

EFFECT OF PHENYLALANINE-TYROSINE RELATIONSHIPS  
ON THE GROWTH AND NITROGEN METABOLISM OF RATS

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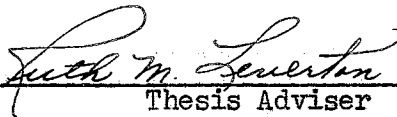
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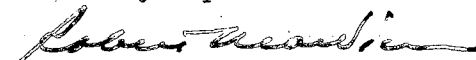
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## CHAPTER I

### INTRODUCTION

#### Amino Acid Requirements

Amino acids which must be supplied preformed in the diet to support life and promote growth are termed "essential". Other amino acids which are needed for these purposes but which can be synthesized by the body from protein moieties are termed "non-essential". The essentiality of various amino acids has been determined for several species.

Work by Rose (14, 15) has established the following list of ten "essential" and nine "non-essential" amino acids for the growing rat:

<u>Essential</u>	<u>Non-Essential</u>
Lysine	Serine
Tryptophan	Alanine
Histidine	Asparagine
Phenylalanine	Tyrosine
Leucine	Glutamic Acid
Isoleucine	Proline
Threonine	Hydroxyproline
Methionine	Cystine
Valine	Glycine
Arginine	

Phenylalanine is an "essential" and tyrosine a "non-essential" amino acid. Rose (14, 15) found that the absence of phenylalanine and tyrosine from the diet of the weanling rat resulted in complete nutritive failure. Addition of these two amino acids to the diet immediately induced rapid growth. Later Rose and Womack (16) and Womack and Rose (23) found that rats which received phenylalanine without tyrosine grew as well as animals which ingested both amino acids. However, it was shown by Ramasarma,

Henderson and Elvehjem (13) and by Womack and Rose (23, 25) that the substitution of tyrosine for phenylalanine resulted invariably in a rapid loss of body weight. These facts indicate that phenylalanine and tyrosine are not mutually interchangeable in metabolism and verify the essentiality of phenylalanine in the diet of the growing rat.

Following the establishment of the qualitative requirement of rats for phenylalanine, work was begun on the quantitative requirement. The phenylalanine requirement of the growing rat was first set by Rose (14) at 0.7% of the diet, but was later found by Rose and Womack (16) and confirmed by Ramasarma, Henderson and Elvehjem (13) to be 0.9% of the diet. Rose and Womack (16) and Womack and Rose (23, 25) showed that this requirement for phenylalanine could be supplied by either the L- or the DL- isomer because both are equally well utilized by the rat.

Various workers have reported that phenylalanine can undergo oxidation to tyrosine in vivo. Moss and Schoenheimer (12) and Womack and Rose (23, 25) have shown that the inclusion of tyrosine in the diet of the rat will lower the phenylalanine requirement by sparing the conversion of phenylalanine to tyrosine. Rose and Womack (16) and Rose, Oesterling and Womack (17) found that the amount of phenylalanine needed for maximum increases in weight was reduced from 0.9% to 0.4% when 0.5% tyrosine was included in the diet. More recent work by Rose and Wixom (19) with young men and by Leverton et al. (9) with young women has shown that the presence of tyrosine in the diet exerts a sparing action on phenylalanine. Rose and Wixom (19) reported that as much as 70 to 75% of the phenylalanine requirement could be replaced by tyrosine and the subjects could still maintain nitrogen equilibrium.

Since the phenylalanine and tyrosine replacement values were published for growing rats, much has been learned about the additional nutritive needs of these animals. Consequently, present-day experimental diets produce more rapid growth than the diets which were formerly fed. For this reason, the quantitative requirement of phenylalanine and the extent to which tyrosine spares phenylalanine in the diet of the weanling rat seem to warrant further investigation.

#### Utilization of Purified Diets

Frazier, Wissler and Steffee (7), Ramasarma, Henderson and Elvehjem (13), and Womack and Rose (24) have reported that rats which received amino acids as the principal source of nitrogen in the diet did not grow as well as rats which received casein at an equivalent level of nitrogen intake. Recently, however, Maddy and Swift (11) have formulated a diet, in which amino acids were the source of nitrogen and which permitted weanling rats to grow at a rate comparable to that of rats on a diet in which casein was the source of nitrogen.

Maddy and Swift (11) have reported that rats utilize the nitrogen and energy from amino acids less efficiently than from casein. This was shown by a lower average weight gain, less efficient utilization of the diet, decreased deposition of nitrogen and fat in the body, increased urinary excretion of energy, and a significantly lower metabolizable energy value for the amino acid diet.

Swift, Kahlenberg, Voris and Forbes (21) have shown that rats on a diet which was deficient in essential amino acids used a greater proportion of the metabolizable energy for body gain than rats which received a diet adequate in all amino acids. The rats on the diets deficient in



essential amino acids also synthesized less body protein and eliminated more nitrogen in the urine than the rats on the diets which were adequate in amino acids.

#### Purpose of the Work

The purpose of the work which will be reported here was to study further the quantitative requirement of phenylalanine and the replacement value of tyrosine for phenylalanine in the growing rat.

Data on the efficiency of diets which contain casein as compared with diets which contain amino acids as the source of nitrogen, with adequate and inadequate amounts of phenylalanine, were also sought. This information is needed to increase our understanding of the effects of amino acid deficiencies and to aid in the evaluation of highly purified diets.

## CHAPTER II

### METHODS AND PROCEDURE

The methods used to accomplish the previously stated purposes involved: 1) the use of weanling rats as experimental subjects, 2) feeding these animals four purified diets which contained different levels of phenylalanine plus a fifth diet which contained casein and served as a control, and 3) studying the growth and nitrogen metabolism of these rats on the different diets.

#### Animals

Weanling female, albino rats of the Sprague-Dawley strain with initial weights of 41.9 to 50.3 gm. were used for the study. Following a 12-hour period during which they received no food, the rats were paired by weight. Six rats were killed and their carcasses were analyzed to give representative data on the initial status of the experimental rats at the time they were placed on the various diets.

Twenty-five rats were placed in individual metabolism cages. These cages were 11-in. high and 9-in. wide. They were placed on a 10-in. pyrex pie plate which contained 1 ml. of concentrated HCl. Each pie plate was covered with a fine mesh screen which served to collect the feces and to allow the urine to drop through into the dish. The top of each cage was closed with an inverted tin pie pan.

The feeders were constructed of galvanized iron and suspended from the upper edge of the cages. A 50-ml. beaker was used as the feed container. It was held in place by a cup which was soldered to the floor of the feeder. This apparatus was satisfactory in preventing scattering of food.

Although the work reported here was carried out between June and August, the room was maintained by cooled air at  $27 \pm 2^{\circ}\text{C}$ . for the 60-day experimental period.

All of the rats were killed at the end of the 60-day feeding period and the nitrogen and fat contents of the carcasses were determined.

#### Diets

Five diets were fed and five rats received each diet. On the basis of previous work the following levels of phenylalanine and tyrosine were chosen:

- (1) Diet A  
0.9% DL-phenylalanine  
0.6% L-tyrosine  
This represents an adequate intake of phenylalanine with tyrosine.
- (2) Diet B  
0.6% DL-phenylalanine  
0.3% L-tyrosine
- (3) Diet C  
0.3% DL-phenylalanine  
0.6% L-tyrosine

Diets B and C represent two intermediate levels of phenylalanine and tyrosine to help establish the replacement value of tyrosine for phenylalanine.

- (4) Diet D  
0 Phenylalanine  
0.9% L-tyrosine  
This was the negative control diet.
- (5) Diet E  
0.69% L-phenylalanine  
0.69% L-tyrosine  
This was the control diet which contained casein.

The mixture of purified amino acids which was used in all of the diets is shown in Table I. It was patterned after the Amino Acid Mix IV used by Maddy and Swift (11). All five of the diets were made isonitrogenous by adjusting the level of glycine fed.

