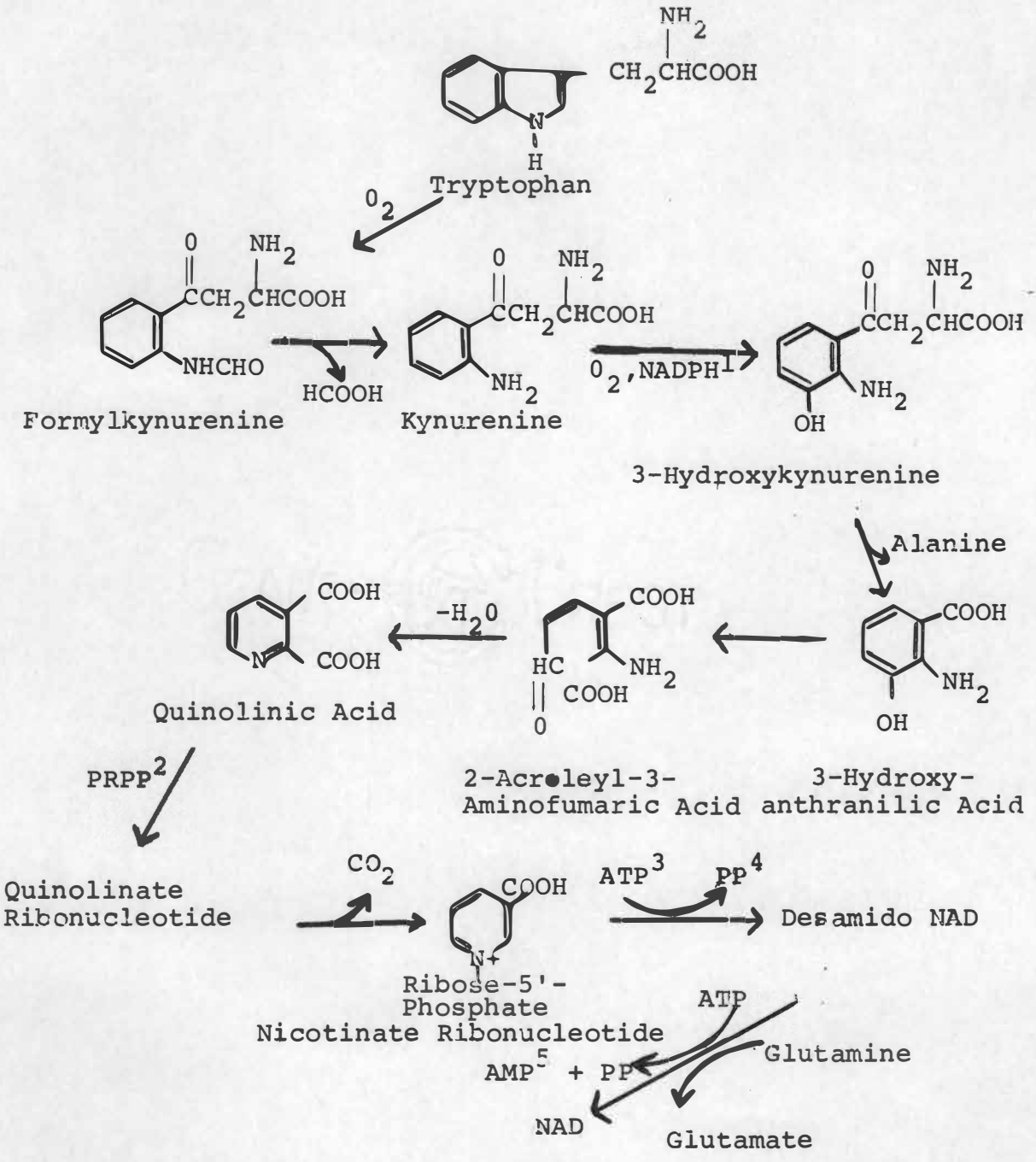


Figure 1. Serotonin pathway of tryptophan metabolism.

Only 1 to 2 percent of the tryptophan utilized normally enters the serotonin pathway of tryptophan metabolism (6). Serotonin occurs in many tissues but most prominently in the intestine, brain, and platelets. According to Erspamer (7) serotonin is released from the enterochromaffin tissue in the intestine into the circulation and is concentrated by the platelets. The relation of serotonin to the nervous system has been demonstrated in many instances. The action of serotonin on smooth muscle was found to be antagonized by the hallucinogenic agent lysergic acid diethylamide (LSD). The action of the drug reserpine causes a loss of serotonin from brain tissue with a very slow return to normal and this fall in cerebral serotonin has been associated with the sedative action of reserpine. Other implications concerning serotonin include the presence of large amounts of serotonin in extracts from carcinoid tumor and the low serum levels of serotonin found in phenylketonuria (8).

The dominant or most prominent route of tryptophan metabolism is that which leads to the formation of nicotinamide adenine dinucleotide (NAD). The degradation of tryptophan in animals by this pathway involves the series of reactions in Figure 2 (4).



[1]Reduced Nicotinamide Adenine Dinucleotide Phosphate
[2]Phosphoribosyl-pyrophosphate
[3]Adenosine Triphosphate
[4]Pyrophosphate
[5]Adenosine Monophosphate

Figure 2. Kynurenine (NAD) pathway of tryptophan metabolism.

NAD, synthesized primarily in the liver, functions as a hydrogen acceptor (transport systems) for a large number of dehydrogenases, especially those catalyzing the removal of hydrogen from primary and secondary alcohol groups. In all cases the reaction of the nicotinamide is the same, involving the addition of one hydrogen atom, the rearrangement of electron pairs, and the formation of a proton. NAD usually works in conjunction with the respiratory chain, concluding with the union of hydrogen atoms with molecular oxygen to form water and release energy (8, 9).

Influence of Excess Amounts of Amino Acids on Tryptophan Metabolism

Serotonin pathway. Certain levels of dietary L-phenylalanine have been shown to lower the concentration of serotonin in rat brain and liver (10-16). Wang et al. (14) observed that feeding weanling rats diets containing either 2, 3, 5 or 7 percent L-phenylalanine for periods of one to three weeks reduced both the concentration of brain and liver serotonin and that the degree of reduction depended on the level of the amino acid rather than on the duration of the experiment. These same co-workers demonstrated that the level of tryptophan in the diet also affected the serotonin concentration of brain and liver. The serotonin concentration of both tissues increased in rats fed diets supplemented with 1 percent L-tryptophan.

Weanling rats of the Long-Evans strain weighing from 35-45 grams were fed diets for 15-17 days by Green and co-workers (15) containing nine times the amount of L-phenylalanine and five times the amount of tryptophan in the basal diets. The basal diet was designed to permit maintenance but not to support growth. Brains from two rats of each group of six were pooled for the estimation of serotonin. The results showed that the amount of serotonin varied directly with the relative concentration of tryptophan and inversely with phenylalanine.

Boggs et al. (12) investigated whether phenylalanine per se, its metabolites, or related amino acids were responsible for the decreased concentration of serotonin. Various levels of L-phenylalanine (6-9 percent), tyrosine (6-9 percent), an amino acid structurally related to phenylalanine, and phenylacetic acid (6-9 percent), the final metabolite formed in the degradation of phenylalanine, were fed to 18-day old male Holtzman albino rats. The results showed that only in rats fed phenylalanine was the serotonin concentration in the brain reduced. Rats fed phenylacetic acid had an increase in brain serotonin and rats fed tyrosine showed no significant changes. Valine, an amino acid structurally unrelated to the aromatic amino acids, was fed at 6-9 percent levels. The data demonstrated that L-valine caused a decrease in brain serotonin and served to illustrate the problem of interdependence of amino acid metabolism in the brain.

Culley and co-workers (13) fed Wistar rats diets supplemented with 7 percent L-phenylalanine, 4.5 percent phenylpyruvic acid and 4 percent phenylacetic acid. Analysis of brain samples for serotonin after two weeks on these diets showed that phenylalanine feeding effectively lowered brain serotonin, phenylpyruvic acid feeding did so less effectively and phenylacetic acid feeding caused no significant change. It was suggested by these workers that the reduction of brain serotonin by phenylalanine but not by its metabolites might be attributed to the fact that transport of the precursor of serotonin, 5-hydroxytryptophan, into the brain was inhibited by phenylalanine.

