STUDIES ON THE THREONINE-TRYPTOPHAN AMINO ACID IMBALANCE: CONVERSION OF TRYPTOPHAN INTO CELLULAR TRYPTOPHAN AND NIACIN

By

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Bachelor of Science

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1957

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
September, 1959

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ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr.'s Duane A. Benton, LaVell M. Henderson, and Franklin R. Leach for their counsel throughout this investigation. He also wishes to thank the National Institute of Health for the financial support of this research, the Department of Biochemistry for supplying laboratory facilities, and the members of the Department of Biochemistry for their valuable assistance. Special thanks are due to Carol Lewis, Lousia Bane, and Mrs. Jordon Tang for analyses.

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INTRODUCTION

Elvehjem (1) has suggested three classes of amino acid imbalance. 1) The amino acid may be an antagonist of a structurally similar amino acid. When the amount of the first amino acid is increased in the diet, a similar increase in the amount of the second amino acid is required to prevent the development of an amino acid imbalance and the resulting growth depression. Usually this type of imbalance requires levels of amino acids far above the dietary requirements. 2) Certain amino acids, for example, methionine (2) and tyrosine (3), cause a specific toxic effect when their level is increased. This toxic effect cannot be reversed by structurally similar amino acids or single amino acids. 3) This type of an amino acid imbalance occurs when the level of the growth-limiting amino acid is increased and this increase precipitates the deficiency of the next most limiting amino acid. The increase in concentration of the specific amino acid required to produce this imbalance is very small. In the well established cases of this type, one of the limiting amino acids serves a function other than protein synthesis.

The study reported in this thesis involves only the last type of amino acid imbalance, which may be called an amino acid imbalance at physiological concentrations. Krehl *et al.* (4) have demonstrated that the 9% casein diet is limiting in cystine or methionine and threonine as well as tryptophan. The addition of 0.2% cystine leaves threonine

as the most limiting amino acid with tryptophan as the next most limiting. These workers also demonstrated that tryptophan can be utilized for both protein synthesis and pyridine nucleotide formation. Hankes et al. (5) have suggested that on the 9% casein diet certain amino acids are the factors which limit growth and that the amount of tryptophan present is sufficient to supply the needs of protein and niacin synthesis for a slow rate of growth. Addition of threonine causes a stimulation of protein synthesis which then results in the depletion of the next most limiting amino acid, tryptophan, and precipitates a niacin deficiency, since the amount of tryptophan is not sufficient to support both protein and niacin synthesis at the increased growth rate.

The purpose of the study reported here was to determine the effect of the threonine amino acid imbalance on the tryptophan-niacin interrelationship using isotopically labeled tryptophan.

HISTORICAL

In 1937 Elvehjem and coworkers (6) demonstrated the nutritional significance of nicotinic acid when they reported that it would cure canine blacktongue. Later in that year Spies et al. (7), Fouts et al. (8) and Smith et al. (9) almost simultaneously published results indicating that nicotinic acid would cure pellagra in man. These results, at least on the surface, appeared to establish that pellagra in man and blacktongue in the dog were caused by a deficiency of niacin in the diet.

After the development of a microbiological method for the determination of niacin by Snell and Wright (10), food and diets were analyzed for their niacin content. Much to the surprise of these investigators it was found that the corn was not particularly low in niacin, in fact, it contained more niacin than milk which had long been used for the treatment of pellagra.

In view of the contradiction of the niacin level in diet and the effect of pellagra, Krehl and coworkers looked for the complicating factor(s). The addition of corn grits to replace 60% of the sucrose in the diets of dogs was found by Krehl et al. (11) to increase the niacin requirement threefold in order to maintain the same growth rate. When the corn grits supplementation was reduced to 36%, the niacin requirement was less. These results establish that addition of corn increases the amount of niacin required to prevent pellagra and blacktongue.

Rats do not normally require a dietary source of niacin; however, Krehl et al. (12) demonstrated a growth response with the addition of niacin when rats were fed the usual niacin-free diet (70% sucrose, 15% casein, 3% corn oil, 5% salts IV (13) and 0.2% cystine) supplemented to the extent of 40% of the entire ration with corn or corn grits. A significant response was obtained with 0.4 mg. %, while 1.5 mg. % niacin was required to obtain the maximum growth rate for this diet. At this point it had been established that in two experimental species high levels of corn would produce a niacin deficiency.

Corn is known to be deficient in lysine and tryptophan and when Krehl et al. (4) added these amino acids to the diet, they found that as little as 50 mg. % of L-tryptophan produced a growth response while lysine was without effect. Thus Krehl and coworkers demonstrated the interchangeability of niacin and tryptophan in supporting growth on these corn diets. Other sources of amino acids, such as acid hydrolyzed fibrin, egg albumin and casein and wheat gluten, all of which are deficient in tryptophan, caused a growth suppression when added to a 9% casein diet (14). In 1947 Henderson et al. (15) demonstrated that this growth suppression was not a result of the natural proteins or some degradation product, when they produced the growth suppression by adding crystalline amino acids at the level as present in 2% acid hydrolyzed casein. The addition of tryptophan to this diet completely alleviated this effect.

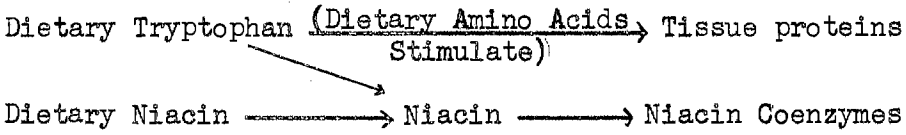
Krehl et al. (16) found that addition of 6% gelatin or 3% zein to the 9% casein diet caused a decrease in the growth rate which could be reversed by the addition of either niacin or tryptophan. From the above results and the fact that the actual amount of tryptophan required for

maximum growth varies with the source of amino acid, Krehl et al. (19) postulated that corn exerts its effect on a low-niacin diet because the amino acid imbalance of the major protein of corn, that is, zein.

Hankes et al. (17) demonstrated that the amino acids which were most effective in producing the niacin-tryptophan deficiency with 9% casein diets were threonine and to a lesser extent phenylalanine. Singal et al. (18) confirmed the above findings with the additional observation that there was not a decreased amount of stored niacin in the liver or muscle during the growth inhibition.