TABLE I  
PERCENTAGE OF AMINO ACIDS INCLUDED IN THE DIETS

Amino Acid	Diet Code				
	A	B	C	D	E-casein <sup>1</sup>
	%	%	%	%	%
Constant in all diets					
L-lysine monohydrochloride	1.50	1.50	1.50	1.50	0.90
DL-tryptophan	0.40	0.40	0.40	0.40	0.13
L-histidine monohydrochloride	0.86	0.86	0.86	0.86	0.34
L-leucine	1.20	1.20	1.20	1.20	1.10
DL-isoleucine	1.82	1.82	1.82	1.82	0.82
DL-threonine	1.43	1.43	1.43	1.43	0.53
DL-methionine	0.80	0.80	0.80	0.80	0.37
DL-valine	2.00	2.00	2.00	2.00	0.85
L-arginine monohydrochloride	0.50	0.50	0.50	0.50	0.45
DL-serine	0.20	0.20	0.20	0.20	0.69
DL-alanine	0.40	0.40	0.40	0.40	0.33
L-asparagine	0.20	0.20	0.20	0.20	0.78
L-glutamic acid	2.00	2.00	2.00	2.00	2.45
L-proline	0.20	0.20	0.20	0.20	1.23
L-hydroxyproline	0.10	0.10	0.10	0.10	—
L-cystine	0.20	0.20	0.20	0.20	0.03
Variable in all diets					
DL-phenylalanine	0.90	0.60	0.30	0.00	0.69
L-tyrosine	0.60	0.30	0.60	0.90	0.69
Glycine	0.10	0.70	0.70	0.70	0.29

<sup>1</sup>R. J. Block and D. Bolling., The Amino Acid Composition of Proteins and Foods (Springfield, Illinois, 1951): these figures are for the L-isomer.

The percentage composition of the complete basal diets is found in Table II. The composition of the salt mixture is identical to that used by Lichstein, McCall, Kearney, Elvehjem and Clark (10) which is shown

in Table III. The composition of the vitamin mixture is identical to that used by Tillman (22) and it is listed in Table IV.

TABLE II  
PERCENTAGE COMPOSITION OF THE BASAL DIETS

Ingredients	Diets	Diet
	A, B, C, D	E
	%	%
Cerelose	68.56	71.57
Salka Flax (cellulose)	3.00	3.00
Vegetable Oil	6.70	6.70
Salt Mix	4.00	4.00
Choline Chloride	0.10	0.10
Vitamin Mix	0.03	0.03
Amino Acid Mix	17.61	---
Casein	---	14.85

In compounding the diets, the phenylalanine and tyrosine were weighed on an analytical balance. The other amino acids were weighed on a small torsion balance which had an accuracy of 15 mg. These were thoroughly mixed and ground in a ball mill. The remaining components were added to the amino acids and each complete diet was then blended in a large Hobart mixer. The diets were stored in a refrigerator.

Each of the diets was assayed microbiologically to check its content of phenylalanine and tyrosine. Acid hydrolysis and Leuconostoc mesenteroides P-60 were used to determine the total L-phenylalanine content. Alkaline hydrolysis and Leuconostoc citrovorum ATCC 8081 were used to determine the total L-tyrosine content. The method of Henderson and Snell (8) was followed. Nitrogen determinations were made on all of the diets by the use of a macro-Kjeldahl method (1).

TABLE III  
COMPOSITION OF THE SALT MIX

Salt	Amount in diet
	mg./100 gm.
NaCl	600.0
$K_2HPO_4 \cdot 3H_2O$	1516.0
$Ca_2H_2(PO_4) \cdot 4H_2O$	340.4
$MgSO_4 \cdot 7H_2O$	365.6
$CaCO_3$	1076.0
$Fe(C_6H_5O_7)_2 \cdot 6H_2O$	100.0
KI	28.0
$MnSO_4 \cdot 6H_2O$	1.2
$ZnCl_2$	0.9
$CuSO_4 \cdot 5H_2O$	1.1

TABLE IV  
COMPOSITION OF THE VITAMIN MIX

Vitamin	Amount in 100 gm. of diet	
	Thiamine·HCl	0.5
Riboflavin	0.8	mg.
Niacin	4.0	mg.
Pyridoxine·HCl	0.5	mg.
Ca-Pantothenate	4.0	mg.
Biotin	0.04	mg.
Folic Acid	0.2	mg.
Menadione	0.5	mg.
Vitamin B <sub>12</sub>	0.003	mg.
Inositol	10.0	mg.
p-Aminobenzoic acid	10.0	mg.
Vitamin E	10.0	mg.
Vitamin A	2000	IU
Vitamin D	200	IU

## Growth and Metabolism Studies

All of the animals were on their respective diets for 60 days. The rats were weighed daily and the food intakes were recorded for each 24-hour period.

The study consisted of 22 days, designated as Period I, during which pair-feeding was carried out. This was followed by 38 days, designated as Period II, during which ad libitum feeding was allowed.

The urine for each 24-hour period was acidified, brought to volume, and stored under toluene. Collections for each 10-day period were made into a composite from which aliquots were taken for nitrogen determinations. The feces were also collected daily, acidified, refrigerated and made into 20-day composites. This material was dried in an oven at 50°C for 12 hours, weighed, ground in a mortar, and aliquots were taken for nitrogen determinations.

At the end of the 60-day period, each rat was killed. The contents of the alimentary tract were removed and discarded and the body was cut into small pieces. Each carcass was dried in a vacuum desiccator until there was no further weight loss. It was then extracted with ether by placing the pieces of carcass in three or four fat extraction tubes and extracting with anhydrous diethyl ether for 16 hours. These samples were dried in vacuum desiccators and weighed. The ether soluble material was estimated from the loss of weight during extraction. This method is a modification of the method given in A.O.A.C. (1).

The fat-free residue of each carcass was ground first in a Waring blender and then in a Wiley mill in preparation for nitrogen analysis. Nitrogen determinations by a macro-Kjeldahl method (1) were made in duplicate on the 10-day urine composites, 20-day fecal composites, and the carcass of each rat.

## CHAPTER III

### RESULTS

The findings will be presented in the following order: 1) weight gains and food intakes, 2) food efficiency, 3) nitrogen retention, 4) nitrogen efficiency, and 5) body composition of the rats on the different diets.

The results of the daily food intakes and weight gains of the five individual rats on each diet were combined and averaged. The means of the five rats which received each of the five diets will be discussed and compared. The casein diet, which was fed as the control, will be used for purposes of comparison.

Data on the gains in weight, comparison of these gains by the rats on the purified diets with those of the rats on the control diet, the food intake, and the food efficiency, or weight gain per gram of food consumed, are given in Table V. The data were analyzed statistically by the Student "t" test (20) to determine the significance of differences between results secured on different diets. Differences are reported as significant if they exhibited a probability of 1 percent or less.

#### Weight Gains and Food Intakes

The means and standard deviations of the changes in weight of the rats on the five diets are shown in Table V. The results are presented separately for Period I and Period II and then combined to show results



TABLE V  
THE GROWTH RESPONSE, FOOD INTAKE, AND EFFICIENCY  
OF FOOD UTILIZATION OF RATS

Diet A 0.9% DL-phenylalanine + 0.6% L-tyrosine				
Rat Code	Average wt. gain gm./day	Per cent of wt. gained by control rats %	Average food intake gm./day	Food efficiency
<u>Period I</u> <u>Days 1 - 22</u>				
A <sub>1</sub>	0.88	98.9	6.5	0.14
A <sub>2</sub>	0.93	104.5	6.5	0.14
A <sub>3</sub>	1.37	153.9	6.5	0.21
A <sub>4</sub>	0.72	80.9	6.5	0.11
A <sub>5</sub>	0.92	103.4	6.5	0.14
Mean	0.96	108.3	6.5	0.15
S.D. <sup>1</sup>	0.24	27.19	0	0.04
<u>Period II</u> <u>Days 23 - 60</u>				
A <sub>1</sub>	1.69	183.7	8.5	0.20
A <sub>2</sub>	1.35	146.7	8.9	0.15
A <sub>3</sub>	1.45	157.6	9.8	0.15
A <sub>4</sub>	1.13	122.8	7.8	0.14
A <sub>5</sub>	1.77	192.4	8.9	0.20
Mean	1.48	160.6	8.8	0.17
S.D.	0.26	28.18	0.71	0.03
<u>Total</u> <u>Days 1 - 60</u>				
A <sub>1</sub>	1.40	153.8	7.8	0.18
A <sub>2</sub>	1.20	131.9	8.1	0.15
A <sub>3</sub>	1.42	156.0	8.6	0.17
A <sub>4</sub>	0.98	107.7	7.3	0.13
A <sub>5</sub>	1.46	160.4	7.8	0.19
Mean	1.29	142.0	7.9	0.16
S.D.	0.20	22.09	0.45	0.02