Another disorder that has been associated with excess amounts of the amino acid L-phenylalanine and its relationship to the serotonin pathway of tryptophan metabolism is that of phenylketonuria, an inherited metabolic disorder which may lead to severe degrees of mental retardation. Considerable brain damage in individuals suffering from phenylketonuria is thought to result from the inability to metabolize phenylalanine. Polidora et al. (16) fed 5 and 7 percent levels of L-phenylalanine to rats which at the end of this experimental period exhibited elevated blood phenylalanine levels and had symptoms typical of phenylketonuria. The L-phenylalanine fed group learned to negotiate a T-Maize but at a slower rate and made more errors than the control group. The severity of

phenylketonuria in rats has been linked to a high plasma level of phenylalanine with decreased serotonin in brain (17, 18). This decrease in brain serotonin may be responsible for the mental defects associated with phenylketonuria (18).

Kynurenine or NAD pathway. Feeding excess amounts of certain amino acids has also resulted in alteration of the kynurenine pathway of tryptophan metabolism. Raghuramulu et al. (19) showed that adult and young rats fed a 1.5 percent leucine supplement to a 9 percent casein diet had a significant increase in the urinary excretion of N'-methylnicotinamide and quinolinic acid. Pearson and Song (20) by feeding rats excess threonine found that the excretion of N'-methylnicotinamide, 3-hydroxyanthranilic acid, kynurenine, and quinolinic acid was significantly reduced. Glycine at levels of 3.08 and 4.62 percent in a 0.1 percent L-tryptophan supplemented diet depressed pyridine nucleotide concentration below that obtained with rats fed a 6 percent niacin-free, casein diet + 0.1 percent L-tryptophan (21). Rosen and Perlzweig (22) have reported an increased excretion of N'-methylnicotinamide in rats fed gelatin.

The literature cited has concerned itself entirely with the effect of excess amounts of various amino acids on either one or the other of the two major pathways of tryptophan metabolism. Few, if any, workers have made an attempt to examine the effect of various amino acids fed in excess

on both the serotonin and NAD pathways in the same animal. The study undertaken here was designed to investigate (1) the effect of high levels of L-phenylalanine on the serotonin pathway of tryptophan metabolism, (2) the effect of high levels of L-phenylalanine on the kynurenine (NAD) pathway of tryptophan metabolism and (3) the relationship between the two major metabolites of tryptophan metabolism, serotonin and NAD, in the same animal as affected by high levels of L-phenylalanine.

CHAPTER III

EXPERIMENTAL PROCEDURE

Diets

The basal diet employed in this study contained by percentage: vitamin-free casein, 12.0; choline chloride, 0.15; corn oil, containing fat-soluble vitamin mixture, 5.0; niacin-free, water-soluble vitamin mixture, 0.25; Hubbell, Mendel and Wakeman salt mixture (23), 5.0; L-tryptophan, 0.8; and sucrose, 76.8. All additions to the basal diet were made at the expense of sucrose. The components of the fat-soluble vitamin mixture are shown in Table I and of the niacin-free, water-soluble vitamin mixture in Table II. The three experimental diets were prepared by calculating the amount of phenylalanine (0.62 percent) already present in the casein of the basal diet and adding L-phenylalanine to bring the levels of phenylalanine to 3, 5, and 7 percent respectively.

General Plan

Weanling male rats[1] were fed a niacin-free, 12 percent casein basal diet for one week. During this pre-experimental period, the animals were housed individually with feed and

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[1] "Long-Evans-Sprague-Dawley"; Nutrition Department, The University of Tennessee, Knoxville, Tennessee.

TABLE I

COMPONENTS OF THE FAT-SOLUBLE VITAMIN MIXTURE

Component	Amount g
Calciferol	0.006
α-DL-Tocopherol	12.750
Halibut Liver Oil	8.500
Mazola Corn Oil	616.000

TABLE II

COMPONENTS OF THE NIACIN-FREE, WATER-SOLUBLE VITAMIN MIXTURE

Component	Amount
	g
Thiamine HCl	0.250
Riboflavin	0.250
Calcium Panthothenate	1.000
Pyridoxine HCl	0.130
Folic Acid	0.010
Menadione	0.030
Biotin	0.005
Vitamin B_{12} (1 g triturate) 0.1 percent with mannitol	0.001
Inositol	5.000
Ascorbic Acid	2.500
Sucrose	115.824

water supplied ad libitum. The rats were weighed at the beginning and at the end of this pre-experimental adjustment period.

At the end of the adjustment period each rat was assigned to one of seven groups (see Table III) so that the average weight per group did not differ by more than one or two grams. Three of the seven groups ("A," "B," and "C") were fed the experimental diets. Each rat fed an experimental diet had a pair-fed control rat ("AA," "BB," and "CC"). The rats were paired between these two groups on the basis of body weight. One group of rats ("BC") was fed the basal diet ad libitum. Distilled water was given ad libitum to all rats. Feed consumption of all rats fed the experimental diets was measured daily and rations of basal diet equal to the amounts eaten by experimental rats were given to the pair-fed control rats. Paired feeding was necessary to account for any differences that might arise in feed consumption due to the high levels of phenylalanine used. Paired feeding was also used to keep the intake of tryptophan comparable. If this varied, so would the concentration of NAD and serotonin in the tissues. Weight gains were recorded weekly and at the end of the two-week experimental period the rats were sacrificed by decapitation. Immediately the whole brain was removed and cut into two sections. The right hemisphere was used for the analysis of NAD and the left hemisphere for

TABLE III

DISTRIBUTION OF ANIMALS INTO GROUPS

Groups	Diet	No. of Animals
BC	Basal (fed ad libitum) (0.62% L-phenylalanine)	10
A	3% L-phenylalanine	7
AA	Basal (pair-fed to A)	7
B	5% L-phenylalanine	7
BB	Basal (pair-fed to B)	7
C	7% L-phenylalanine	8
CC	Basal (pair-fed to C)	8

serotonin assay. Liver (two separate lobes) samples were also taken for analysis. The tissues to be analyzed for serotonin were frozen immediately using a mixture of dry ice and acetone, weighed using an O'Haus balance, and placed in a freezer until the actual analyses were carried out the same day. Those tissues taken for serotonin determinations were treated as described below.

Serotonin Extraction and Analysis

The extraction of serotonin was carried out following the method of Udenfriend et al. (24). Previously frozen and weighed brain and liver samples were homogenized in 3.0 ml of 0.1 N HCl. After transferring a 3.0 ml aliquot to a chilled beaker, the pH was adjusted to 10 by the addition of anhydrous sodium carbonate. Five milliliters of borate buffer (pH 10) were added and the volume adjusted to 15 ml. The solutions were transferred quantitatively to screw-cap polyethylene centrifuge tubes and 5.0 g of NaCl and 15.0 ml of purified n-butanol were added. Several centrifuge tubes were then taped together and placed horizontally in an Eberbach[2] shaker for 10 minutes after which time they were centrifuged[3] (10 minutes at 550 x g). The fluid was decanted from the solid

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[2]Eberbach Corporation, Ann Arbor, Michigan.

[3]International Centrifuge Model V, No. 3602D, International Equipment Corporation, Boston, Massachusetts.

material into glass stoppered centrifuge tubes and centrifuged (five minutes at 550 x g). The aqueous layer was drawn off and the butanol phase washed by shaking (two minutes by hand) with an equal volume of borate buffer (pH 10). The tubes were then centrifuged (five minutes at 550 x g) and two more washings (one minute each by hand) with equal volumes of borate buffer, followed by centrifugation (five minutes at 550 x g), were carried out. After the removal by aspiration of the last washing phase, the volume of the butanol phase was measured and this phase was transferred to another tube containing 20.0 ml of purified heptane and 1.5 ml of 0.1 N HCl, shaken (one minute by hand) and centrifuged (five minutes at 550 x g). The supernatant fluid was removed and the aqueous phase (HCl solution) containing the extracted serotonin was transferred to quartz micro cuvettes and the optical density determined using the Beckman model DU spectrophotometer at 275 mμ. The instrument was zeroed using a reagent blank. The concentration of serotonin in μmoles per tube was calculated from the equation $C = OD_{275}/E$ where

C = μ moles of serotonin per tube

OD_{275} = optical density value at 275 mμ

E = molar extinction coefficient of serotonin which is 5.7×10^3 liters $mole^{-1}$ cm^{-1} as determined in this laboratory

The concentration of serotonin in µg /g of tissue was calculated using the following relationship:

$$X = \frac{C \times K}{W}$$

X = µg /g serotonin in volume of butanol analyzed after three washings

C = µmoles serotonin per tube

K = constant value of 1.7×10^5 µg /ml derived from

$$\frac{\text{m.wt. serotonin g /l}}{\text{1000 ml /l}} \times 1 \times 10^6 \text{ µg /g}$$

The concentration of serotonin (µg /g) was then corrected using the following equation:

$$\frac{X}{V} = \frac{X'}{V'}$$

X = µg /g serotonin in volume of butanol analyzed after three washings

V = volume of butanol phase after three washings

X' = µg /g serotonin in 15.0 ml butanol extract

V' = 15.0 ml (original volume of butanol phase before three washings

NAD Extraction

The brain and liver samples for NAD determinations were blotted dry and weighed on an O'Haus balance. The samples were placed in homogenizing tubes containing 2.0 ml of water and 6.0 ml of a 0.05 M phosphate buffer, pH 5.4, which had been previously heated one and a half minutes in a boiling water bath. After incubation for one minute in a boiling water bath, they were homogenized with a Potter-Elvehjem teflon pestle and temporarily stored in an ice bath. The samples were centrifuged[4] at 8,500 x g for 15 minutes at 0°C, the supernatant fluid was decanted into test tubes and then frozen for analysis at a later time.