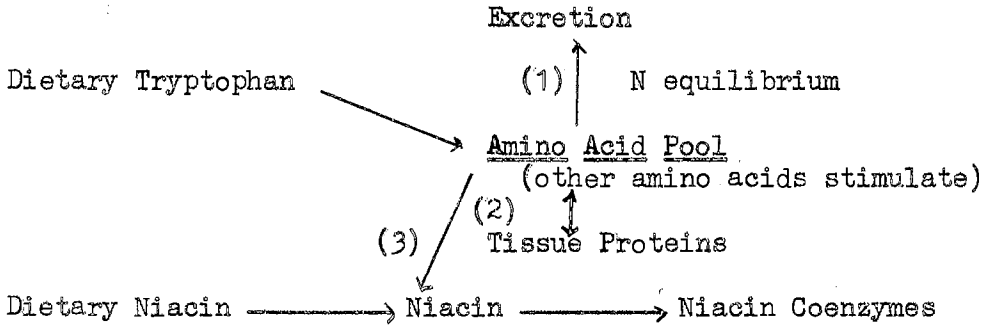
If the amino acid imbalance, per se, is responsible for the production of niacin deficiency, then an inhibition of growth would be produced by any of the essential amino acids when they are raised from the status of the most limiting amino acid to an adequate amount, leaving tryptophan as the most limiting amino acid. Henderson et al. (19) found that a dietary level of 0.10 to 0.11% of DL tryptophan would be marginal for the rats. With this level of tryptophan on a 18% amino acid-purified diet Koeppe and Henderson (20) were able to demonstrate that an increase in the level of lysine, leucine, isoleucine, valine or threonine from marginal to adequate or less marginal amounts made tryptophan the most limiting amino acid which produced a niacin deficiency and growth suppression.

The reaction sequence for the conversion of tryptophan to niacin in rats and Neurospora is known (21). Hankes et al. (5) considered that the two-purpose role of tryptophan, shown in the following scheme, is responsible for its interrelation to the niacin and the amino acids.



Tryptophan is thought to be directed to both routes under normal conditions or when some other dietary factor is limiting growth; however, when tryptophan becomes the most limiting amino acid on a relatively low-niacin diet, then the quantities of niacin produced from tryptophan is not adequate to maintain growth. An increase in amino acids stimulates protein synthesis and draws the tryptophan away from niacin synthesis and into protein synthesis. This causes a decrease in growth rate resulting from a niacin deficiency which can be relieved by increasing either tryptophan or niacin.

However, other investigators (22, 23) have reported that niacin does not completely reverse the growth depression caused by an amino acid imbalance which make tryptophan the most limiting amino acid. This observation coupled with the observation that there was a rise in the excretion of the tryptophan in the urine of rats suffering from an amino acid imbalanced diet in which tryptophan was the most limiting amino acid led Chaloupka et al. (24) to propose the following scheme with three functions for dietary tryptophan. These authors



believe that the maintenance of the nitrogen balance is the most critical factor in the tryptophan deficient animal. Thus under an amino acid imbalance there was a large non-discriminate excretion of amino acids in an attempt to maintain the nitrogen equilibrium. This could

be reversed by the addition of small quantities of tryptophan. The second way in which tryptophan could be utilized was the synthesis and repair of tissue proteins. Only after the first two requirements have been met can tryptophan be converted to niacin. However, the authors do not differentiate between the conditions of positive nitrogen balance and growth.

The purpose of the experiments reported in this thesis was to delineate which reaction(s) in an amino acid imbalanced animal was concerned with the increased requirement for tryptophan or niacin. The general experimental design was to produce an amino acid imbalance by increasing the level of threonine to where it was no longer the limiting amino acid in the low-niacin, 0.10% DL-tryptophan diet and to determine the metabolic fate of isotopically labeled tryptophan under these dietary conditions.

MATERIALS AND METHODS

Care of Animals

Weanling rats from the Holtzman Animal Company weighing between 35 and 45 gm., average weight 40 gm., were used in these experiments. For not more than three days after their arrival the rats were given free access to Purina stock chow and water, then they were transferred to one of the diets listed in Table I. The animals were housed in metal screen bottom cages in the Home Economics Department's small animal room which was kept at 78° Fahrenheit. The rats were fed ad libitum and weighed daily.

In order to be certain that the rats received all of the radioactive tryptophan it was fed to them by a stomach tube with a portion of the normal diet. The level of the unlabeled tryptophan in the diet was adjusted so that the rats received a total of 0.10% DL-tryptophan. It was shown that rats of the size used in these experiments were killed when fed four or more grams of the diet by a stomach tube. In the subsequent experiments two grams of diet was given to each animal by this method.

Radioactive Compounds

Tritium Labeled Tryptophan

Tritium labeled tryptophan was prepared by the Wilzbach procedure (25) in the laboratories of the University of Illinois and at Oklahoma

TABLE I

Experimental Diets

All diets had the following composition:

Amino Acid Mixture (20) (minus threonine and tryptophan)	18.00%
Vitamin Mixture (20) (niacin free)	2.00%
Salts IV (13)	4.00%
Corn Oil	5.00%
Sucrose	71.00%
Total	100.00%

Dietary supplements:

	0.8% DL-threonine	0.6% DL-threonine	Complete
DL-tryptophan	0.10	0.10	0.20
DL-threonine	0.8	0.6	1.80

Fat soluble vitamins were fed weekly.

State University. Paper chromatography of the tritium-labeled tryptophan in pyridine, methyl alcohol and water (4:80:20) showed a single ninhydrin positive spot of R_f 0.6. By assaying the developed chromatograms with the automatic open window chromatogram scanner it was noted that there were three radioactive areas; (1) a spot at the origin, (2) the tryptophan, and (3) a spot at the solvent front. The material at the origin was insoluble in both polar and non-polar solvents and thus was apparently irreversibly adsorbed on the paper. The tryptophan spot was eluted from a strip of paper by washing with water and when rechromatographed, it contained the same impurities as shown in the first chromatogram although in lesser quantities. The original tryptophan was used without further purification. The specific activity of the tryptophan used was 36.5 mc/mM.

Tryptophan-7a-C^{14}

Carbon-14 labeled tryptophan prepared from aniline-1-C^{14} (26) was obtained from Dr. L. M. Henderson. The recrystallized product, specific activity 153uc/mM., contained no radioactive impurities separable by paper chromatography.

Isolation of Niacin and Tryptophan

Niacin and tryptophan were released from whole rat livers by 12 to 18 hours autoclaving (15 psi) in three volumes of 3.0 N NaOH. The hydrolyzate was neutralized with 3.0 N HCl, filtered and made up to a total volume of 50 ml. The niacin and tryptophan present in the hydrolyzate were determined by microbiological assay on a 10 ml. aliquot. To the remaining solution 100 mg. of nicotinic acid and 200 mg. of DL-tryptophan were routinely added to serve as carrier.

The solution was made to approximately 0.3 N in HCl by the addition of 1 ml. of concentrated HCl. Five grams of Lloyd's reagent was then added to adsorb the pyridine like compounds. The suspension was centrifuged and the clear supernatant decanted. A total volume of 150 ml. of 0.3 N HCl in three 50 ml. fractions was used to remove all of the tryptophan from the Lloyd's reagent. The adsorbed pyridine compounds were then eluted with 150 ml. of 3.0 NaOH in three 50 ml. portions.