(continued on next page)

TABLE V (continued)

Diet B 0.6% DL-phenylalanine + 0.3% L-tyrosine				
Rat Code	Average wt. gain	Per cent of wt. gained by control rats	Average food intake	Food efficiency
	gm./day	%	gm./day	
<u>Period I</u>		<u>Days 1 - 22</u>		
B <sub>1</sub>	0.78	87.6	6.5	0.12
B <sub>2</sub>	1.05	118.0	6.5	0.16
B <sub>3</sub>	0.98	110.1	6.5	0.15
B <sub>4</sub>	1.03	115.7	6.5	0.16
B <sub>5</sub>	0.87	97.7	6.5	0.13
Mean	0.94	105.8	6.5	0.14
S.D.	0.11	12.87	0	0.02
<u>Period II</u>		<u>Days 23 - 60</u>		
B <sub>1</sub>	1.04	113.0	7.7	0.13
B <sub>2</sub>	1.00	108.7	8.3	0.12
B <sub>3</sub>	1.37	148.9	8.4	0.16
B <sub>4</sub>	1.08	117.4	8.7	0.12
B <sub>5</sub>	1.15	125.0	8.2	0.14
Mean	1.13	122.6	8.3	0.13
S.D.	0.15	15.89	0.38	0.02
<u>Total</u>		<u>Days 1 - 60</u>		
B <sub>1</sub>	0.95	104.4	7.2	0.13
B <sub>2</sub>	1.02	112.1	7.6	0.13
B <sub>3</sub>	1.23	135.2	7.7	0.16
B <sub>4</sub>	1.06	116.5	7.9	0.13
B <sub>5</sub>	1.05	115.4	7.6	0.14
Mean	1.06	116.7	7.6	0.14
S.D.	0.10	11.36	0.24	0.01

(continued on next page)

TABLE V (continued)

Diet C 0.3% DL-phenylalanine + 0.6% L-tyrosine				
Rat Code	Average wt. gain	Per cent of wt. gained by control rats	Average food intake	Food efficiency
	gm./day	%	gm./day	
<u>Period I</u> <u>Days 1 - 22</u>				
C <sub>1</sub>	0.85	95.5	6.0	0.14
C <sub>2</sub>	0.53	59.5	6.5	0.08
C <sub>3</sub>	0.63	70.8	6.5	0.10
C <sub>4</sub>	0.79	88.8	6.5	0.12
C <sub>5</sub>	0.67	75.3	6.5	0.10
Mean	0.69	80.0	6.4	0.11
S.D.	0.12	14.35	0.20	0.02
<u>Period II</u> <u>Days 23 - 60</u>				
C <sub>1</sub>	1.24	134.8	7.4	0.17
C <sub>2</sub>	0.99	107.6	8.4	0.12
C <sub>3</sub>	1.39	151.1	9.7	0.14
C <sub>4</sub>	1.86	202.2	8.2	0.23
C <sub>5</sub>	1.06	115.2	9.3	0.11
Mean	1.31	142.2	8.6	0.15
S.D.	0.35	37.62	0.93	0.05
<u>Total</u> <u>Days 1 - 60</u>				
C <sub>1</sub>	1.10	120.9	6.9	0.16
C <sub>2</sub>	0.82	90.1	7.7	0.11
C <sub>3</sub>	1.12	123.1	8.5	0.13
C <sub>4</sub>	1.47	161.5	7.5	0.19
C <sub>5</sub>	0.92	101.1	8.2	0.11
Mean	1.09	119.3	7.8	0.14
S.D.	0.24	27.30	0.65	0.03

(continued on next page)

TABLE V (continued)

Diet D				
0 Phenylalanine + 0.9% L-tyrosine				
Rat Code	Average wt. gain	Per cent of wt. gained by control rats	Average food intake	Food efficiency
	gm./day	%	gm./day	
<u>Period I</u> <u>Days 1 - 22</u>				
D <sub>1</sub>	-0.33	-37.1	5.7	-0.06
D <sub>2</sub>	-0.39	-43.8	5.2	-0.07
D <sub>3</sub>	-0.46	-51.7	4.8	-0.10
D <sub>4</sub>	-0.36	-40.4	5.8	-0.06
D <sub>5</sub>	-0.33	-37.1	5.3	-0.06
Mean	-0.37	-42.0	5.3	-0.07
S.D.	0.05	6.08	0.40	0.02
<u>Period II</u> <u>Days 23 - 35</u>				
D <sub>1</sub>	-0.14	-15.2	2.9	-0.05
D <sub>2</sub>	-0.16	-17.4	2.9	-0.05
D <sub>3</sub>	-0.21	-22.8	3.5	-0.06
D <sub>4</sub>	-0.10	-10.9	3.3	-0.03
D <sub>5</sub>	-0.18	-19.6	2.9	-0.06
Mean	-0.16	-17.2	3.1	-0.05
S.D.	0.04	4.49	0.28	0.01
<u>Total</u> <u>Days 1 - 35</u>				
D <sub>1</sub>	-0.26	-25.5	4.7	-0.05
D <sub>2</sub>	-0.30	-29.4	4.3	-0.07
D <sub>3</sub>	-0.25	-24.5	4.3	-0.06
D <sub>4</sub>	-0.25	-24.5	4.8	-0.05
D <sub>5</sub>	-0.19	-18.6	4.4	-0.04
Mean	-0.25	-24.5	4.5	-0.05
S.D.	0.04	3.87	0.23	0.01

(continued on next page)

TABLE V (continued)

Diet E - Control 0.69% L-phenylalanine + 0.69% L-tyrosine				
Rat Code	Average wt. gain gm./day	Per cent of wt. gained by control rats %	Average food intake gm./day	Food efficiency
<u>Period I</u> <u>Days 1 - 22</u>				
E <sub>1</sub>	0.77		6.5	0.12
E <sub>2</sub>	1.14		6.5	0.17
E <sub>3</sub>	0.86		6.5	0.13
E <sub>4</sub>	0.81		6.4	0.13
E <sub>5</sub>	0.85		6.4	0.13
Mean	0.89	100.0	6.5	0.14
S.D.	0.15		0.05	0.02
<u>Period II</u> <u>Days 23 - 60</u>				
E <sub>1</sub>	1.11		8.5	0.13
E <sub>2</sub>	1.11		7.6	0.15
E <sub>3</sub>	0.59		7.1	0.08
E <sub>4</sub>	1.04		7.9	0.13
E <sub>5</sub>	0.73		7.7	0.09
Mean	0.92	100.0	7.8	0.12
S.D.	0.24		0.47	0.03
<u>Total</u> <u>Days 1 - 60</u>				
E <sub>1</sub>	0.99		7.7	0.13
E <sub>2</sub>	1.12		7.2	0.15
E <sub>3</sub>	0.69		6.9	0.10
E <sub>4</sub>	0.96		7.3	0.13
E <sub>5</sub>	0.77		7.2	0.11
Mean	0.91	100.0	7.3	0.12
S.D.	0.18		0.30	0.02

<sup>1</sup>Standard Deviation

for the entire time of the study. During Period I, the 22 days of pair-feeding, the food intakes of the rats were low because the rats on the phenylalanine-free diet had no appetite. This low food intake resulted in retarded weight gains for Period I by all of the rats. During Period II, the 38 days of ad libitum feeding, the rats on each diet which contained phenylalanine gained more rapidly than during Period I.