NAD Analysis

The concentration of NAD was determined according to the method of Jedeikin and Weinhouse (25) using the Beckman model DU spectrophotometer. For the analysis of NAD an 0.5 ml aliquot of the supernatant fluid, 1.5 ml of 0.1 M $Na_4P_2O_7$ buffer (pH 9), 0.1 ml of 10 percent ethanol, and 0.8 ml of distilled water were put into a Beckman Corex cuvette. After two inversions of the cuvette an optical density reading was taken at 340 mμ using a reagent blank to zero the instrument. Three minutes after the addition of 0.1 ml of yeast alcohol dehydrogenase (ADH)[5] a second optical density reading was

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[4] "Beta-Fuge," Model A, Lourdes Instrument Corporation, Brooklyn, New York.

[5] Worthington Biochemical Corporation, Freehold, New Jersey.

taken. Another 0.1 ml of ADH was added immediately and a third optical density reading was taken to obtain a correction for the dilution made by the first addition of 0.1 ml of ADH. From the increase in optical density readings the concentration of NAD was calculated using a change of one optical density unit as equivalent to 318 µg of NAD (25).

$$\text{Concentration of NAD } (\mu g/g) = \frac{OD \times 318\ \mu g/OD\ \text{unit} \times V}{V' \times W}$$

OD = (2nd OD reading - 1st OD reading) + (2nd OD reading - 3rd OD reading) ± correction factor for cell

V = volume of supernatant fluid

V' = volume of supernatant fluid analyzed

W = weight of tissue sample

Statistics

The mean ± standard error and Student's *t* test for nonpaired experiments as described by Hoel (26) were used to determine statistical significance of the data.

CHAPTER IV

RESULTS AND DISCUSSION

Feed Consumption and Weight Gain

In order to carry out research that involved the feeding of diets containing an excess amount of a particular amino acid, the experimental design of paired feeding was utilized. It has been demonstrated by others (27, 28) that rats fed diets containing excess amounts of amino acids show a decrease in feed consumption. The data in Tables IV and V are in agreement with these findings in that as the percentage of L-phenylalanine in the diet increased to 5 and 7 percent the feed consumption decreased respectively in relation to the amount eaten by rats fed the basal control diet. The decrease was significant ($P < 0.005$) for rats fed the 7 percent L-phenylalanine diet and approached significance for rats fed the diet containing 5 percent L-phenylalanine. Thus it can be seen that it would be impossible to relate any differences in results obtained to the levels of L-phenylalanine in the diet alone since there is the factor of decreased feed consumption. In order to compensate for this decreased feed consumption due to the excess amounts of amino acid fed, rats were paired as described in the experimental plan. This allowed the determination of whether the experimental results

TABLE IV

MEAN FEED CONSUMPTION AND MEAN WEIGHT GAIN OF RATS

Group	Diet	Feed Consumed	Weight Gain	Pair-Fed Basal Group	Feed Consumed	Weight Gain
		g/14 days	g/14 days		g/14 days	g/14 days
("BC")	Basal Control (0.62% L-phenyl-alanine)	152 ± 12[a]	37 ± 4			
("A")	3% L-Phenylalanine	154 ± 14	36 ± 4	("AA")	150 ± 10	33 ± 4
("B")	5% L-Phenylalanine	125 ± 6	20 ± 3	("BB")	123 ± 5	30 ± 8
("C")	7% L-Phenylalanine	94 ± 5	4 ± 3	("CC")	94 ± 4	17 ± 3

[a]Mean ± S.E.

TABLE V

SIGNIFICANCE OF DIFFERENCE IN MEAN FEED CONSUMPTION AND MEAN WEIGHT GAIN OF RATS

Groups[a] Compared	Mean Feed Consumption	Mean Weight Gain
A, AA	ns[b]	ns
B, BB	ns	$P < 0.05$
C, CC	ns	$P < 0.005$
A, B	$P < 0.10$	$P < 0.02$
A, C	$P < 0.005$	$P < 0.005$
A, BC	ns	ns
B, C	$P < 0.005$	$P < 0.005$
B, BC	ns	$P < 0.10$
C, BC	$P < 0.005$	$P < 0.005$
AA, BB	$P < 0.10$	ns
AA, CC	$P < 0.005$	$P < 0.005$
AA, BC	ns	ns
BB, CC	$P < 0.005$	$P < 0.01$
BB, BC	$P < 0.10$	ns
CC, BC	$P < 0.005$	$P < 0.01$

[a]Code for groups: ("BC"), basal control; ("A"), 3 percent L-phenylalanine; ("B"), 5 percent L-phenylalanine; ("C"), 7 percent L-phenylalanine; ("AA"), pair-fed basal to "A"; ("BB"), pair-fed basal to "B"; ("CC"), pair-fed basal to "C."

[b]ns = not significant

were attributable to the relationship being investigated or to a smaller feed intake.

Those rats fed, ad libitum, diets containing 3, 5 and 7 percent levels of L-phenylalanine showed no significant difference in feed consumption from the correspondingly paired rats fed the basal control diet. Paired feeding as previously discussed serves as a control factor to compensate for decreased feed consumption due to the high levels of the amino acid. No explanation is available on how an excess of an amino acid depresses feed intake or whether or not it is a secondary effect accompanying a basic underlying mechanism. According to Kumta and Harper (29) reduction in feed intake as a result of a high level of amino acid in the diet might reflect a protective mechanism which discourages the animal from ingesting more of the excess amino acid which would increase the deleterious effects. Another possible explanation for decreased feed intake might be the abnormal amino acid composition of the blood plasma as a result of the single excess amino acid.

Daniel and Waisman (30) demonstrated that plasma levels of the amino acid being fed can be significantly elevated and the extent of the elevation correlated directly with the growth depression and decreased feed consumption. These findings are in agreement with the results recorded in Tables IV and V. As the level of L-phenylalanine in the diet increased

from 5-7 percent, the feed consumption decreased and growth was depressed with the greatest growth depression demonstrated at the 7 percent level of L-phenylalanine. The significant difference of the mean weight gain of "B" rats (5 percent L-phenylalanine) to their pair-fed "BB" rats serves to illustrate this point further (Figure 3). Even though this particular pair-fed group ("BB") was getting significantly less feed than those paired to rats ("A") fed the diet containing 3 percent L-phenylalanine or the rats fed the basal control diet, the mean weight gain of "BB" rats was not significantly different from the mean weight gain of rats "AA" or "BC." However, the mean weight gain of "CC" rats pair-fed to "C" rats (7 percent L-phenylalanine) was significantly decreased with respect to the rats fed the basal control diet and the other pair-fed rats ("AA" and "BB") but yet the mean weight gain was significantly higher than the corresponding experimental group ("C"). Thus the growth depression demonstrated in rats fed the diet containing 5 percent L-phenylalanine was not due to feed restriction alone. Feed restriction did affect the weight gain of rats pair-fed with group "C" rats but there was still a significant difference in weight gain between "CC" and "C" indicating that this level of L-phenylalanine was also a factor in causing depressed growth. It has been suggested that the effect of the excess amino acid itself on growth depression may be attributed to several