The tryptophan containing solution was neutralized immediately to eliminate the danger of decomposition of the tryptophan by the acid and the volume of the solution was reduced to less than 50 ml. under reduced pressure on a rotary evaporator. The solution was made to 50 ml. and 5% acetic acid by the addition of 2.5 ml. of concentrated acetic acid. This solution was then passed through a charcoal column which had been prepared by the saturation of 5 grams of Darco-G-60 charcoal with glacial acetic acid. The tryptophan was adsorbed along with other amino acids from the 5% acetic acid solution. The column was washed with 2% pyridine, 5% acetic acid until the eluate was ninhydrin negative which usually required 100 ml. to remove all of the amino acids except tryptophan. The tryptophan was then eluted from the column by the addition of 10% phenol, 20% acetic acid solution. Usually 150 ml. of this eluant was required to completely remove the tryptophan as judged by the Hopkins Cole test (27).

The tryptophan fractions were then extracted with diethyl ether which removed the phenol and most of the acetic acid. The total volume of the water layer was reduced to 5 to 10 ml. on the rotary evaporator and 20 ml. of concentrated acetic acid were then added. The

volume was again reduced to 5 to 10 ml. and then 25 ml. of dry, redistilled benzene were added to precipitate tryptophan acetate. The precipitate was collected on a sintered glass filter stick, washed with benzene and then ether. The sample was then redissolved by the addition of a small amount of 0.3 N NaOH and the volume was reduced to less than 10 ml. The pure tryptophan was recrystallized from the neutralized solution by the addition of absolute ethyl alcohol until the alcohol content was 65%.

The eluate from the Lloyd's reagent was neutralized with 3.0 N HCl and the volume reduced to 10 ml. The niacin and other alcohol extractable materials were separated from the sodium chloride by their solution in ethyl alcohol, filtering, reducing the solution to dryness, and then repeating the process. The alcohol solution was reduced to dryness and a small amount of water was added to dissolve the solids.

The water solution was placed on a 1 x 10 cm. Dowex-50 x 8 column in the H ion form. Then the column was washed with 0.1 N HCl and the nicotinic acid eluted with 200 ml. of 0.3 N HCl as determined by the cyanogen bromide test (28). The nicotinic acid solution was reduced to 10 ml. in vacuo on the rotary evaporator and then transferred to a sublimation apparatus in which the solution was reduced to dryness. Sublimation was effected at water aspirator vacuum, 110° and for 12 hours. The melting point of the sublimate was the same as the melting point of pure nicotinic acid.

Radioactive Determinations

Analysis of the tritium labeled compounds was via the Wilzbach zinc-fusion method (29), counting the gas in a 250 cc. ionization

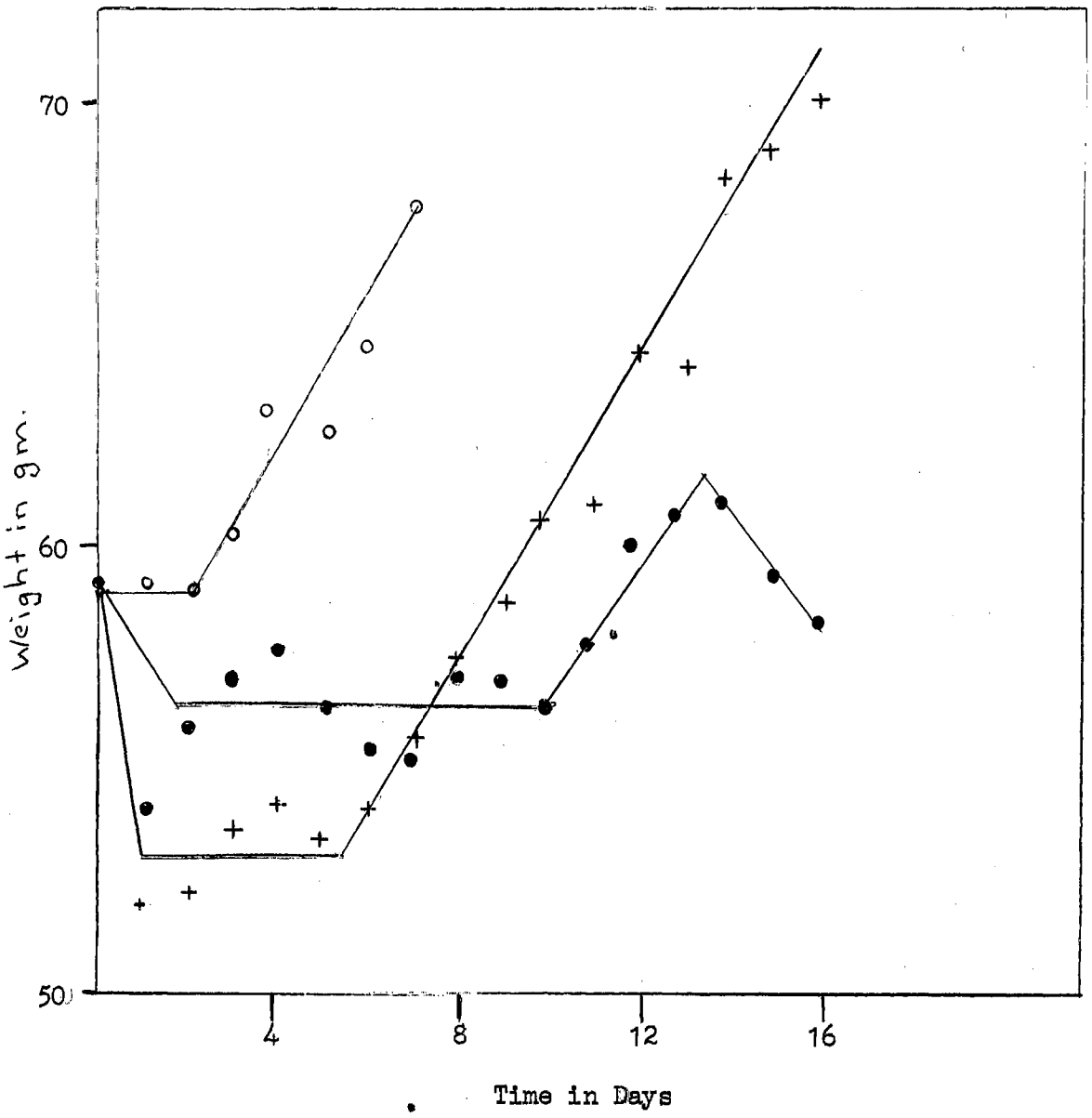
chamber using the model 315 Cary Vibrating Reed Electrometer. Samples of the C^{14} labeled compounds were analyzed by the wet combustion method of Van Slyke, Steel and Plazin (30). The $C^{14}O_2$ was collected in a 250 cc. ionization chamber and counted with the Vibrating Reed Electrometer.

