Amino Acid Imbalance [1] and Tryptophan-niacin Metabolism

I. EFFECT OF EXCESS LEUCINE ON THE URINARY EXCRETION OF TRYPTOPHAN-NIACIN METABOLITES IN RATS

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ABSTRACT The effect of feeding excess leucine on the urinary excretion of tryptophan-niacin metabolites and nitrogen was studied in young and adult rats. Urinary excretion of quinolinic acid and N′methylnicotinamide was increased in both young and adult rats when L-leucine was added at 1.5% level to a 9% casein diet. Quinolinic acid excretion was more markedly affected in young rats, whereas N′-methylnicotinamide excretion was more affected in adult rats. Isoleucine counteracted the effect of leucine in young rats. Nitrogen excretion increased on leucine feeding in adult rats but not in young rats. Adult rats fed a jowar (*Sorghum vulgare*) diet tended to excrete relatively more N′-methylnicotinamide and niacin than when fed a wheat diet.

The effect of leucine feeding on the urinary excretion of tryptophan-niacin metabolites in human subjects and pellagrins has been reported by Gopalan and Srikantia (1) and Belavady et al. (2). Feeding leucine caused a significant increase in quinolinic acid excretion and a decrease in 6-pyridone of N′-methylnicotinamide excretion in normal subjects besides altering the excretion of other metabolites to varying degrees (2). Leucine is present in a relatively high concentration in the millet jowar (*Sorghum vulgare*). Amino acid imbalance due to an excess of leucine in jowar has been suggested as a possible factor in the development of pellagra which is endemic in certain population groups that subsist principally on this millet (1).

Amino acid imbalance in certain types of diets has been shown to cause an increase in niacin requirement in rats and chicks (3). Addition of relatively low levels of gelatin, acid hydrolyzed protein or certain amino acids to a niacin-free casein diet has been shown to cause growth retardation in rats (4–8), which can be corrected by niacin or tryptophan. These observations have been extended to non-casein diets (9) and diets based on amino acid mixtures by Koeppe and Henderson (10). Lyman and Elvehjem (11) have shown that niacin is more effective in correcting the imbalance than tryptophan itself, indicating that imbalance affects the efficient conversion of tryptophan to niacin. Sauberlich and Salmon (12) showed, however, that growth retardation in animals due to imbalance caused by feeding casein-gelatin diet could be corrected by tryptophan and not by niacin alone.

These observations would point to a disturbance in tryptophan-niacin metabolism brought about by an amino acid imbalance in the diet, the mechanism of which is not clear. A detailed investigation of the effect of feeding excess leucine on tryptophan-niacin metabolism in experimental animals was considered necessary to throw some light on the mechanism of the observed action of leucine in humans. The present paper reports such an investigation with rats.

EXPERIMENTAL

Experiment 1. Weanling rats of either sex, distributed at random into 7 groups of 6 each were used in this experiment. They were fed ad libitum for 4 weeks a basal ration (diet A) containing casein (9% protein) either alone, or supplemented with leucine, niacin or tryptophan in different combinations. The composition of the basal

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[1] The term "amino acid imbalance" has been used in a general sense indicating adverse effect of an excess of an amino acid.

ration is shown in table 1, and additions to the diets are shown in table 2. Weekly weights and food intakes were recorded. At the end of this period the animals were transferred to metabolic cages and urine was collected for 3 consecutive days. An accurate record of food intake during the collection period was maintained.

Experiment 2. Six adult female rats weighing between 130 and 160 g housed individually in metabolic cages were fed in 3 periods the following diets: basal ration (diet A, table 1) containing 9% protein derived from casein during the first and the third periods and basal ration plus 1.5% L-leucine during the middle period. The duration of each period was 10 days. The first 7 days were allowed for stabilization, and urine was collected during the last 3 days. A record of food intake was kept during the collection period.

Experiment 3. In another metabolic experiment similar to experiment 2, 6 adult rats (3 males and 3 females) with an average weight of 200 g were given 2 diets (diets B and C) in which the proteins were derived from jowar (*Sorghum vulgare*) or wheat, respectively. The compositions of the diets are shown in table 1. These 2 diets were fed to 3 rats each during the first period and the diets were interchanged during the second period. The purpose of this experiment was to study tryptophan-niacin metabolism in rats fed these 2 cereals which differ in their leucine content, jowar having a much higher content than wheat.

Methods. Urine was collected in toluene and glacial acetic acid, and stored in cold. Urine was pooled for three days, made up to a known volume, filtered and kept cold. Total and free niacin, N′-methylnicotinamide and quinolinic acid were estimated in all samples of urine. Total urinary nitrogen was estimated in all the samples except those collected in experiment 3.