The greatest mean weight gain for the entire study was 1.29 gm. per day, which was achieved by the rats on the highest level of phenylalanine, diet A with 0.9% DL-phenylalanine plus 0.6% L-tyrosine. This gain in weight was significantly greater, as measured by the Student "t" test, than the mean weight gain of 1.06 gm. per day by the rats on diet B with 0.6% DL-phenylalanine plus 0.3% L-tyrosine, and also greater than the mean weight gain of 1.09 gm. per day by the rats on diet C with only 0.3% DL-phenylalanine plus 0.6% L-tyrosine.

The animals which received diet D with no phenylalanine and 0.9% L-tyrosine showed weight losses beginning with the second day of feeding, and these continued throughout the study. The mean weight loss for these rats was 0.25 gm. per day. The rat which showed the greatest loss of weight, 15 gm., died on the 59th day of the study.

The results for the rats on diet D are presented for Days 1 to 35 only. On Day 36 each rat which normally received diet D was offered, by mistake, 7 gm. of diet C. Over a 24-hour period these rats consumed 55.2 to 69.0 mg. DL-phenylalanine and gained from 3.1 to 4.6 gm. in weight. If this consumption of phenylalanine had not occurred, it is possible that these rats which were the negative controls would not have lived the entire 60 days of the experiment.

The rats which consumed the control diet with 0.69% L-phenylalanine plus 0.69% L-tyrosine from casein showed a mean weight gain of 0.91 gm. per day. This was less than the gain in weight by the rats which received diets A, B, or C.

The mean growth response of the rats on each of the diets for Periods I and II is graphed in Figure 1. The slow growth during Period I for the rats which received phenylalanine in their diets is followed by more rapid growth during Period II. The rats which consumed diet A showed the greatest amount of growth. The rats on the phenylalanine-free diet showed a continual decrease in weight for Days 1 to 36.

A comparison of the mean weight gain of each group of rats with that of the control rats is found in Table V. The rats which received the highest level of phenylalanine, diet A, gained 142% of the amount gained by the rats on the control diet. This was significantly higher than the gains by the rats on diets B and C. The rats which received no phenylalanine in the diet lost weight and at the end of 35 days weighed only 57% of the mean weight of the control rats.

A deficient condition in the rats which received no phenylalanine in the diet was manifested by an impairment of appetite, emaciated appearance, bloody mouth and paws, a general sluggish condition, loss of hair, and lack of coordination. No such effects were evident in the rats which received phenylalanine in the amounts fed in this study.

The food intake was controlled during the first 22 days at 6.5 gm. per rat per day for the rats on all of the diets except the one which included no phenylalanine. A mean of only 5.3 gm. per day was consumed by each rat on diet D. During Period II, when ad libitum feeding was allowed, the food intakes by the rats on all of the diets, except the

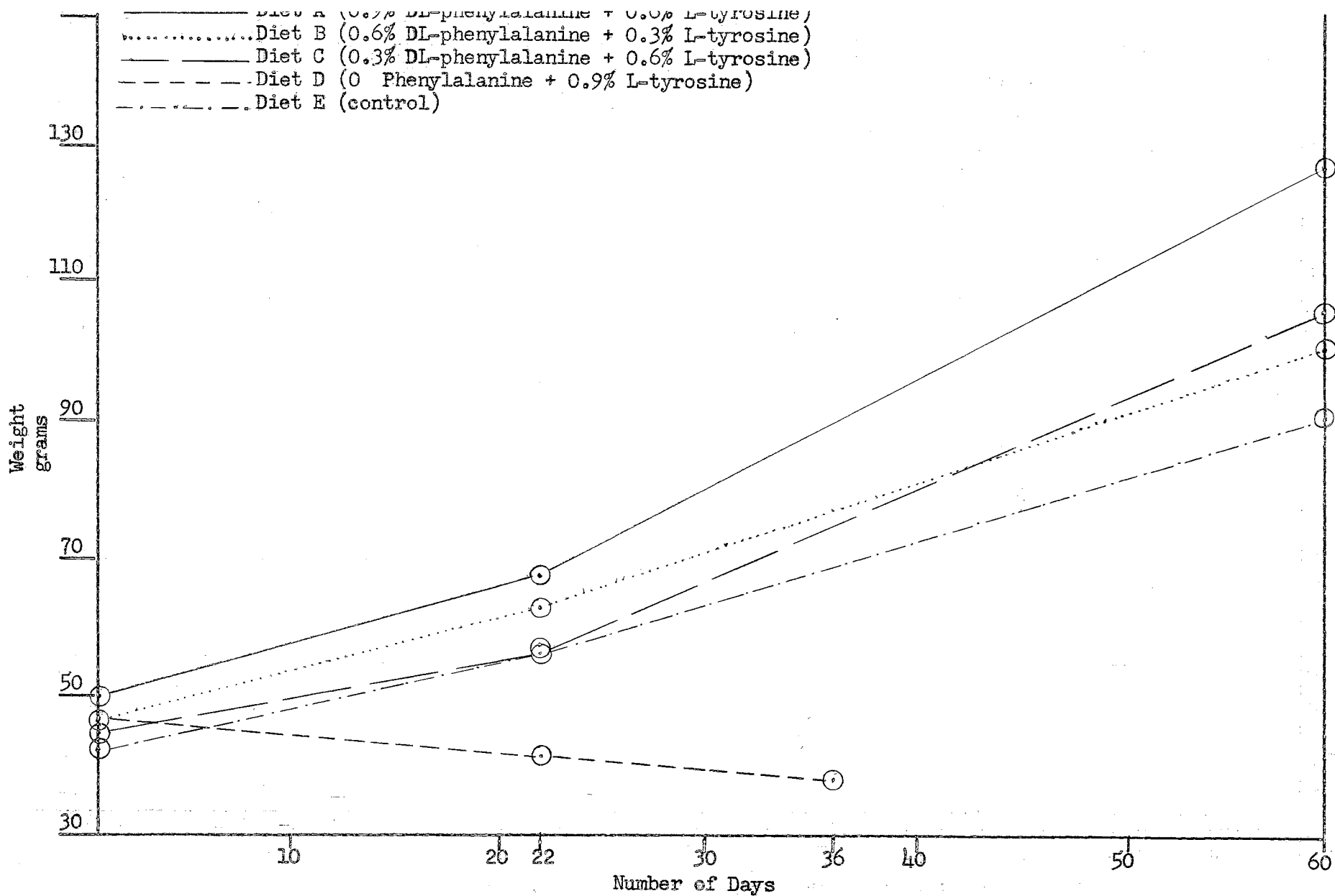


Figure 1 - MEAN GROWTH OF RATS ON DIETS WITH DIFFERENT LEVELS OF PHENYLALANINE AND TYROSINE



one which contained no phenylalanine, immediately increased. No significant differences were found in the food intakes of the rats on diets A, B, and C. Thus, differences in weight gains may be attributed to the quality of the diets.

The data for the food intakes for the entire period show a 4% to 8% lower food consumption by the rats on the control diet than by the rats on diets A, B, or C. At the same time, the control animals showed a 17% to 42% lower gain in weight than the rats on diets A, B, or C.

#### Food Efficiency

The means and standard deviations of the food efficiency, as measured by the grams of weight gain per gram of food intake, are also presented in Table V. No significant differences in food efficiency were seen in the rats which received any of the diets that supplied phenylalanine. The food efficiency of the rats on diet C increased from a mean of 0.11 during Period I to a mean of 0.15 during Period II. The rats which received the phenylalanine-free diet showed a mean food efficiency of only -0.05 gm.

#### Nitrogen Retention

The means and standard deviations of the daily intakes of phenylalanine and tyrosine together with the nitrogen retentions of the rats on each of the diets are summarized in Table VI. As the rats on each diet grew in size, the food consumption became greater, and thus the phenylalanine and tyrosine intakes increased. This was not true for the rats which received no phenylalanine in their diets. The L-tyrosine intake by these rats continued to decrease throughout the study.