RESULTS

Production of an amino acid imbalance

Figure 1 shows the general growth response of rats on the various diets. When rats are on a balanced diet as shown in curve 1, there is only a slight lag of two days before they become adapted to the diet and then their growth progresses at a rate of 1.7 grams per day. Rats on the 0.6% and 0.8% threonine amino acid-sucrose diets showed an initial loss in weight then a stabilized period of little weight change while they were adapting to the new diet. After adaptation the growth rate on the 0.6% threonine diet was 1.7 gm. per day (curve 2) and on the 0.8% threonine diet was 1.5 gm. per day (curve 3). The rats on the 0.8% threonine diet showed a rapid loss of 1.5 gm. per day after approximately thirteen days had elapsed. This was the growth depression due to the niacin deficiency produced by an amino acid imbalance.

In other experiments it was shown that the growth depression could be reversed by the addition of either tryptophan or niacin to the diets. The experiments reported above establish that when the level of threonine in the amino acid-sucrose diet is increased an amino acid imbalance results which makes tryptophan the limiting amino acid and produces a growth depression. In all subsequent experiments it was established that the animals used were showing growth depression from an amino acid imbalance.


70
60
50
Weight in gm.
4
8
12
16
Time in Days

Radioactive Experiments

Tritium Labeled Tryptophan

Figure 2 shows the growth response of the rats used in this experiment. At the end of eight days the expected amino acid imbalance had been produced and the rats from each of the two diets were divided into two groups. One group was fed the 0.6% threonine diet by stomach tube while the second group was fed the 0.8% threonine diet also by stomach tube. The rats were given 2 grams of diet which contained0.1% labeled DL-tryptophan-H^3, specific activity 18.25 mc/mM, by stomach tube and were sacrificed ten hours after forced feeding. They were anesthetized with ether and killed by heart puncture. The liver was removed and stored under refrigeration until analyzed as described in the Materials and Methods section.

Table II summarizes the results of the analysis of rat liver for tryptophan-H^3 incorporation into tryptophan and niacin. It is seen that the diet had very little effect on the total amount of tryptophan or niacin contained in the liver as determined by microbiological assay. The specific activity of the isolated tryptophan shows that more label was incorporated on the 0.6-0.6% threonine diet than under any other condition. With the rat changed from 0.8 to 0.6% threonine diet, the incorporation of isotope into cellular tryptophan is increased.

C^{14} Labeled Tryptophan

0.75 µc Tryptophan-7a-C^{14}

A similar experiment was performed using tryptophan-7a-C^{14} instead of the tritium labeled compound. Figure 3 shows the growth response of these animals. At the end of the fifteenth day the food was removed, the animals were fasted for 24 hours and the animals on each diet were

Figure 2. Growth Response on the DL-Tryptophan-H^3 Diets.

- ● Average daily body weight of the 2 animals maintained on the .8% DL-threonine diets.
- o Average daily body weight of the 2 animals maintained on the .6% threonine diets.

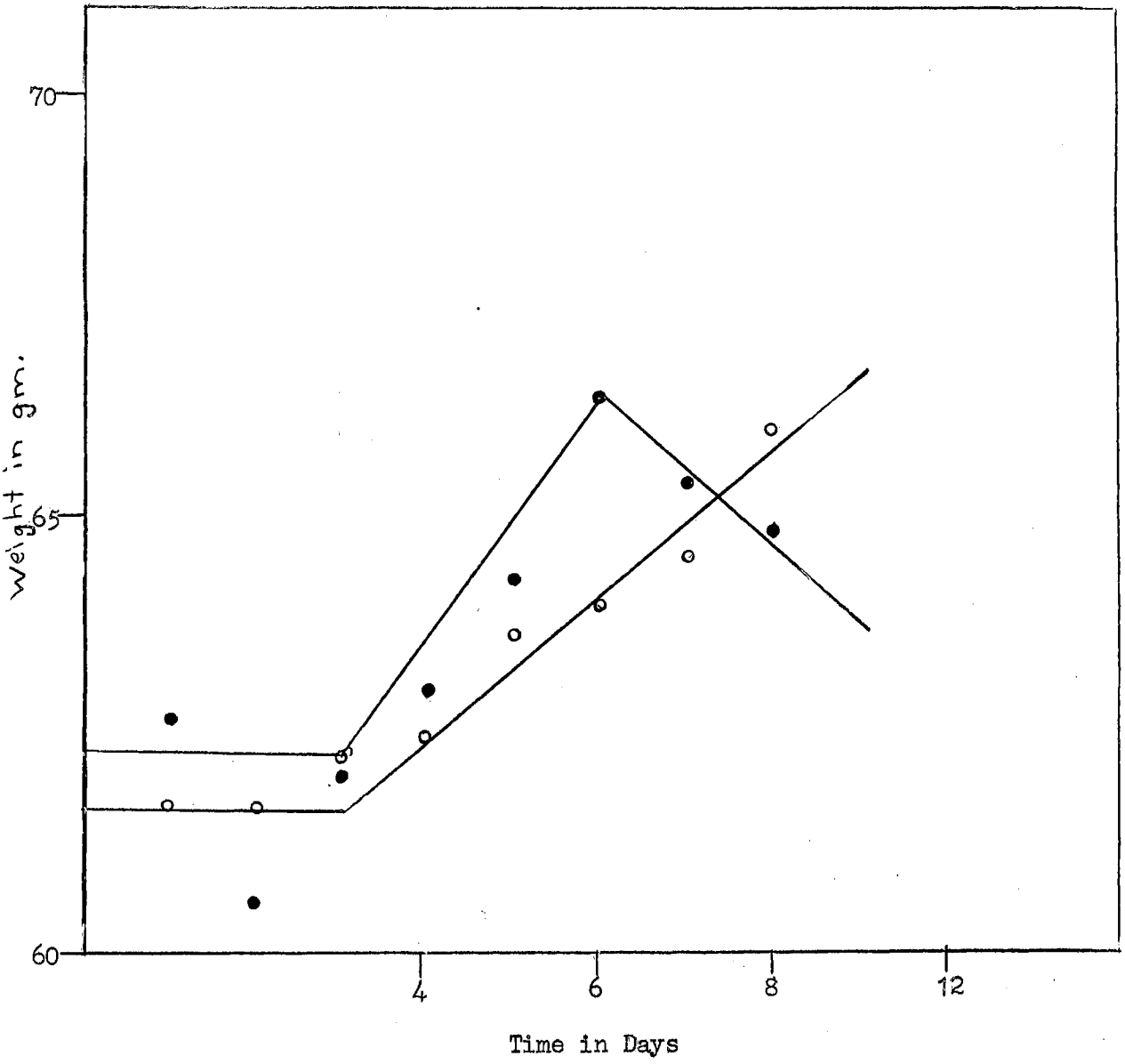

70
65
60
Weight in gm.
4
6
8
12
Time in Days

TABLE II

Tryptophan-H^3 Incorporation into Liver Tryptophan and Niacin
(180.0 µc Tryptophan-H^3)