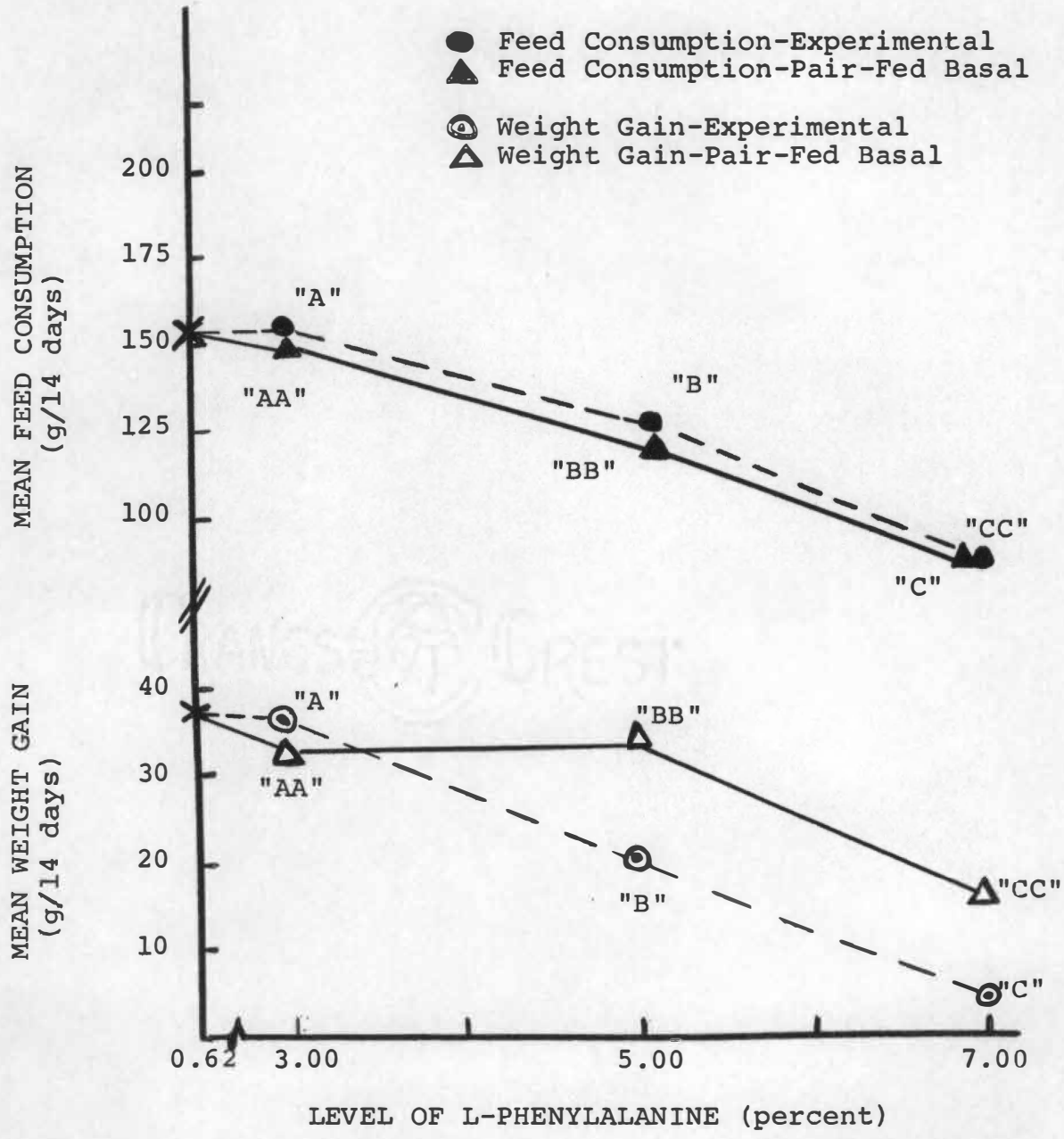

Figure 3. Mean feed consumption and mean weight gain of rats.

circumstances (27, 31). A disruption of the normal balance of the amino acid pools may prevent normal protein synthesis, toxic metabolites may be formed or antagonism of the cellular transport of other amino acids may be influencing factors. The latter case will be further discussed in relation to tryptophan metabolism and its metabolites, serotonin and NAD.

Brain and Liver Serotonin Concentration

Measurement of the concentration of brain serotonin demonstrated a direct relationship between the level of L-phenylalanine fed and the amount of serotonin per gram of tissue analyzed. As the level of L-phenylalanine in the diets increased from 3 percent to 5 and 7 percent the concentration of brain serotonin decreased with the rats fed the 7 percent level of L-phenylalanine showing the greatest difference from those fed ad libitum the basal control diet (Table VI). The lack of significance noted between the concentration of brain serotonin of pair-fed rats ("BB" and "CC") as compared to rats fed the basal control diet (Table VII) further substantiates that the level of L-phenylalanine fed and not decreased feed consumption (Table IV, page 22) is directly responsible for decreased brain serotonin in those rats ("B" and "C") fed high levels of L-phenylalanine. No significant decrease was noted at the 3 percent level of L-phenylalanine but the pair-fed "AA" group was significantly higher than the "BC" group.

TABLE VI

MEAN CONCENTRATION OF BRAIN SEROTONIN

Group	Diet	Serotonin Concentration	Pair-Fed Basal Group	Serotonin Concentration
		µg/g		µg/g
("BC")	Basal Control (0.62% L-phenyl-alanine)	6.4 ± 0.6[a]		
("A")	3% L-Phenylalanine	6.0 ± 0.6	("AA")	8.0 ± 0.6
("B")	5% L-Phenylalanine	3.6 ± 0.3	("BB")	7.3 ± 1.2
("C")	7% L-Phenylalanine	2.9 ± 0.4	("CC")	5.9 ± 0.8

[a]Mean ± S.E.

TABLE VII

SIGNIFICANCE OF DIFFERENCE IN MEAN CONCENTRATION OF BRAIN SEROTONIN

Groups Compared[a]	Significance of "t"
A, AA	$P < 0.005$
B, BB	$P < 0.005$
C, CC	$P < 0.005$
A, B	$P < 0.005$
A, C	$P < 0.005$
A, BC	ns[b]
B, C	ns
B, BC	$P < 0.005$
C, BC	$P < 0.005$
AA, BB	ns
AA, CC	$P < 0.10$
AA, BC	$P < 0.10$
BB, CC	ns
BB, BC	ns
CC, BC	ns

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed basal to "A"; ("BB"), pair-fed basal to "B"; ("CC"), pair-fed basal to "C."

[b]ns = not significant

All values of brain serotonin reported in Table VI are high in relation to those reported in the literature. Garrattini and Valzelli (4) reported values for serotonin using the spectrophotometer as being several times higher than those obtained by fluorometric determinations. Bogdanski et al. (32) using a spectrophotofluorometer reported the range for the concentration of rat brain serotonin as 0.40 - 1.00 µg/g. Although spectrophotometric determinations of serotonin gave values higher than those reported by Bogdanski et al. (32), the results of this research still show the relative effects of an excess amount of the amino acid L-phenylalanine on brain serotonin concentration. Sample recoveries of a standard solution of serotonin creatinine sulfate in the range of 85 to 90 percent were found using the extraction procedure of Udenfriend et al. (24). These recoveries were done with brain and liver samples and were found to be consistent.

The values reported in Table VIII for the concentration of liver serotonin exhibited this same trend of being several times higher than the average value of 0.30 µg/g reported by Udenfriend et al. (24). It is believed that this discrepancy might be related not only to the spectrophotometric determination but also to the serotonin content of the blood of the liver samples analyzed since the technique of perfusion with saline was not employed. Several workers (33-35) have demonstrated the serotonin content of whole blood from rats to be

TABLE VIII

MEAN CONCENTRATION OF LIVER SEROTONIN

Group	Diet	Serotonin Concentration	Pair-Fed Basal Group	Serotonin Concentration
		µg/g		µg/g
("BC")	Basal Control (0.62% L-phenyl-alanine)	7.8 ± 0.4[a]		
("A")	3% L-Phenylalanine	6.9 ± 0.6	("AA")	8.1 ± 0.7
("B")	5% L-Phenylalanine	4.9 ± 0.6	("BB")	8.6 ± 0.7
("C")	7% L-Phenylalanine	3.7 ± 0.3	("CC")	7.5 ± 0.7

[a]Mean ± S.E.

0.87, 0.73, and 1.04 µg/ml respectively. Bogdanski et al. (32) reported little or no effect of blood content on brain serotonin since such a small quantity of blood is found in the brain in comparison to liver tissue.

The mean concentration of liver serotonin decreased as the level of L-phenylalanine increased from 3 to 7 percent with the greatest differences demonstrated at the 5 and 7 percent levels of L-phenylalanine in comparison to "BC" rats fed ad libitum the basal control diet (Table IX). Those rats ("AA," "BB" and "CC") pair fed to rats ("A," "B" and "C") showed no significant decrease in liver serotonin concentration from the "BC" rats fed the basal control diet. This again demonstrates that the level of L-phenylalanine fed and not decreased feed consumption (Table IV, page 22) was responsible for the decreased concentration of liver serotonin found. Figure 4 shows that with an increase (3-7 percent) of L-phenylalanine in the diet the concentration of both brain and liver serotonin decreased in relation to the value obtained for "BC" and pair-fed basal ("AA," "BB" and "CC") rats with the greatest differences found at the 5 and 7 percent levels of L-phenylalanine. It should also be noted that at higher levels (5 and 7 percent) of L-phenylalanine the effect on serotonin concentration of brain and liver levels off with the diet containing 7 percent L-phenylalanine producing no significantly greater decrease in serotonin concentration than did the diet containing 5 percent L-phenylalanine.