TABLE VI  
 THE PHENYLALANINE AND TYROSINE INTAKE, NITROGEN RETENTION  
 AND NITROGEN EFFICIENCY OF RATS

Diet A 0.9% DL-phenylalanine + 0.6% L-tyrosine				
Rat Code	DL-phenylalanine intake mg./day	L-tyrosine intake mg./day	Nitrogen retention mg./day	Nitrogen efficiency
<u>Period I    Days 1 - 22</u>				
A <sub>1</sub>	66.9	44.9	76.0	1.01
A <sub>2</sub>	66.9	44.9	76.9	1.07
A <sub>3</sub>	66.9	44.9	78.0	1.57
A <sub>4</sub>	66.9	44.9	81.3	0.82
A <sub>5</sub>	66.9	44.9	83.5	1.06
Mean	66.9	44.9	79.1	1.11
S.D. <sup>2</sup>	0	0	3.15	0.28
<u>Period II    Days 23 - 60</u>				
A <sub>1</sub>	87.5	58.7	98.1	1.47
A <sub>2</sub>	91.7	61.4	86.4	1.12
A <sub>3</sub>	100.9	67.6	110.3	1.10
A <sub>4</sub>	80.3	53.8	82.2	1.07
A <sub>5</sub>	91.7	61.4	102.1	1.48
Mean	90.4	60.6	95.8	1.25
S.D.	7.48	5.00	11.50	0.21
<u>Total        Days 1 - 60</u>				
A <sub>1</sub>	80.3	53.8	90.0	1.33
A <sub>2</sub>	83.4	55.9	82.9	1.10
A <sub>3</sub>	88.6	59.3	98.5	1.23
A <sub>4</sub>	75.2	50.4	81.9	0.99
A <sub>5</sub>	80.3	53.8	95.3	1.35
Mean	81.6	54.6	89.7	1.20
S.D.	4.89	3.27	7.34	0.15

(continued on next page)

TABLE VI (continued)

Diet B 0.6% DL-phenylalanine + 0.3% L-tyrosine				
Rat Code	DL-phenylalanine intake mg./day	L-tyrosine intake mg./day	Nitrogen retention mg./day	Nitrogen efficiency
<u>Period I Days 1 - 22</u>				
B <sub>1</sub>	44.9	22.1	75.8	0.86
B <sub>2</sub>	44.9	22.1	79.4	1.16
B <sub>3</sub>	44.9	22.1	82.6	1.08
B <sub>4</sub>	44.9	22.1	66.2	1.13
B <sub>5</sub>	44.9	22.1	43.3	0.96
Mean	44.9	22.1	69.5	1.04
S.D.	0	0	15.85	0.13
<u>Period II Days 23 - 60</u>				
B <sub>1</sub>	53.1	26.2	84.9	0.97
B <sub>2</sub>	57.3	28.2	96.7	0.86
B <sub>3</sub>	58.0	28.6	91.9	1.16
B <sub>4</sub>	60.0	29.6	98.5	0.89
B <sub>5</sub>	56.6	27.9	92.8	1.00
Mean	57.0	28.1	92.9	0.98
S.D.	2.52	1.24	5.25	0.12
<u>Total Days 1 - 60</u>				
B <sub>1</sub>	49.7	24.5	81.6	0.94
B <sub>2</sub>	52.4	25.8	90.3	0.95
B <sub>3</sub>	53.1	26.2	88.5	1.14
B <sub>4</sub>	54.5	26.9	86.6	0.96
B <sub>5</sub>	52.4	25.8	74.7	0.99
Mean	52.4	25.8	84.3	1.00
S.D.	1.75	0.87	6.32	0.08

(continued on next page)

TABLE VI (continued)

Diet C				
0.3% DL-phenylalanine + 0.6% L-tyrosine				
Rat Code	DL-phenylalanine intake	L-tyrosine intake	Nitrogen retention	Nitrogen efficiency
	mg./day	mg./day	mg./day	
<u>Period I Days 1 - 22</u>				
C <sub>1</sub>	20.4	41.4	76.1	1.03
C <sub>2</sub>	22.1	44.9	82.2	0.60
C <sub>3</sub>	22.1	44.9	81.4	0.71
C <sub>4</sub>	22.1	44.9	75.7	0.89
C <sub>5</sub>	22.1	44.9	80.3	0.75
Mean	21.8	44.2	79.1	0.80
S.D.	0.76	1.57	3.04	0.17
<u>Period II Days 23 - 60</u>				
C <sub>1</sub>	25.2	51.1	76.8	1.23
C <sub>2</sub>	28.6	58.0	106.0	0.86
C <sub>3</sub>	33.0	66.9	121.0	1.05
C <sub>4</sub>	27.9	56.6	89.1	1.67
C <sub>5</sub>	31.6	64.2	106.2	0.84
Mean	29.3	59.4	99.8	1.13
S.D.	3.09	6.28	17.09	0.34
<u>Total Days 1 - 60</u>				
C <sub>1</sub>	23.5	47.6	76.6	1.17
C <sub>2</sub>	26.2	53.1	97.3	0.78
C <sub>3</sub>	28.9	58.7	106.5	0.96
C <sub>4</sub>	25.5	51.7	84.2	1.43
C <sub>5</sub>	27.9	56.6	96.7	0.82
Mean	26.4	53.5	92.2	1.03
S.D.	2.11	3.87	11.81	0.27

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TABLE VI (continued)

Diet D				
0 Phenylalanine + 0.9% L-tyrosine				
Rat Code	DL-phenylalanine intake	L-tyrosine intake	Nitrogen retention	Nitrogen efficiency
	mg./day	mg./day	mg./day	
<u>Period I Days 1 - 22</u>				
D <sub>1</sub>	0	58.7	77.3	-0.43
D <sub>2</sub>	0	53.6	75.4	-0.56
D <sub>3</sub>	0	49.4	68.4	-0.72
D <sub>4</sub>	0	59.7	90.9	-0.47
D <sub>5</sub>	0	54.6	59.5	-0.47
Mean	0	55.2	74.3	-0.53
S.D.	0	4.15	11.62	0.12
<u>Period II Days 23 - 35</u>				
D <sub>1</sub>	0	30.9	18.9	-0.23
D <sub>2</sub>	0	28.8	22.9	-0.29
D <sub>3</sub>	0	36.1	27.9	-0.27
D <sub>4</sub>	0	30.0	1.7	-0.18
D <sub>5</sub>	0	27.8	1.9	-0.31
Mean	0	30.7	14.7	-0.26
S.D.	0	3.23	12.16	0.05
<u>Total Days 1 - 35</u>				
D <sub>1</sub>	0	44.8	48.1	-0.30
D <sub>2</sub>	0	41.2	49.1	-0.43
D <sub>3</sub>	0	42.7	48.1	-0.47
D <sub>4</sub>	0	44.9	46.3	-0.34
D <sub>5</sub>	0	41.2	30.7	-0.39
Mean	0	43.0	44.5	-0.39
S.D.	0	1.83	7.76	0.07

(continued on next page)

TABLE VI (continued)

Diet E - Control				
0.69% L-phenylalanine + 0.69% L-tyrosine				
Rat Code	L-phenylalanine intake	L-tyrosine intake	Nitrogen retention	Nitrogen efficiency
	mg./day	mg./day	mg./day	
<u>Period I Days 1 - 22</u>				
E <sub>1</sub>	49.5	49.5	77.1	0.94
E <sub>2</sub>	49.5	49.5	74.4	1.39
E <sub>3</sub>	49.5	49.5	81.1	1.05
E <sub>4</sub>	48.8	48.8	75.7	1.00
E <sub>5</sub>	48.8	48.8	76.2	1.05
Mean	49.2	49.2	76.9	1.09
S.D.	0.39	0.39	2.54	0.17
<u>Period II Days 23 - 60</u>				
E <sub>1</sub>	64.8	64.8	76.7	1.04
E <sub>2</sub>	57.9	57.9	81.6	1.15
E <sub>3</sub>	54.1	54.1	64.6	0.65
E <sub>4</sub>	60.2	60.2	73.4	1.04
E <sub>5</sub>	58.7	58.7	63.7	0.75
Mean	59.1	59.1	72.0	0.93
S.D.	3.88	3.88	7.75	0.21
<u>Total Days 1 - 60</u>				
E <sub>1</sub>	58.7	58.7	74.8	1.01
E <sub>2</sub>	54.9	54.9	78.9	1.23
E <sub>3</sub>	52.6	52.6	70.7	0.79
E <sub>4</sub>	55.6	55.6	74.3	1.03
E <sub>5</sub>	54.9	54.9	68.3	0.84
Mean	55.3	55.3	73.4	0.98
S.D.	2.19	2.19	4.10	0.17

<sup>2</sup>Standard Deviation

Positive nitrogen balances were observed in the rats which received each of the diets. The largest mean positive balance, which was 92 mg. of nitrogen per day, was found in the rats on diet C. Nitrogen retentions by the rats on diet A (0.9% DL-phenylalanine plus 0.6% L-tyrosine), diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine), and diet C (0.3% DL-phenylalanine plus 0.6% L-tyrosine) were significantly greater than the nitrogen retentions by the rats which received diet D (no phenylalanine) or diet E (the control).