Dietary Threonine	Liver Weight gm.	Tryptophan mg./liver	Niacin mg./liver	Radioactive Measurement: Tryptophan Count Rate Times Background	Tryptophan µc/mM	Niacin Count Rate Times Background	Niacin µc/mM
0.6-0.6	3.26	4.20	0.273	160*	3,910.0	4	90.8
0.6-0.8	3.02	3.80	0.256	---**	---**	18	183.5
0.8-0.8	2.26	4.00	0.268	16*	94.4	33	299.5
0.8-0.6	2.75	4.90	0.295	32	183.0	29	2,325.0

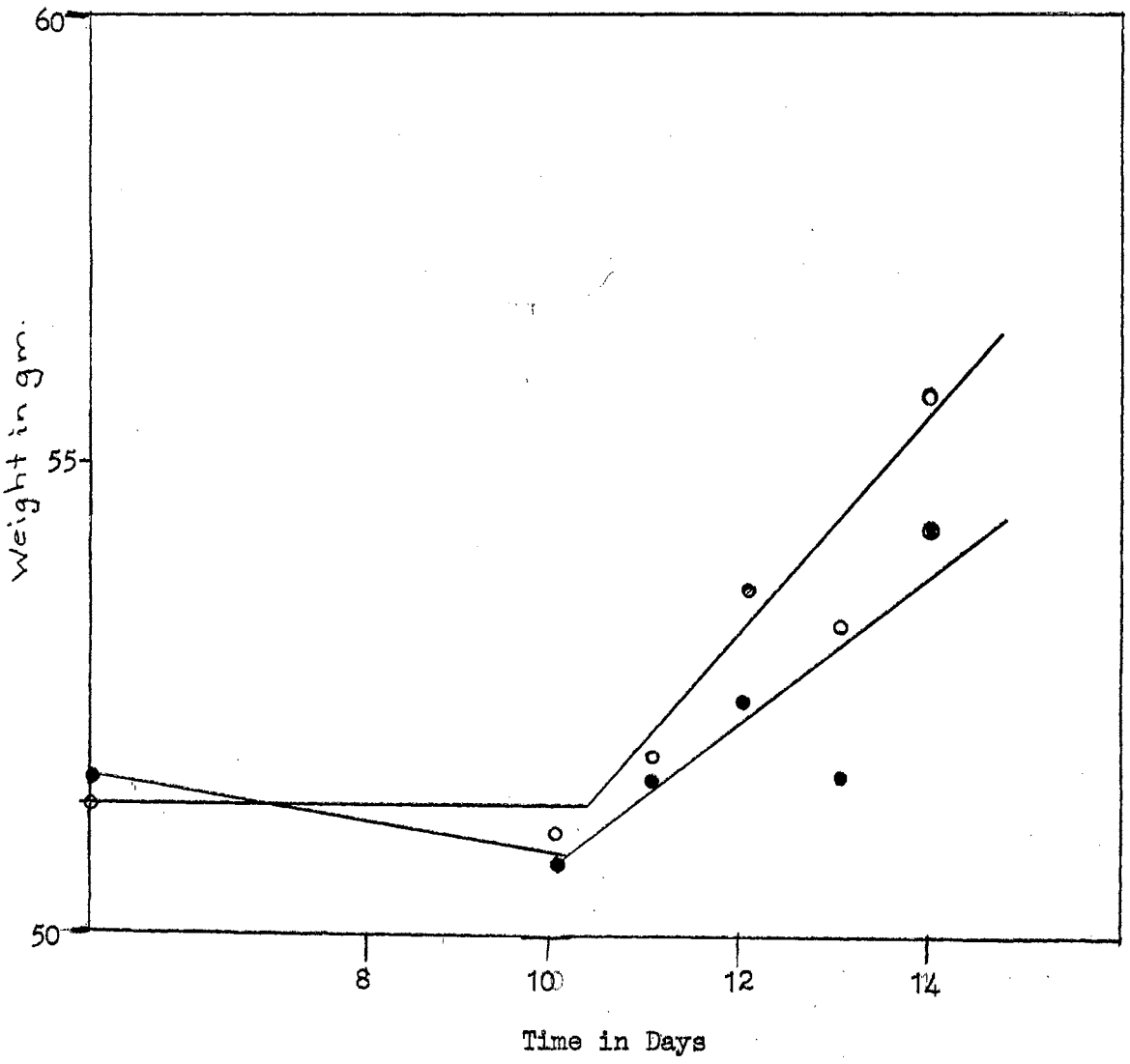
*Samples counted by static method on the vibrating reed electrometer.

**Sample lost in isolation.

Figure 3. Growth Response on the DL-Tryptophan-7a-C^{14} Diets (.75 µc Tryptophan-7a-C^{14}.)

● Average daily body weight of 4 animals maintained on the .8% DL-threonine diets.

o Average daily body weight of 4 animals maintained on the .6% DL-threonine diets.


60
55
50
Weight in gm.
8
10
12
14
Time in Days

divided into two groups which were given 2 gm. of either the 0.6 or 0.8% threonine diet containing .1% DL-tryptophan-7a-C^{14}, specific activity 76.5 μc/mM. The rats were killed by heart puncture after 12 hours, during which time they had free access to water. Blood plasma, thigh muscle, liver and gastro-intestinal contents were collected for analysis. Table III summarizes the results of the analysis of the livers for incorporation of tryptophan-7a-C^{14} into liver tryptophan. The level of radioactivity in the isolated liver niacin, in the niacin and tryptophan of muscle, in the blood plasma, and in the gastro-intestinal contents was not of sufficient magnitude to allow accurate determination; therefore, these results are not presented.

1.5 μc Tryptophan-7a-C^{14}

An experiment similar to the one reported above was done, but the specific activity of the tryptophan was doubled to obtain more incorporated radioactivity. Figure 4 shows the growth responses of these animals and establishes that the amino acid imbalance was produced on the eleventh day. At this time the animals on each diet were divided into two groups which were given either 0.6 or 0.8% threonine diet. The feeding of the diets was staggered over four days to allow the use of the glass metabolism chamber for collection of respiratory carbon dioxide, urine and feces. All rats received two grams of the experimental amino acid-sucrose diet containing 0.1% DL-tryptophan-7a-C^{14}. The animals were in the metabolism chamber for six hours and were then placed in screened bottomed cages for six hours, after which the rats were sacrificed.