TABLE IX

SIGNIFICANCE OF DIFFERENCE IN MEAN CONCENTRATION OF LIVER SEROTONIN

Groups Compared[a]	Significance of "t"
A, AA	ns[b]
B, BB	$P < 0.005$
C, CC	$P < 0.005$
A, BC	ns
A, B	$P < 0.05$
A, C	$P < 0.005$
B, C	ns
B, BC	$P < 0.005$
C, BC	$P < 0.005$
AA, BB	ns
AA, CC	ns
AA, BC	ns
BB, CC	ns
BB, BC	ns
CC, BC	ns

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed basal to "A"; ("BB"), pair-fed basal to "B"; ("CC"), pair-fed basal to "C".

[b]ns = not significant

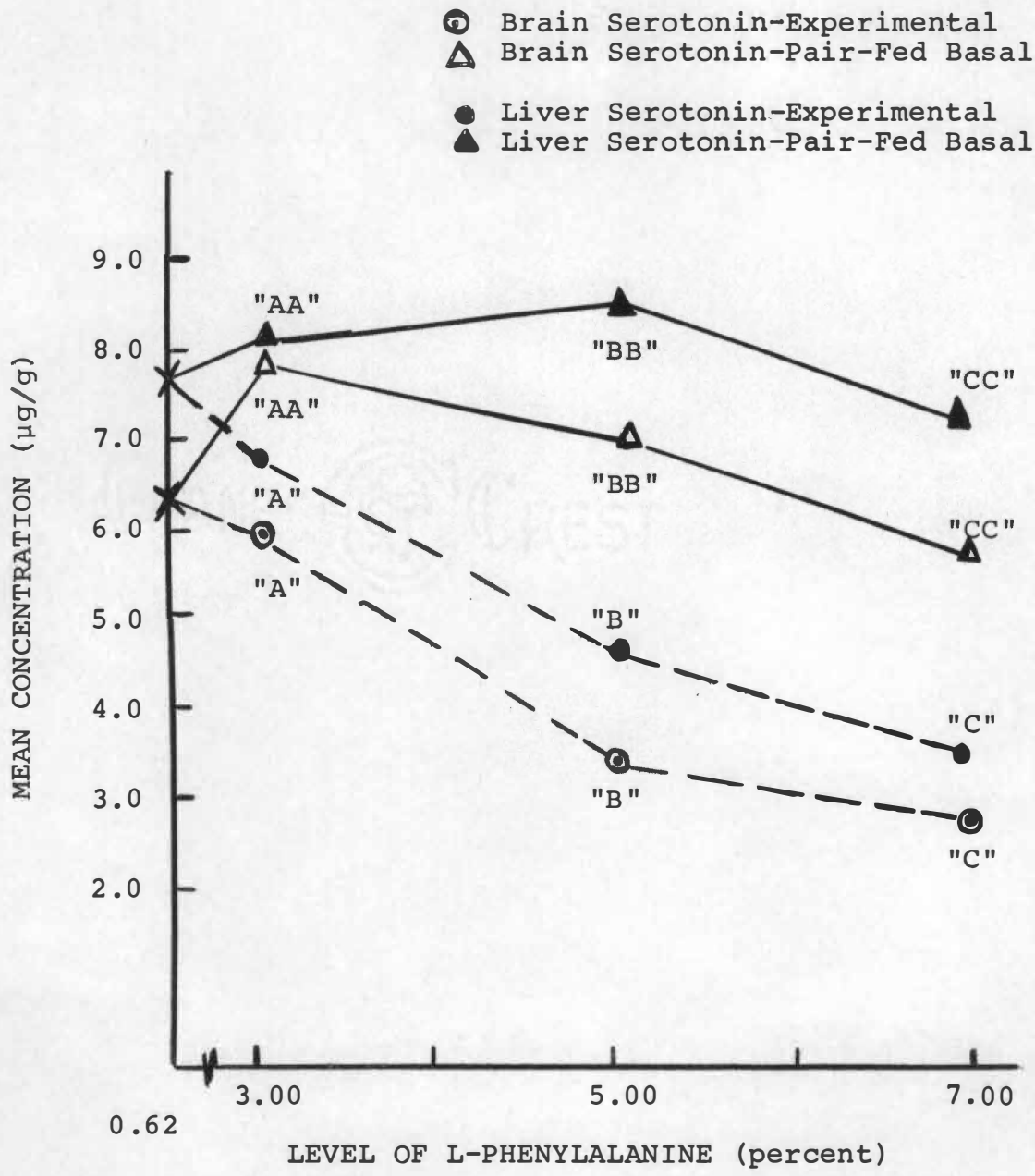

Figure 4. Mean concentration of brain and liver serotonin.

Brain and Liver NAD Concentration

The second major concern of this research involved the determination of the concentrations of both brain and liver NAD in the same animals on which the serotonin determinations were performed. Through this investigation a relationship might be demonstrated between the two major pathways of tryptophan metabolism (kynurenine and serotonin pathways) as affected by the presence of a dietary excess of the amino acid L-phenylalanine.

Determination of the mean concentrations of brain NAD found in rats ("A," "B," and "C") fed 3, 5 and 7 percent levels of L-phenylalanine, the pair-fed basal rats ("AA," "BB," and "CC"), and rats fed ad libitum the basal control diet gave the data reported in Table X. As the level of L-phenylalanine increased from 3-7 percent of the diet, the concentration of brain NAD increased from the low concentration of 48.7 µg/g at the 3 percent level of L-phenylalanine to values comparable to that for rats ("BC") fed the basal control diet. The only significant difference ($P < 0.005$) in the concentration of brain NAD of the experimental rats as compared to the rats fed the basal control diet was the decrease demonstrated by rats "A" fed the 3 percent level of L-phenylalanine (Table XI). It might appear that L-phenylalanine fed at the 3 percent level caused more tryptophan to enter the serotonin pathway than the kynurenine pathway in the brain, but no significant

TABLE X

MEAN CONCENTRATION OF BRAIN NAD

Group	Diet	NAD Concentration	Pair-Fed Basal Group	NAD Concentration
		µg/g		µg/g
("BC")	Basal Control (0.62% L-phenyl-alanine)	104.9 ± 13.4[a]		
("A")	3% L-Phenylalanine	48.7 ± 5.5	("AA")	77.9 ± 11.1
("B")	5% L-Phenylalanine	74.3 ± 10.8	("BB")	96.2 ± 13.4
("C")	7% L-Phenylalanine	81.9 ± 10.4	("CC")	105.0 ± 9.3

[a]Mean ± S.E.

TABLE XI

SIGNIFICANCE OF DIFFERENCE IN MEAN CONCENTRATION OF BRAIN NAD

Groups Compared[a]	Significance of "t"
A, AA	$P < 0.10$
B, BB	$P < 0.10$
C, CC	ns[b]
A, B	$P < 0.02$
A, C	$P < 0.02$
A, BC	$P < 0.005$
B, C	ns
B, BC	ns
C, BC	ns
AA, BB	$P < 0.10$
AA, CC	$P < 0.10$
AA, BC	ns
BB, CC	ns
BB, BC	ns
CC, BC	ns

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed to "A"; ("BB"), pair-fed to "B"; ("CC"), pair-fed to "C."

[b]ns = not significant

increase in brain serotonin was found in rats fed the diet containing 3 percent L-phenylalanine. The low concentration of NAD found in "A" rats might also be attributed to failure of NAD already formed in the liver to pass from the blood stream to the brain.

The concentration of brain NAD of rats "B" and "C" fed diets containing 5 and 7 percent levels of L-phenylalanine was significantly higher ($P < 0.02$) than the concentration of brain NAD of rats "A" fed a diet containing 3 percent L-phenylalanine. This was true in spite of the fact that the "B" and "C" rats were eating significantly less tryptophan (89 mg/day and 67 mg/day) as compared to "A" rats (108 mg/day). The greater NAD concentration might be attributed to the 5 and 7 percent levels of L-phenylalanine fed and to a resultant shift of more tryptophan to the kynurenine pathway of tryptophan metabolism. However, a situation similar to the one just described existed between the corresponding pair-fed rats ("BB" and "CC") and the group "AA." Group "BB" and "CC" rats had a mean tryptophan intake of 89 mg/day and 67 mg/day respectively as compared to 108 mg/day for group "AA" rats, yet "BB" and "CC" rats had a significantly higher concentration of brain NAD than did "AA" rats. Perhaps some protective mechanism of the brain was operative in relation to the tryptophan intake of groups "BB" and "CC" causing the more efficient metabolism of tryptophan to NAD in these groups as

compared to group "AA" rats. This explanation is only a suggestion and admittedly questionable since the significance demonstrated between the brain NAD concentration of groups "AA" and "BB" and groups "AA" and "CC" was only $P < 0.10$.