Negative nitrogen balance was not shown in these data by the rats which received the phenylalanine-free diet. However, when the nitrogen excretions of the individual animals on diet D were presented, negative nitrogen balances of 7 to 10 mg. per day were found in three rats during Days 30 to 40. Following Day 40 (the period after these rats mistakenly received phenylalanine), return to negative nitrogen balances was never seen.

In considering the effects of an amino acid deficiency upon nitrogen balance, it is necessary to consider the level of nitrogen in the diet and the amount of diet consumed. Figure 2 shows the nitrogen intake and Figure 3 the nitrogen retention of the rats on each diet. While the nitrogen intake increased on all diets which contained phenylalanine during Period I, the nitrogen retention decreased. When ad libitum feeding was allowed in Period II, nitrogen intake and retention increased in diets A, B, and C. Those animals which received the phenylalanine-free diet showed decreasing nitrogen intake and retention throughout the study. The rats which consumed the control diet showed increased nitrogen intake but decreased nitrogen retention during Period II.

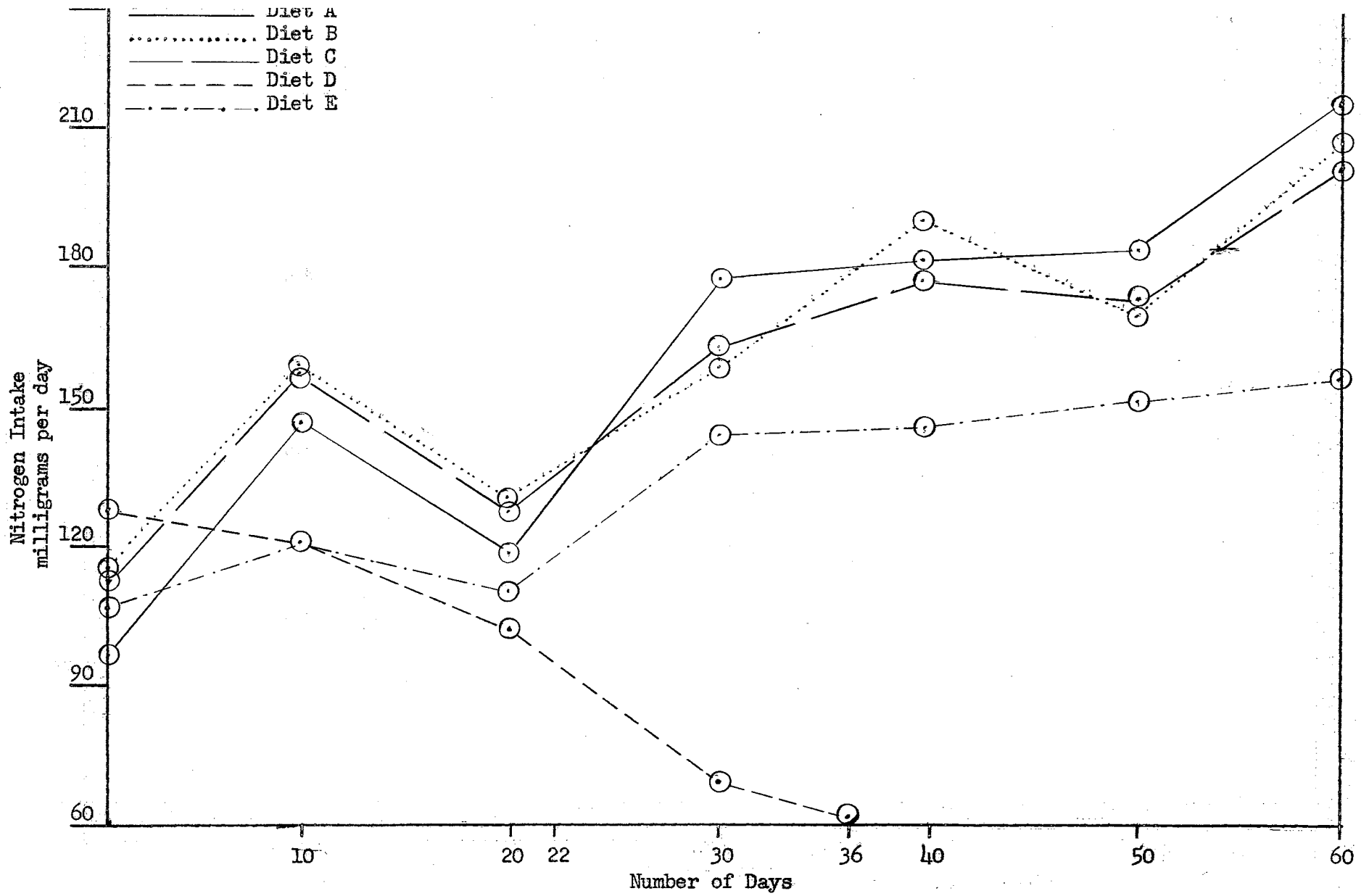


Figure 2 - MEAN NITROGEN INTAKE OF RATS ON DIETS WITH DIFFERENT LEVELS OF PHENYLALANINE AND TYROSINE



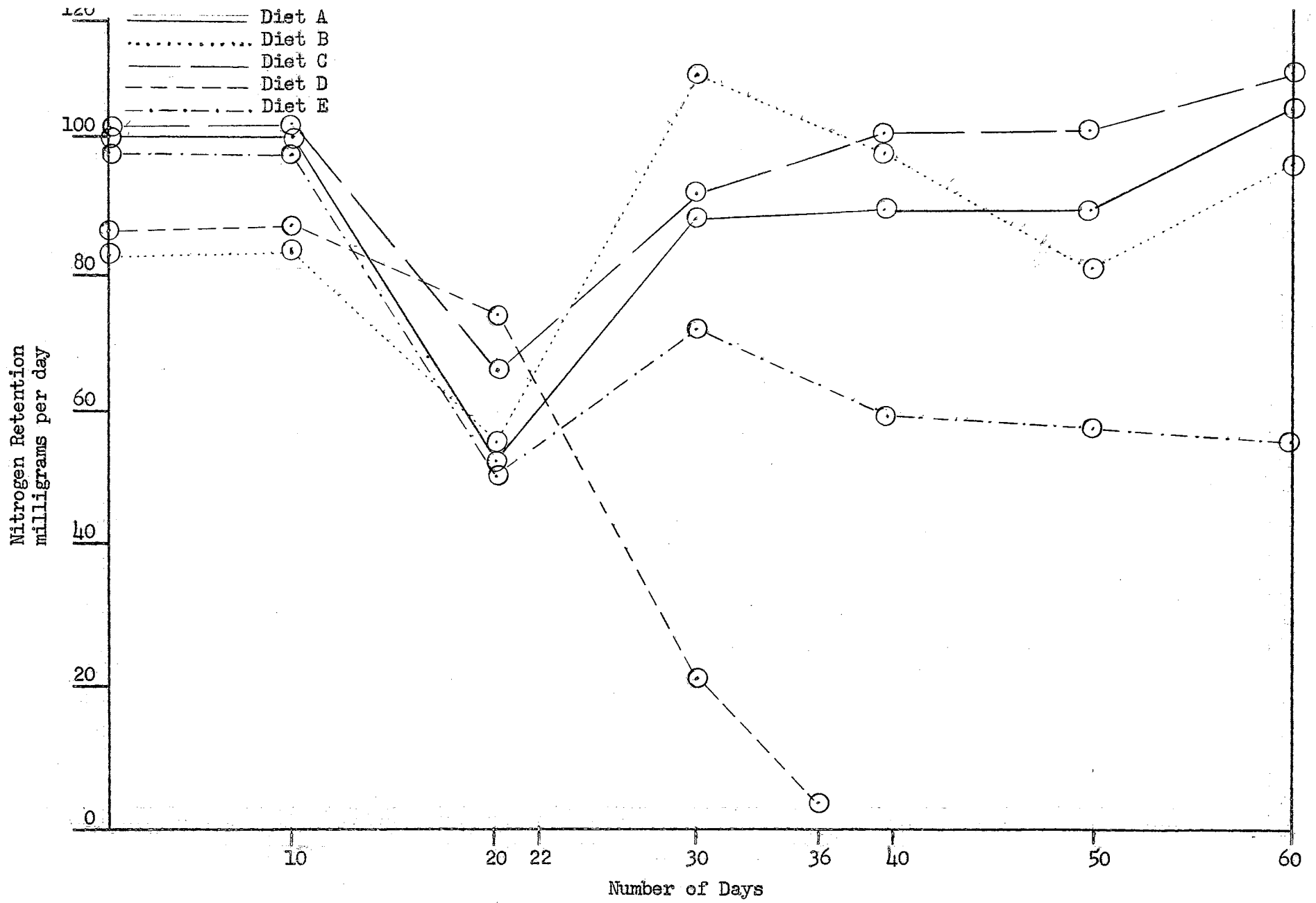


Figure 3 - MEAN NITROGEN RETENTION OF RATS ON DIETS WITH DIFFERENT LEVELS OF PHENYLALANINE AND TYROSINE

### Nitrogen Efficiency

Data on nitrogen efficiency, or grams of weight gain per gram of nitrogen intake, are presented in Table VI.

The rats which received diet A (0.9% DL-phenylalanine plus 0.6% L-tyrosine) showed the greatest nitrogen efficiency for both periods. The mean of 1.20 for the rats on diet A was significantly higher than the mean nitrogen efficiency of 1.00 for the rats on diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine) or that of 1.03 for the rats on diet C (0.3% DL-phenylalanine plus 0.6% L-tyrosine). A mean nitrogen efficiency of only 0.80 was found in the rats which received diet C during the pair-feeding period. This figure was increased to 1.13 during the ad libitum feeding period.

A mean nitrogen efficiency of -0.39 was observed in the rats which received no phenylalanine. This low value was less severe during Period II than during Period I.

A significantly higher nitrogen efficiency was observed in the rats on diet A than in the rats on the control diet, but no differences were noted among the rats on diets B, C, and the control.

### Body Composition

Table VII shows the gains or losses in body nitrogen and fat for each group of rats for the 60-day period. These values are the difference between the final nitrogen and fat content of the carcass of each rat and the mean nitrogen and fat content of the carcasses of the six rats killed at the beginning of the study. Rats on diet A (0.9% DL-phenylalanine plus 0.6% L-tyrosine) showed a mean gain in body nitrogen of 5.27 gm. and in body fat of 12.03 gm. Slightly lower gains were made by the

TABLE VII  
GAINS IN BODY NITROGEN AND FAT OF RATS  
DURING THE 60-DAY EXPERIMENTAL PERIOD

Code	Diet		Rat Code	Body Gain	
	DL-phenylalanine	L-tyrosine		In Nitrogen	In Fat
	%	%		gm.	gm.
A	0.9	0.6	A <sub>1</sub>	5.31	12.79
			A <sub>2</sub>	4.79	10.95
			A <sub>3</sub>	5.83	12.90
			A <sub>4</sub>	4.73	10.13
			A <sub>5</sub>	5.70	13.37
			Mean	5.27	12.03
			S.D. <sup>3</sup>	0.50	1.41
B	0.6	0.3	B <sub>1</sub>	4.83	8.70
			B <sub>2</sub>	5.37	9.30
			B <sub>3</sub>	5.29	10.75
			B <sub>4</sub>	5.13	9.73
			B <sub>5</sub>	4.41	6.57
			Mean	5.01	9.01
			S.D.	0.39	1.55
C	0.3	0.6	C <sub>1</sub>	4.52	10.05
			C <sub>2</sub>	5.16	7.50
			C <sub>3</sub>	6.08	10.20
			C <sub>4</sub>	4.99	13.50
			C <sub>5</sub>	5.08	7.43