Table IV shows the analysis of the liver for the incorporation of the C^{14} labeled tryptophan into the liver tryptophan and niacin.

TABLE III

Incorporation of Tryptophan-7a-C^{14} into Liver Tryptophan
(0.75 μc Tryptophan-7a-C^{14})

Dietary Threonine	Liver Weight gm.	Tryptophan mg./liver	Radioactivity Measurement	
			Counting Rate Times Background	mμc/mM
0.6-0.6	2.58	4.42	5.0	688.0
	2.38	6.42	1.2*	426.5
0.6-0.8	2.97	2.98	8.0	632.5
	2.91	6.94	1.2*	1,995.0
0.8-0.8	2.33	4.88	5.0	477.5
	2.18	5.34	1.2*	216.0
0.8-0.6	2.64	3.85	8.0	283.0
	2.19	6.09	1.1*	183.0

*Due to the low radioactivity of these samples the data are questionable.

Figure 4. Growth Response on the DL-Tryptophan-7a-C^{14} Diets (1.50 μc Tryptophan-7a-C^{14}.)

o Average daily body weight of 4 rats fed on .6% DL-threonine diet.

● Average daily body weight of 4 rats fed on .8% DL-threonine diet.

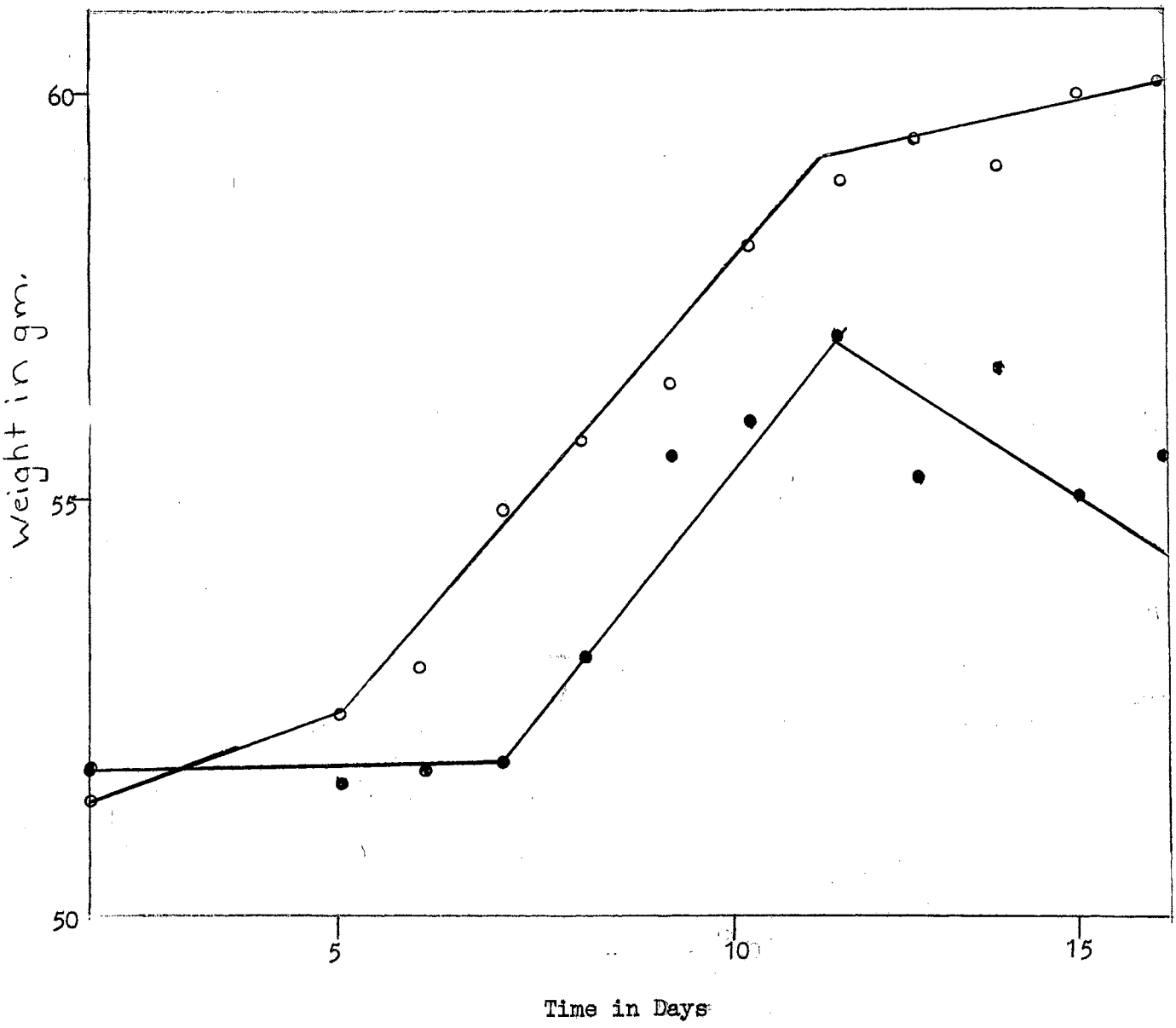

60
55
50
Weight in gm.
5
10
15
Time in Days

TABLE IV

Tryptophan-7a-C^{14} Incorporation into Liver Tryptophan and Niacin
(1.50 μc Tryptophan-7a-C^{14})

Dietary Threonine	Liver Weight gm.	Tryptophan mg./liver	Niacin mg./liver	Radioactivity Measurement			
				Tryptophan		Niacin	
				Count Rate Times Background	mμc/mM	Count Rate Times Background	mμc/mM
0.6-0.6	1.80	2.92	0.214	3.4	2,202.0	2.0	860.0
0.6-0.8	2.25	4.04	0.230	1.6	127.0	2.0	2,575.0
0.8-0.8	1.78	3.65	0.212	1.7	139.0	1.5	1,750.0
0.8-0.6	2.01	2.98	0.218	3.8	1,262.0	1.5	803.0

There was a much greater incorporation of tryptophan into cellular tryptophan when the animal received the 0.6% threonine diet. Table V shows the total amount of radioactivity excreted in the urine of the animals on the various diets. It appears that the metabolic stress of changing diet causes an increased excretion of labeled material.