The tendency for the concentration of brain NAD of rats "A," "B" and "C" to increase as the level of L-phenylalanine increased from 3 percent to 5 and 7 percent of the diet is shown in Figure 5. Rats "BB" and "CC" had significantly higher ($P < 0.10$) concentrations of brain NAD than did "AA" rats even though rats "AA" were eating significantly more tryptophan. Rats "B" and "C" also had significantly higher ($P < 0.02$) concentrations of brain NAD than did group "A" rats. The significant increases in the concentrations of brain NAD of rats "B" and "C" as compared to "A" rats seems to be attributable to some protective mechanism of the brain favoring more efficient use of the tryptophan consumed for NAD production or absorption of NAD across the blood-brain barrier even though "B" and "C" rats consumed significantly less tryptophan than did "A" rats. This same relationship was noted when the concentrations of brain NAD of "BB" and "CC" rats were compared to the concentrations of brain NAD of "AA" rats when no excess amounts of L-phenylalanine were present in their diets.

The mean concentration of liver NAD (Table XII) illustrates an effect opposite to that found in the mean concentration

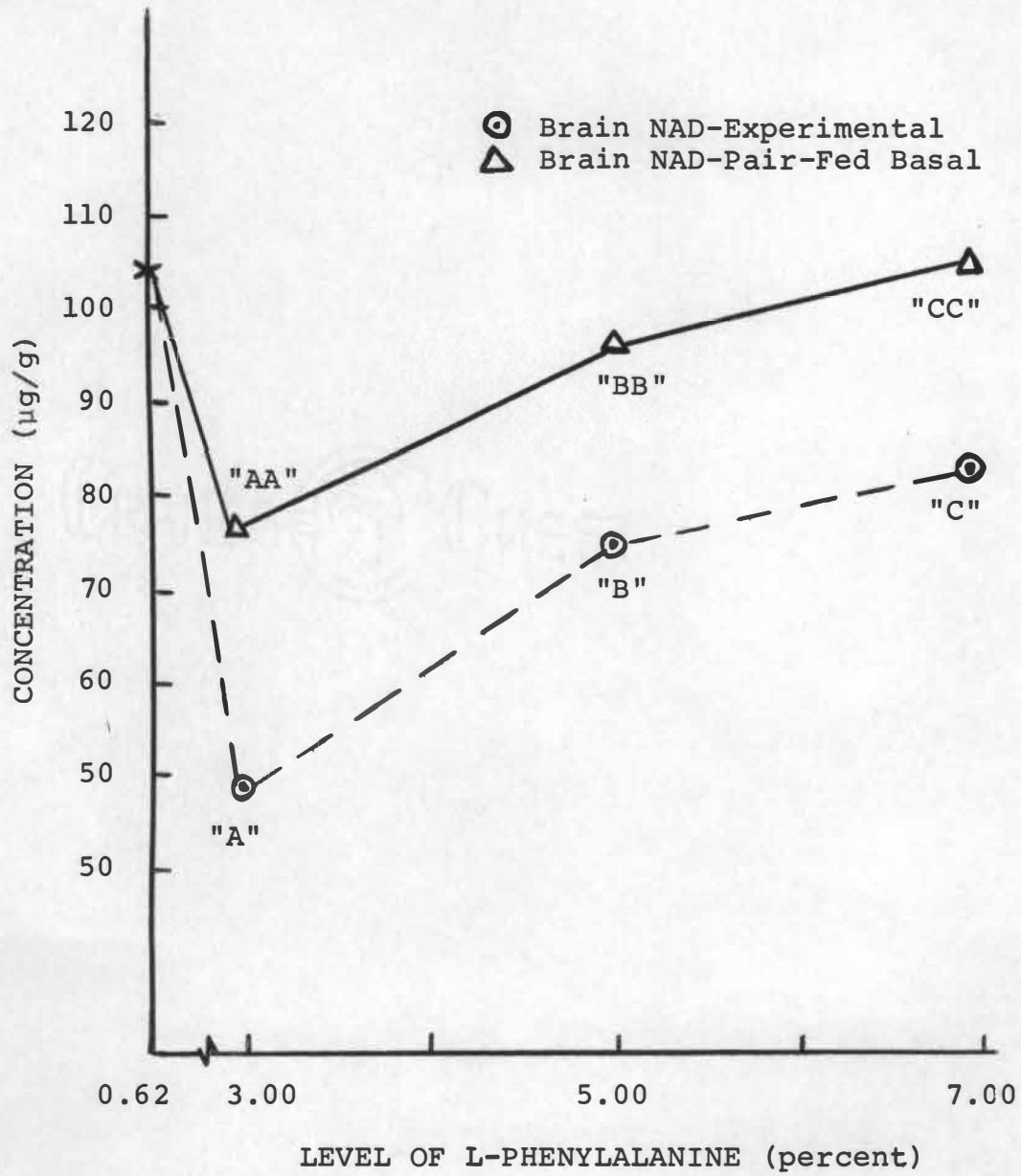

Figure 5. Mean concentration of brain NAD.

TABLE XII

MEAN CONCENTRATION OF LIVER NAD

Group	Diet	NAD Concentration	Pair-Fed Basal Group	NAD Concentration
		µg/g		µg/g
("BC")	Basal Control (0.62% L-phenyl-alanine)	782.6 ± 60.8[a]		
("A")	3% L-Phenylalanine	711.5 ± 76.2	("AA")	659.2 ± 69.6
("B")	5% L-Phenylalanine	656.4 ± 63.2	("BB")	547.9 ± 93.1
("C")	7% L-Phenylalanine	502.6 ± 25.2	("CC")	423.7 ± 47.2

[a]Mean ± S.E.

of brain NAD. When the data obtained for the concentration of liver NAD of the pair-fed basal rats were examined the significant decreases in the concentration of the liver NAD of "BB" and "CC" as compared to "BC" rats might be attributable to reduced tryptophan intake. "BB" and "CC" rats ate 87 mg/day and 67 mg/day of tryptophan as compared to 107 mg/day eaten by "BC" rats. Thus "BB" and "CC" rats would have less tryptophan available to enter the kynurenine pathway of tryptophan metabolism resulting in the decreased concentrations of NAD reported for "BB" and "CC" as compared to the rats fed the basal control diet. The mean tryptophan intake (108 mg/day) of group "AA" was not significantly different from that of the "BC" group (107 mg/day) and no significant difference in the concentration of liver NAD between "AA" and "BC" rats was noted.

Examination of the data obtained for rats "A," "B" and "C" indicated that as the level of L-phenylalanine increased from 3-7 percent of the diet the concentration of liver NAD decreased with the only significant decrease ($P < 0.005$) demonstrated in "C" rats fed the 7 percent level of L-phenylalanine as compared to the basal control rats (Table XIII). One plausible explanation for the effect that different levels of L-phenylalanine have on the concentration of liver NAD would lie in the reasoning that NAD, which is mainly synthesized in the liver, would be influenced by tryptophan intake. That is,

TABLE XIII

SIGNIFICANCE OF DIFFERENCE IN MEAN CONCENTRATION OF LIVER NAD

Groups Compared[a]	Significance of "t"
A, AA	ns[b]
B, BB	ns
C, CC	ns
A, B	ns
A, C	$P < 0.02$
A, BC	ns
B, C	$P < 0.05$
B, BC	ns
C, BC	$P < 0.005$
AA, BB	ns
AA, CC	$P < 0.02$
AA, BC	ns
BB, CC	ns
BB, BC	$P < 0.05$
CC, BC	$P < 0.005$

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed to "A"; ("BB"), pair-fed to "B"; ("CC"), pair-fed to "C."

[b]ns = not significant

rats fed the 5 and 7 percent levels of L-phenylalanine ("B" and "C") decreased their feed consumption below that of the rats fed the basal control diet, thus less tryptophan (89 mg/day and 67 mg/day) as compared to "BC" rats (107 mg/day) would be available to enter the kynurenine pathway resulting in a decreased production of NAD. Thus it appears that the decreased concentration of liver NAD in rats fed the diet containing 7 percent L-phenylalanine might be attributed to decreased feed intake attributed to the excess of the amino acid in the diet.