			Mean	5.17	9.74
			S.D.	0.57	2.49
D	0	0.9	D <sub>1</sub>	-1.37	-1.50
			D <sub>2</sub>	-1.31	-1.95
			D <sub>3</sub>	-1.61	-2.23
			D <sub>4</sub>	-1.40	-1.62
			D <sub>5</sub>	-0.98	-1.79
			Mean	-1.33	-1.82
			S.D.	0.22	0.28
E-Control	0.69	0.69	E <sub>1</sub>	4.39	9.01
			E <sub>2</sub>	4.66	10.33
			E <sub>3</sub>	4.17	6.34
			E <sub>4</sub>	4.36	8.87
			E <sub>5</sub>	4.00	7.02
			Mean	4.32	8.31
			S.D.	0.24	1.61

<sup>3</sup>Standard Deviation

rats on diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine), and the control diet to average losses of 1.33 gm. of nitrogen and 1.82 gm. of fat by the rats which received no phenylalanine.

## CHAPTER IV

### DISCUSSION

#### Weight Gains and Food Intakes

From the data on weight gain presented in Table V and Figure 1, it appears that the levels of phenylalanine and tyrosine fed in diet A (0.9% DL-phenylalanine plus 0.6% L-tyrosine), diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine), and diet C (0.3% DL-phenylalanine plus 0.6% L-tyrosine) are adequate to maintain growth of weanling rats. The levels fed in diet A supported the greatest growth. Lower gains in weight were found with lower levels of phenylalanine intake; and there were marked weight losses when this amino acid was omitted.

During the pair-feeding period, retarded weight gains from Days 1 to 10 and little or no gains from Days 10 to 22 by all of the rats resulted from the low food intake. In order to avoid further inhibition of growth of the rats which received adequate diets, ad libitum feeding was allowed for the remainder of the study. Immediate increases in food intake and weight, as shown in Table V and Figure 1, occurred in all of the rats which received diets that contained phenylalanine.

The data on the percentage of weight gained by the rats on the various diets as compared to the weight gained by the rats on the control diet show that diet A was superior to the other diets. There were no significant differences between diets B and C when considered for the total 60-day period. However, during pair-feeding, the rats which consumed

the lowest level of phenylalanine, diet C, showed significantly lower weight gains than the rats which received diets A and B. When the rats on diet C were allowed food ad libitum in Period II, the food intakes rose, and the daily weight gains doubled. From these data, it appears that the caloric needs for normal growth are increased when the phenylalanine intake is below optimum levels.

#### Food Efficiency

The results of the food efficiency of the rats on all of the diets do not show values as high as those reported in the literature for male weanling rats. However, no figures for female weanling rats were available. The low food efficiency reported here for the rats which received casein, as well as for the rats which received purified amino acids in their diets, indicates that something other than purified amino acids as a nitrogen source led to this poor food utilization.

#### Nitrogen Retention

Even though the rats on diet D were sick and one died because of a phenylalanine deficiency, they were never in negative nitrogen balance. Bothwell and Williams (3, 4, 5) and Denton, Williams and Elvehjem (6) have had difficulty in obtaining negative nitrogen balances in young animals and have found positive balances appearing until death from lysine and histidine deficiencies. Rose, Smith, Womack and Shane (18) have shown that the exclusion of any one or all of the ten amino acids essential for the growing rat exerts no demonstrable effect upon nitrogen balance. Thus, maintenance of nitrogen balance is no guarantee of protein adequacy



in growing rats. The hypoproteinemic rat may be in nitrogen balance on amino acid deficiencies but continue to lose weight and finally succumb.