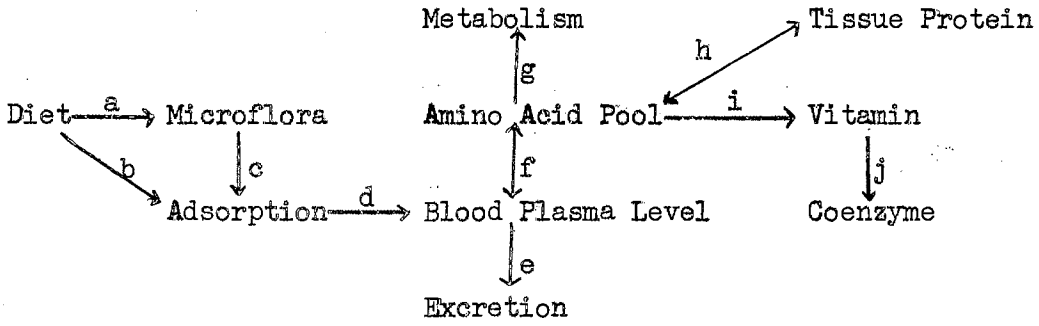
TABLE V

Excretion of Radioactivity from DL-Tryptophan-7a-C^{14} in the Urine (1.50 μc Tryptophan-7a-C^{14})

Dietary threonine	mμc excreted in 12 hours
0.6-0.6	96
0.6-0.8	136
0.8-0.8	54
0.8-0.6	114

DISCUSSION

The actual mechanism by which an amino acid imbalance causes a growth depression has been widely debated and only by a careful consideration of all the data available can a reasonable working hypothesis be proposed. The following scheme illustrates the various factors which have been suggested as possible sites for the manifestations of the imbalance as related to a niacin deficiency.



Studies by other workers have given results which might be interpreted as eliminating certain of the factors of the above scheme from consideration in the mechanism(s) for the effect of the imbalance. Thus Krehl et al. (16), Hall et al. (31), and Tepley et al. (32) have noted that the dietary carbohydrate has definite effects on the amino acid imbalance and growth depression. At first it was thought that this was due to the change in the microflora depending on the carbohydrate used, but recent work by Henderson et al. (33), Schweigert et al. (34), Hundley (35), and Reynier's group at the University of Notre Dame (36) have proven that the microbiological synthesis of niacin

is not the predominate mechanism for the conversion of tryptophan to niacin. Hundley (37) has suggested that the carbohydrate effect was due to requirements of different levels of niacin coenzymes for the metabolism of various sugars.

Although Ebisuzaki et al. in 1952 (38) proposed that threonine was interfering with the adsorption of tryptophan, no direct evidence, pro or con, has been obtained. Earlier Hankes (17) demonstrated a growth depressing effect of D-phenylalanine and attributed this to interference with the adsorption of L-phenylalanine. In the guinea pig, Kamin and Handler (39) noted that various amino acids affected slightly the absorption of other amino acids, but only when they were supplied in large amounts. However, absorption interrelationships of amino acids have been observed in bacteria and the above cited results (39) in no way rule out their occurrence in animals, and only direct experiments can ascertain the contribution of absorption effects to the tryptophan-niacin relationship in an amino acid imbalanced animal.

A correlation between plasma levels of amino acids and their excretion in the urine has been observed by Sauberlich and Salmon (22), Singal (40), and Kamin and Handler (41). These unbalanced diets led to an increase in the excretion of both amino acids and niacin. Kamin and Handler (41) showed that an increase in threonine was the most influential factor on the excretion of other amino acids. Although the results reported in Table V measure the effect of amino acid imbalance on the excretion of radioactivity derived from labeled tryptophan, no trend or significant differences are noted which could not be ascribed to the stimulation of excretion due to metabolic shock. That these animals are sensitive to metabolic shock produced by a change in the

diet is easily seen from the results in Figure 1 which shows that the rats did not gain weight for five days and until the lapse of this time period did not show the typical growth rate after having the diet changed.

Hankes, Henderson and Elvehjem (5) have proposed that the effect of an amino acid imbalance in which tryptophan is made the limiting amino acid is to draw tryptophan into protein synthesis at the expense of niacin formation. The basis for this proposal was the effect of the diets upon the growth of the rat. In Figure 1 are shown our experimental results which are consistent with their hypothesis. The animals responded to the amino acid diets with either 0.8 or 0.6% DL threonine identically until approximately the thirteenth day when the animals on the 0.8% DL-threonine diet showed a rapid loss of weight. This loss could be reversed by the addition of either niacin or tryptophan to the diet. Thus, it appears that the animals are in a niacin deficiency which may be alleviated by either niacin or tryptophan. The hypothesis of Hankes, Henderson and Elvehjem suggest that there would be an increase in reaction h at the expense of reaction i and which could easily be measured using labeled tryptophan (scheme on page 25). Table VI shows the ratio of h to i calculated from the present results. Based upon Hankes' et al. (5) hypothesis the ratio of activity in liver tryptophan to liver niacin would increase on an amino acid imbalanced diet. It is apparent from the results in Table VI that the ratio is not increased, in fact, it is decreased and thus the results of these experiments are not consistent with the hypothesis of Hankes et al. (5).

Table VII summarizes the results of an experiment showing the percent of the isotope which was incorporated into liver tryptophan and

TABLE VI

Ratio of Isotope Incorporation in Tryptophan and Niacin

Dietary Threonine	H^3 Tryptophan / Niacin	Tryptophan-C^{14} Tryptophan / Niacin
0.6-0.6	400.0	21.20
0.6-0.8	---	0.53
0.8-0.8	2.9	0.83
0.8-0.6	0.8	13.00

liver niacin. There is a close correlation, as expected, between the actual growth response of the animals and the incorporation of tryptophan into liver protein. Also, there is an increased formation of niacin under the circumstances of an amino acid imbalance, .8% DL-threonine, It appears that the effect of the imbalance must be in an increased demand for niacin in some other metabolic scheme. To determine the exact mechanism by which an amino acid imbalance causes the niacin deficiency and resulting growth depression, experiments would have to be done which simultaneously measure all of the parameters illustrated in the scheme.

TABLE VII

Percent Incorporation of Labeled Tryptophan into Liver Tryptophan and Niacin

Diet	Isotope	% Incorporation Tryptophan	Niacin
0.6-0.6	H^3	44.700	0.111
	C^{14} (1.50 µc)	2.102	0.100
	C^{14} (.75 µc)	1.980	---
0.6-0.8	H^3	---	0.218
	C^{14} (1.50 µc)	0.157	0.321
	C^{14} (.75 µc)	1.230	---
0.8-0.8	H^3	1.020	0.362
	C^{14} (1.50 µc)	0.166	0.201
	C^{14} (.75 µc)	1.520	---
0.8-0.6	H^3	2.430	3.090
	C^{14} (1.50 µc)	1.225	0.096
	C^{14} (.75 µc)	0.710	---

SUMMARY

Studies on the threonine induced amino acid imbalance were performed in which the conversion of H^3- and C^{14}-labeled tryptophan into cellular tryptophan and niacin was measured. White rats, average weight 40 gm., were grown on low niacin 18% amino acid diets containing either 0.6 or 0.8% DL-threonine and 0.1% tryptophan. After the rats on the 0.8% DL-threonine diet developed the amino acid imbalance, the rats from both diets, were divided into two groups and were then given isotopically labeled tryptophan which replaced an equal quantity of unlabeled tryptophan in two grams of the diets, given by stomach tube. The rats were killed 12 hours after the administration of the diet containing the labeled material and samples of liver, thigh muscle, blood plasma, urine, gastro-intestinal contents and respiratory carbon dioxide were taken for analysis. Isotope analyses are reported for liver niacin and tryptophan, and for urine. All other samples contained so little radioactivity that meaningful interpretation could not be made.