The mean concentration of the liver NAD (656.4 μg/g) of group "B" was maintained at a higher concentration than was anticipated (Figure 6). Since the tryptophan intake of this group ("B") was significantly decreased in comparison to "A" rats, it seems that the concentration of liver NAD of group "B" rats might decrease significantly as did the liver NAD of their pair-fed rats. This assumption was based upon the fact that rats "BB" (pair-fed to "B" and eating the same amount of tryptophan) demonstrated a significant decrease ($P < 0.05$) in the concentration of liver NAD as compared to rats fed the basal control diet as a result of decreased tryptophan intake. However, this was not observed in "B" rats. It is possible that with less tryptophan entering the serotonin pathway, more entered the kynurenine pathway as a result of the 5 percent level of L-phenylalanine fed to group "B" rats.

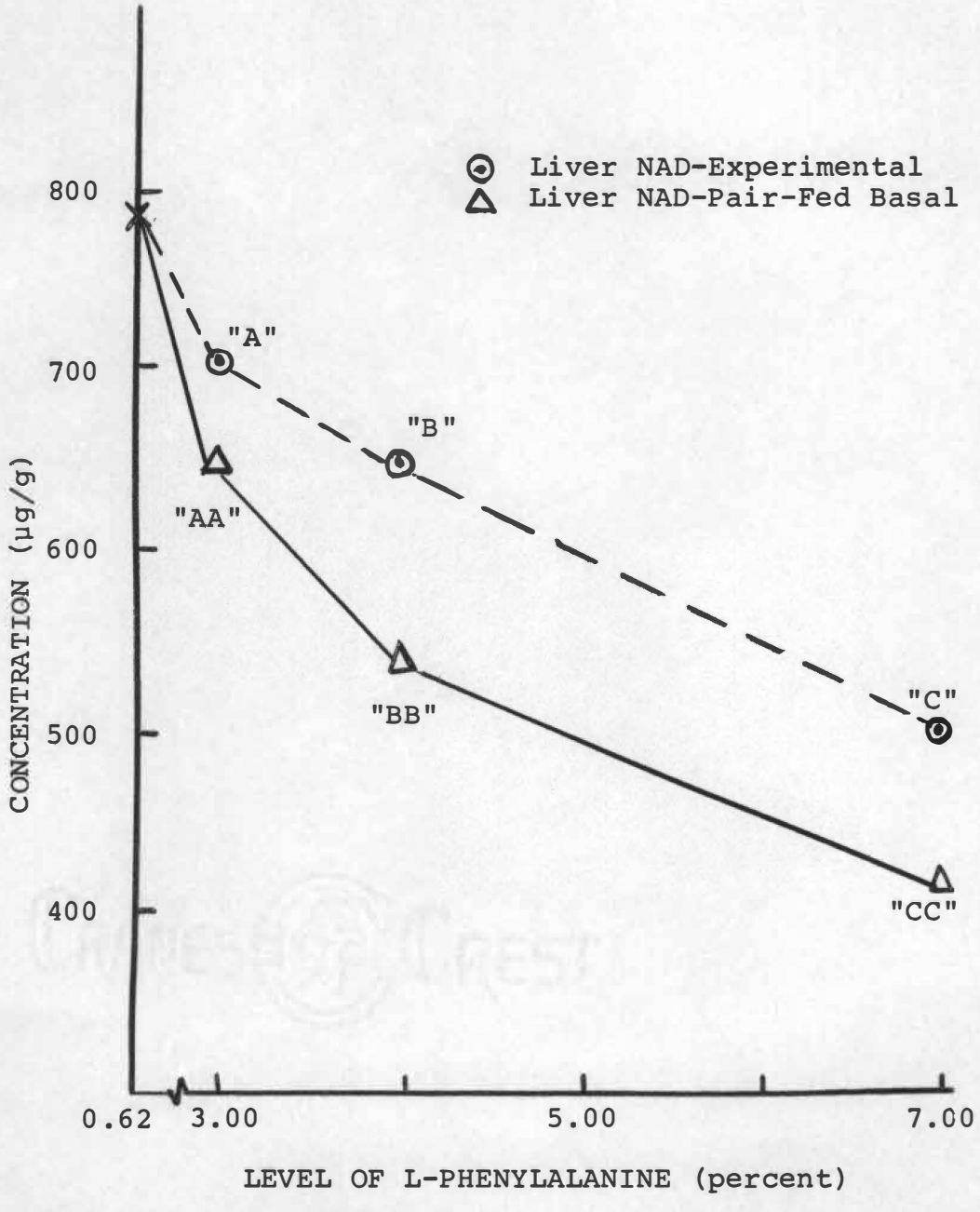

Figure 6. Mean concentration of liver NAD.

Mean Content of Brain Serotonin and NAD

The removal of the whole brain of each animal allowed the calculation of the mean total content of serotonin and NAD in rat brain as reported in Table XIV. It was found that the mean total brain weight of the rats ("B") fed 5 percent L-phenylalanine and the rats ("C") fed 7 percent L-phenylalanine decreased significantly from their respective pair-fed rats as well as groups "A" (3 percent L-phenylalanine), "AA" (rats pair-fed to "A"), and "BC" (basal control) (Table XV). The total brain weights of rats "AA," "BB" and "CC" did not differ significantly from those of the rats fed ad libitum the basal control diet. Therefore, any reduction in brain size was not attributed to decreased feed consumption alone, but rather to the level of L-phenylalanine in the experimental diets fed.

The total content of brain serotonin decreased as the level of L-phenylalanine increased with the greatest differences demonstrated at the 5 and 7 percent levels of L-phenylalanine. Rats ("BB" and "CC") pair-fed to rats ("B" and "C") demonstrated no significant difference in total brain serotonin as compared to rats fed ad libitum the basal control diet. Once again this emphasized that reduction in the concentration of brain serotonin may be attributed to the levels of L-phenylalanine fed and not to reduced feed consumption.

TABLE XIV

MEAN TOTAL BRAIN WEIGHT, MEAN TOTAL CONTENT OF BRAIN SEROTONIN AND NAD

Group[a]	Total Brain Weight	Total Serotonin Content	Total NAD Content	Pair-Fed Basal Group	Total Brain Weight	Total Serotonin Content	Total NAD Content
	g	µg	µg		g	µg	µg
("BC")	1.48±0.03[b]	12.7±1.1	213.2±28.0				
("A")	1.43±0.05	11.6±1.2	100.2±11.0	("AA")	1.46±0.03	15.9±1.4	159.3±24.2
("B")	1.30±0.04	7.1±0.7	152.9±22.9	("BB")	1.49±0.02	14.7±2.6	192.9±27.0
("C")	1.31±0.02	5.6±0.7	161.9±15.2	("CC")	1.45±0.02	11.0±1.4	187.3±17.6

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed basal to "A"; ("BB"), pair-fed basal to "B"; ("CC"), pair-fed basal to "C."

[b]Mean ± S. E.

TABLE XV

SIGNIFICANCE OF DIFFERENCE IN TOTAL BRAIN WEIGHT AND MEAN TOTAL CONTENTS OF BRAIN SEROTONIN AND NAD

Groups[a] Compared	Total Brain Weight	Total Serotonin Content	Total NAD Content
A, AA	ns[b]	P < 0.005	P < 0.10
B, BB	P < 0.005	P < 0.01	P < 0.10
C, CC	P < 0.005	P < 0.005	ns
A, B	P < 0.10	P < 0.01	P < 0.05
A, C	P < 0.02	P < 0.005	P < 0.01
A, BC	ns	ns	P < 0.01
B, C	ns	ns	ns
B, BC	P < 0.005	P < 0.005	ns
C, BC	P < 0.005	P < 0.005	ns
AA, BB	ns	ns	ns
AA, CC	ns	P < 0.02	ns
AA, BC	ns	P < 0.10	ns
BB, CC	ns	ns	ns
BB, BC	ns	ns	ns
CC, BC	ns	ns	ns

[a]Code for groups: ("BC"), basal control; ("A"), 3% L-phenylalanine; ("B"), 5% L-phenylalanine; ("C"), 7% L-phenylalanine; ("AA"), pair-fed basal to "A"; ("BB"), pair-fed basal to "B"; ("CC"), pair-fed basal to "C."