The nitrogen intakes were almost identical for the rats on diet A (0.9% DL-phenylalanine plus 0.6% L-tyrosine), diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine), and diet C (0.3% DL-phenylalanine plus 0.6% L-tyrosine). Less nitrogen, however, was excreted by the rats on diet C. This indicates a need by the body, during a lowered intake of this essential amino acid, for more nitrogen to produce gains equal to those found on higher intakes of phenylalanine.

As the nitrogen intakes and the nitrogen retentions of the rats on all of the diets follow almost exactly the same trends, it appears that fluctuations in the nitrogen retentions were due to the amount of diet consumed. Therefore, variations in the nitrogen intakes must cause fluctuations in the nitrogen retentions. It appears then that the level of nitrogen in the diet, as well as the actual amount of food consumed, must be considered when observing the effects of an amino acid deficiency on the nitrogen retention of the growing rat (3, 4, 5).

#### Nitrogen Efficiency

The significantly higher nitrogen efficiency by the rats which received the highest level of phenylalanine shows the superiority of this diet to the diets which supplied lower levels of phenylalanine. In order to build body tissues to the fullest advantage, it appears that a level of 0.9% DL-phenylalanine is desirable.

In spite of continued, although slight, positive nitrogen balances by the rats which received no phenylalanine, the figure for nitrogen efficiency was a negative one, -0.39. It appears that, because of the

absence of an essential amino acid from the diet, these rats made poor use of the nitrogen supplied to them.

### Body Composition

The results which show the gains in body nitrogen and fat again point out the superiority of the 0.9% level of phenylalanine. Fat constituted a greater percentage of the gain of the rats on diets A, B, and C than of the rats on the control diet. However, the bodies of all of the rats which received phenylalanine in their diets contained approximately 12% fat.

The low values for gains in both body nitrogen and fat by the rats on the control diet indicate some failure of this diet to supply food value comparable to that supplied by the diets which contained purified amino acids.

### Utilization of Purified Diets

As was previously noted, greater total weight gains were found in the rats which received the three purified diets that contained phenylalanine than in the rats which received the control diet. The 13% to 34% greater weight gain per gram of food consumed by the rats on the purified diets shows more efficient utilization of the purified diets than of the casein diet.

The nitrogen intakes and balances of the rats which consumed diets A, B, and C were also higher than those of the rats on the control diet. Nitrogen retention by the control rats decreased throughout the study, even when the nitrogen intake increased. Apparently less nitrogen was being utilized for growth by the control rats. This manifested itself



in a slower growth rate than that found in the rats which received purified amino acids in their diets.

The rats which received the purified diets excreted a significantly lower amount of nitrogen in the feces (6.3 mg. per day) than the animals which consumed the control diet (10.9 mg. per day). No significant differences were seen in the nitrogen content of the urines. Therefore, values for apparent digested nitrogen are greater for the purified diets than for the control diet.

A greater amount of body nitrogen and fat was stored by the rats which received the purified diets adequate in the essential amino acids than by the rats on the casein diet.

In contrast, then, to the results reported by Maddy and Swift (11), the rats in the present study which received purified diets adequate in the essential amino acids showed greater average weight gains, more efficient utilization of the diet, and deposition of more nitrogen and fat in the body than the control animals which had a comparable amount of amino acids from casein. It is possible that the lysine and/or tryptophan content of the casein may have contributed to its poorer utilization by the rats.

Greater weight gains, nitrogen efficiency, and more deposition of nitrogen and fat in the body of the animals which received 0.9% phenylalanine indicates the higher quality of a diet in which optimum amounts of an essential amino acid are fed.

### Conclusions

From the data presented in Tables V, VI, and VII, it appears that the levels of phenylalanine and tyrosine fed in diet A (0.9% DL-phenylalanine

plus 0.6% L-tyrosine), diet B (0.6% DL-phenylalanine plus 0.3% L-tyrosine), diet C (0.3% DL-phenylalanine plus 0.6% L-tyrosine), and diet E (0.7% L-phenylalanine plus 0.7% L-tyrosine) are adequate to maintain growth and nitrogen balance of the weanling rat when ad libitum feeding is allowed. There is some question whether 0.3% DL-phenylalanine plus 0.6% L-tyrosine would be adequate if the rats were on a limited food intake.

The level of 0.3% DL-phenylalanine plus 0.6% L-tyrosine shows a 67% sparing action by tyrosine for phenylalanine. Previous work with rats by Rose (25) suggests a 50% sparing action. Rose and Wixom's work with young men (19) indicates a 75% sparing action by tyrosine for phenylalanine in humans. Feeding lower levels of phenylalanine will lead to finding the maximum sparing action by tyrosine for phenylalanine in the growing rat.

Loss in weight, low food intake, poor food efficiency, low nitrogen utilization, and abnormal physical manifestations give ample proof of the inadequacy, for the weanling rat, of a diet which contains no phenylalanine.

It appears from this work that the utilization of the nitrogen and energy derived from free amino acids in adequate amounts is more efficient than the utilization of the nitrogen fed in the form of intact casein. The utilization of the nitrogen and energy is also more efficient when phenylalanine is fed at a level of 0.9% of the diet than when it is fed at a level of 0.6% or 0.3% of the diet.

No known deficiency levels of any amino acid, except a diet devoid of phenylalanine, were fed in this experiment. In the absence of this essential amino acid, extremely poor utilization of the diet and losses of body nitrogen and fat appeared. The effects of varying degrees of deficiencies of essential amino acids on the utilization of the diet remain to be determined.

### Suggestions for Future Study

Further studies which are indicated on the basis of the data reported in this thesis would be the determination of the effect of feeding levels lower than 0.3% DL-phenylalanine plus 0.6% L-tyrosine. It is believed that such work would reveal the maximum sparing action by tyrosine for phenylalanine in the growing rat. A diet which supplies no phenylalanine and a casein control diet might again be fed to further substantiate the findings of the present study.

Such studies might also include experimentation to obtain data on nitrogen and energy balances and on amino acid excretions in order to provide a more complete picture of the metabolic fate and utilization of these diets. These data would also be helpful in further assessing the metabolic interrelationships of phenylalanine and tyrosine.

## CHAPTER V

### SUMMARY

The purpose of the work reported here was to study further the quantitative requirement of phenylalanine and the replacement value of tyrosine for phenylalanine in the growing rat. Data on the efficiency of diets which contained casein as compared with diets which contained amino acids as the source of nitrogen, with adequate and inadequate amounts of phenylalanine, were also sought.

The methods used to accomplish the purposes involved: 1) the use of weanling rats as experimental subjects, 2) feeding these animals four purified diets which contained different levels of phenylalanine plus a fifth diet which contained casein and served as a control, and 3) studying the growth and nitrogen metabolism of these rats on different diets.

Pair-feeding for 22 days was followed by ad libitum feeding for the remaining 38 days, because the pair-feeding method did not allow the rats which received the more efficient diets to make adequate growth.

Food and weight records, nitrogen intakes and excretions, plus the initial and final body nitrogen and fat contents of the rats were used to study the effects of various levels of phenylalanine and tyrosine on growth, nitrogen retention, and body balance.

The results showed that a level of 0.9% phenylalanine, when L-tyrosine was fed at the 0.6% level, in the diet of the weanling rat

supported the greatest growth. A sparing action by tyrosine for phenylalanine of at least 67% was shown. As no lower levels of phenylalanine were fed, this value may not represent the maximum sparing action by tyrosine for phenylalanine.

The utilization of the nitrogen and energy derived from intact casein and from purified amino acid diets which were low in phenylalanine content was less efficient than the utilization of the nitrogen and energy from a diet which contained 0.9% phenylalanine.

Further work which might be conducted on the basis of these findings would be to determine the maximum replacement value of tyrosine for phenylalanine and to secure more information on the utilization of diets which are deficient in essential amino acids.



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