Under the conditions of this study there was no increase in the conversion of labeled tryptophan to liver tryptophan in the rats having this specific amino acid imbalance. Tryptophan incorporation into tissue tryptophan was greater in the growing animals than in the imbalanced animals, but the incorporation of tryptophan into niacin was greater in the imbalanced animals. These results are not consistent with the hypothesis of Hankes, Henderson and Elvehjem, which suggested that growth depression resulted from a shunting of tryptophan

from niacin synthesis to protein synthesis and was the major effect of an amino acid imbalance. The accelerated niacin formation suggests that the amino acid imbalance is resulting in an increased demand for niacin in some other metabolic scheme.

Before a definite mechanism for the production of the niacin deficiency by an amino acid imbalance may be established, simultaneous measurements of dietary intake, absorption, free and bound amino acids in the blood plasma, incorporation into tissue protein, incorporation into tissue niacin, metabolism and excretion of labeled tryptophan and its degradation products must be done.

REFERENCES

1. Elvehjem, C. A., Fed. Proc., 15, 965 (1956).

2. Harper, A. E., Ann, New York Acad. Sci., 69, 1025 (1958).

3. Harper, A. E., D. A. Benton, and C. A. Elvehjem, Arch. Biochem., 57, 1 (1955).

4. Krehl, W. A., L. J. Tepley, P. S. Sarma, and C. A. Elvehjem, Science, 101, 489 (1945).

5. Hankes, L. V., L. M. Henderson, C. A. Elvehjem, J. Biol. Chem., 180, 1027 (1949).

6. Elvehjem, C. A., R. J., Madden, F. M. Strong, and D. W. Woolley, J. Am. Chem. Soc., 59, 1767 (1937).

7. Spies, T. D., C. Cooper, and M. A. Blankenhorn, J. Am. Med. Assoc., 110, 622 (1938).

8. Fouts, P. J., D. M. Helmer, S. Lepkovsky, and T. H. Jukes, Proc. Soc. Exptl. Biol. Med., 37, 405 (1937).

9. Smith, D. T., J. M. Ruffin, and S. G. Smith, J. Am. Med. Assoc., 109, 2054 (1937).

10. Snell, E. E. and L. D. Wright, J. Biol. Chem., 140, 535 (1941).

11. Krehl, W. A., L. J. Teply, and C. A. Elvehjem, Proc. Soc. Exptl. Biol. Med., 58, 334 (1945).

12. Krehl, W. A., L. J. Teply, and C. A. Elvehjem, Science, 101, 283 (1945).

13. Hegsted, D. M., R. C. Mills, C. A. Elvehjem, and E. B. Hart, J. Biol. Chem., 138, 459 (1941).

14. Krehl, W. A., L. M. Henderson, De La Huerga, and C. A. Elvehjem, J. Biol. Chem., 166, 531 (1946).

15. Henderson, L. M., T. Deodhar, W. A. Krehl, and C. A. Elvehjem, J. Biol. Chem., 170, 261 (1947).

16. Krehl, W. A., P. S. Sarma, and C. A. Elvehjem, J. Biol. Chem., 162, 403 (1946).

17. Hankes, L. V., L. M. Henderson, W. L. Brickson, and C. A. Elvehjem, J. Biol. Chem., 174, 873 (1948).

18. Singal, S. A., V. P. Sydenstricker, and J. M. Littlejohn, J. Biol. Chem., 176, 1063 (1948).

19. Henderson, L. M., O. J. Koeppe, and H. H. Zimmerman, J. Biol. Chem., 201, 697 (1953).

20. Koeppe, O. J. and L. M. Henderson, J. Nutr., 55, 23 (1955).

21. Fruton, J. S. and S. Simmons, General Biochemistry. New York: John Wiley and Sons, 1958, p. 835.

22. Salmon, W. D., Arch. Biochem. and Biophys., 51, 30 (1954).

23. Sauberlich, H. E. and W. D. Salmon, J. Biol. Chem., 214, 463 (1955).

24. Chaloupka, M. M., J. N. Williams, Jr., May S. Reynolds, and C. A. Elvehjem, J. Nutr., 63, 361 (1957).

25. Wilzbach, K. E., A. R. Van Dyken, and L. Kaplan, Anal. Chem., 26, 880 (1954).

26. Henderson, L. M., D. R. Rao, R. F. Nystrom, in C. Vestlinger, Biochemical Preparations 6, 90 (1958).

27. Hopkins, F. G. and S. W. Cole, J. Physiol., 27, 418 (1902).

28. Waisman, H. A. and C. A. Elvehjem, Ind. and Eng. Chem., Anal. Ed., 12, 221 (1941).

29. Wilzbach, K. E., L. Kaplan, and W. G. Brown, Science, 118, 522 (1953).

30. Van Slyke, D. D., J. Plazin, and J. R. Weisigen, J. Biol. Chem., 191, 299 (1951).

31. Hall, W. K. and V. P. Sydensticker, Arch. Biochem., 12, 147 (1947).

32. Teply, L. J., W. A. Krehl, and C. A. Elvehjem, Am. J. of Physiol., 148, 91 (1947).

33. Henderson, L. M. and L. V. Hankes, Proc. Soc. Exptl. Biol. Med., 70, 26 (1949).

34. Schweigert, B. S., J. M. McIntire, L. M. Henderson, and C. A. Elvehjem, Arch. Biochem., 6, 403 (1945).

35. Hundley, J. M., Fed. Proc., 8, 386 (1949).

36. Luckey, T. D., J. R. Pleasants, M. Wagner, H. A. Gordon, and J. A. Reyniers, J. Nutr., 57, 169 (1955).

37. Hundley, J. M., J. Biol. Chem., 181, 1 (1949).

38. Ebisvzaki, K., J. N. Williams, and C. A. Elvehjem, J. Biol. Chem., 198, 63 (1952).

39. Kamin, H. and P. Handler, Am. J. Physiol., 169, 305 (1952).

40. Singal, S. A., A. P. Briggs, V. P. Sydenstricker, and J. M. Littlejohn, Fed. Proc., 5, 154 (1946).

41. Kamin, H. and P. Handler, Am. J. Physiol., 164, 654 (1951).

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