[b]ns = not significant

Calculations of the mean total content of brain NAD showed a decrease in NAD at all levels of L-phenylalanine (3, 5 and 7 percent) with the only significant reduction demonstrated at the 3 percent level. A tendency was noted for the content of brain NAD to increase as the concentration of L-phenylalanine increased. The significant reduction in brain NAD in rats fed 3 percent L-phenylalanine was not understood. If NAD was synthesized in the brain, the reduction might be attributed to a primary role of producing serotonin and a greater proportion of the tryptophan entered this pathway with the result that less tryptophan was available for NAD production. If the problem was one of passage from blood to brain tissue, NAD produced in the liver might not have been transported from the blood stream into the brain. Another possible explanation might be that NAD was catabolized at a rate greater than it was produced or obtained from the blood stream.

Figure 7 illustrates graphically the relationship between both the total content of brain serotonin and NAD and the levels of L-phenylalanine fed. The total brain serotonin content was decreased significantly in rats ("B" and "C") fed diets containing 5 and 7 percent levels of L-phenylalanine while the total brain NAD content was decreased significantly in rats ("A") fed the diet containing 3 percent L-phenylalanine as compared to rats fed the basal control diet. If the kynurenine

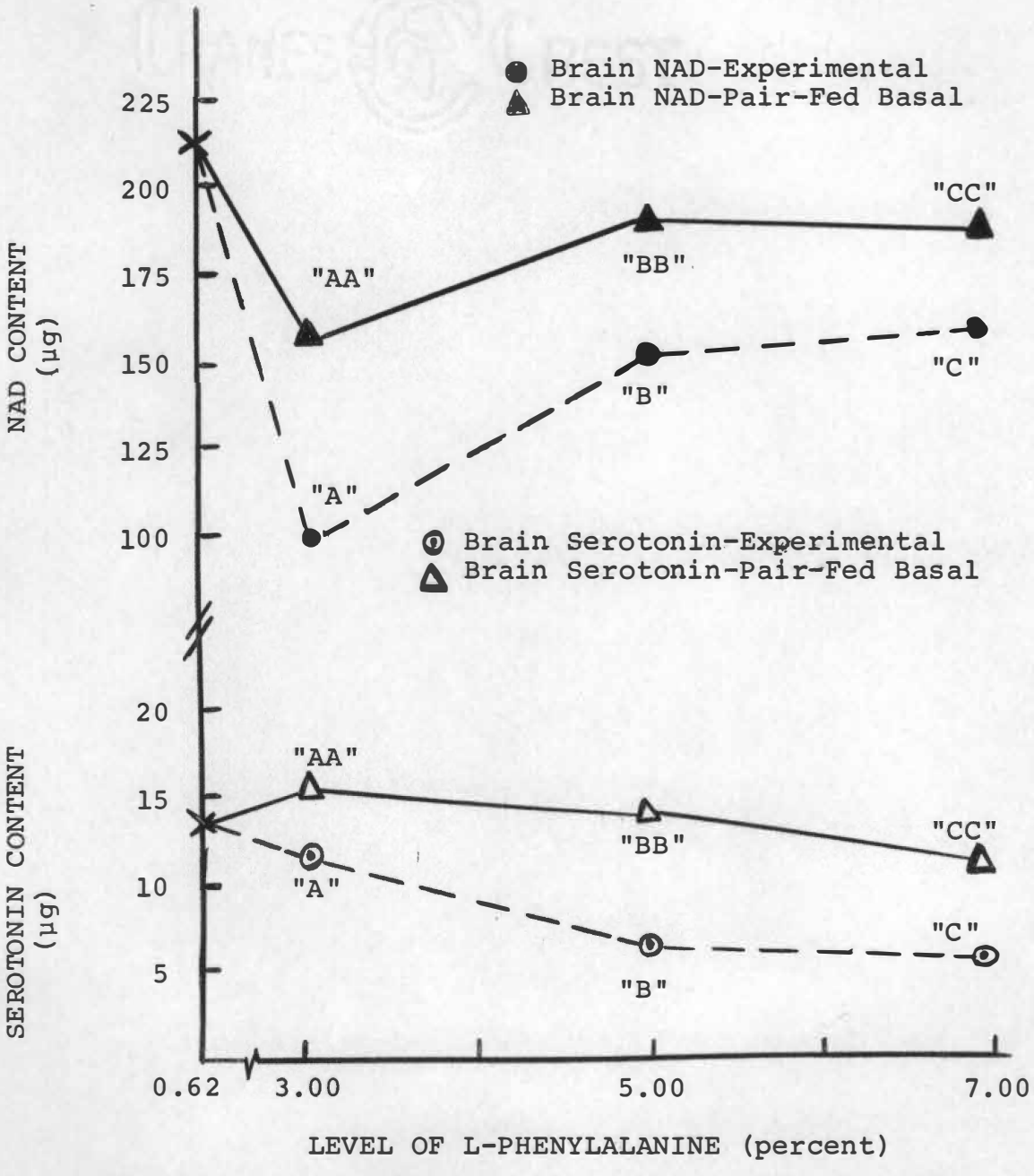

Figure 7. Mean content of brain serotonin and NAD.

and serotonin pathways of tryptophan metabolism are both operative in the rat brain and the NAD content is not a result of NAD produced in the liver and transported to the brain, it appears that the 5 and 7 percent levels of L-phenylalanine caused significant decreases in brain serotonin content. A tendency for the NAD content to increase from the significantly low content found in "A" rats toward values comparable to that found in rats fed the basal control diet was demonstrated. If the mean brain content of NAD was a result of NAD being produced in the liver and transported to the brain, it is possible that the 3 percent level of L-phenylalanine fed may have interfered with the passage of NAD from the blood to the brain with a tendency for the 5 and 7 percent levels to interfere less with this transport. As can be seen from the graph the reduced tryptophan intakes of rats fed 5 and 7 percent levels of L-phenylalanine had no effect on the serotonin content since their pair-fed basal rats "BB" and "CC" showed mean brain serotonin contents not significantly different from the basal control rats. The significant difference found in the content of brain NAD of "A" rats might also be attributed to the effects of excess L-phenylalanine and not to the tryptophan intake since the total content of brain NAD of their pair-fed basal rats ("AA," "BB" and "CC") did not differ significantly from the rats fed the basal control diet.

CHAPTER V

SUMMARY

The effect of the addition of an excess of the amino acid L-phenylalanine on the concentration of serotonin and NAD in the brain and liver of rats fed niacin-free, 12 percent casein diets supplemented with L-phenylalanine to 3, 5, and 7 percent levels was studied. Rats were assigned to one of seven groups so that the average weight per group did not differ by more than one or two grams. Three of the seven groups ("A," "B" and "C") were fed experimental diets. Each rat fed an experimental diet had a pair-fed basal control rat ("AA," "BB," and "CC") paired on the basis of weight. One group ("BC") was fed the basal control diet ad libitum.

Feed consumption of rats fed the experimental diets decreased as the level of L-phenylalanine increased from 3 to 7 percent of the diet with the greatest difference demonstrated at the 7 percent level as compared to rats fed ad libitum the basal control diet. A significant decrease in mean weight gain due to feed restriction was demonstrated only for those rats pair-fed to group "C" (7 percent L-phenylalanine) rats, but the group "CC" rats still gained significantly more weight than did the "C" rats. Feed restriction had no significant effect on the concentration of brain NAD and serotonin, the

concentration of liver serotonin or brain size of rats pair-fed ("AA," "BB" and "CC") to experimental rats ("A," "B" and "C"). Feed restriction (decreased intake of tryptophan) resulted in a decreased concentration of liver NAD in the pair-fed basal rats ("BB," and "CC") as compared to the rats fed the basal control diet.

The 3 percent level of L-phenylalanine decreased both the concentration and total content of brain NAD. There was no observable effect on the concentration of brain and liver serotonin or feed consumption and weight gain. The 5 percent level of L-phenylalanine decreased the concentration of brain and liver serotonin as well as the total serotonin content of the brain. The decrease in feed consumption of those rats fed the 5 percent level of L-phenylalanine approached significance and a significant decrease in brain size was noted. The 7 percent level of L-phenylalanine decreased significantly feed consumption, weight gain, the concentration of brain and liver serotonin, the total content of brain serotonin and the concentration of liver NAD.

From the data reported it appears that an excess of the amino acid L-phenylalanine (5 and 7 percent levels) may have caused more of the tryptophan eaten to enter the kynurenine (NAD) pathway of tryptophan metabolism with a correspondingly smaller amount entering the serotonin pathway. The opposite relationship was indicated for the 3 percent level of

L-phenylalanine. The fact still remains that it is uncertain whether NAD is produced in the liver and transported to the brain or is produced in the brain itself. Further, investigation would be required to determine whether the excess amount of L-phenylalanine was interfering with the passage of NAD from the blood to the brain or was interfering with its production in the brain.

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