Niacin was estimated in the diets and urine by the method of Friedemann, and Frazier (13). Total niacin was determined after hydrolyzing the urine with 40% NaOH as described by Swaminathan (14). The N′-methylnicotinamide was estimated by the method of Carpenter and Kodicek (15). Quinolinic acid was estimated by the method of Henderson (16). The conversion efficiency of quinolinic acid to niacin under the experimental conditions used was determined with pure quinolinic acid.[3] The conversion efficiency was found to be 74.6 ± 4.9% and the conversion factor of 1.87 was used in calculating the quinolinic acid content of the urine samples. Recovery of added quinolinic acid to urine was complete. Nitrogen was determined by the macro-Kjeldahl method. Tryptophan was estimated in the diets by the microbiological assay described by Barton-Wright (16) using *Lactobacillus arabinosus*.

TABLE 1

Composition of the basal diets

	Diet A	Diet B	Diet C
Casein	11.0	—	—
Jowar (*Sorghum vulgare*)	—	90.0	—
Wheat	—	—	71.0
Peanut oil	5.0	5.0	5.0
Salt mixture [1]	4.0	4.0	4.0
Vitamin mixture [2]	1.0	1.0	1.0
Cornstarch	79.0	—	19.0
Cystine	0.2	—	—
Choline chloride	0.1	—	—

[1] Wesson, L. G. 1932 Science, 75: 339.
[2] Vitamin mixture: (mg/100 g) thiamine, 20; riboflavin, 30; Ca pantothenate, 200; biotin, 1; folic acid, 2; inositol, 1000; pyridoxine·HCl, 5; vitamin B_{12}, 0.15; p-aminobenzoic acid, 10; vitamin K, 10. In addition: vitamin A, 100 IU/day; vitamin D, 15 IU/day and, tocopherol, 3 mg/day were fed orally.

RESULTS

Experiment 1. Urinary excretion levels of nitrogen, quinolinic acid, total and free niacin and N′-methylnicotinamide in young rats are shown in table 2. Quinolinic acid excretion increased significantly ($P < 0.01$) by nearly 2.5 times when leucine was added at a 1.5% level to a casein diet. Excretion of quinolinic acid also increased when 1 mg/100 g of niacin or 0.1% tryptophan was added to the basal diet. The former increase was significant ($P < 0.05$) whereas the latter was not. The observation that quinolinic acid excretion was increased when niacin alone was added to the basal diet could possibly be explained as being due to feedback inhibition by the dietary niacin of the conversion of quinolinic acid to niacin.

[3] Obtained from L. Light and Company, Ltd., Colnbrook, England.

TABLE 2

Effect of leucine on the urinary tryptophan-niacin metabolites in young rats [1]

Group	Diet	Diet intake/ rat/day	Wt gain/ 4 weeks/ rat	Avg for 3 days							
				Intake			Excretion				
				Nitrogen	Niacin	Trypto-phan	Nitrogen	Quinolinic acid	Niacin		N'-methyl-nicotinamide
									Total	Free	
		g	*g*	*mg*	*μg*	*mg*	*mg*	*μg*	*μg*	*μg*	*μg*
1	Basal diet A (casein)	9.2	77.9	515.6	534.0	38.6	177.4 ± 35.4 [2]	109.2 ± 17.7	70.1 ± 10.9	40.9 ± 8.1	64.0 ± 5.1
2	+ 1 mg/100 g niacin	9.5	79.9	661.7	1134.0	47.2	181.3 ± 44.6	254.1 ± 51.2	83.1 ± 14.7	40.0 ± 9.5	107.3 ± 15.4
3	+ 0.1% DL-tryptophan	9.6	80.3	618.8	635.0	87.8	141.3 ± 26.0	190.2 ± 56.0	85.9 ± 16.6	51.1 ± 9.7	104.9 ± 5.7
4	+ 1.5% L-leucine	9.7	77.3	612.1	555.0	41.7	183.0 ± 23.9	278.6 ± 61.6	106.8 ± 22.9	42.4 ± 11.7	91.9 ± 12.5
5	+ 1 mg/100 g niacin + 1.5% L-leucine	10.0	77.0	685.7	1019.0	44.6	200.6 ± 28.1	196.2 ± 35.4	63.5 ± 12.5	36.8 ± 6.6	143.5 ± 19.0
6	+ 0.1% DL-trypto-phan + 1.5% L-leucine	10.2	80.6	568.6	567.0	70.0	171.5 ± 43.5	252.4 ± 36.3	99.1 ± 23.9	32.1 ± 11.4	136.4 ± 21.3
7	+ 1.5% L-leucine + 0.2% DL-isoleucine	9.3	78.5	648.1	579.0	41.9	149.8 ± 30.2	141.2 ± 28.8	82.8 ± 8.9	31.4 ± 6.0	41.6 ± 3.3

[1] Six rats in each group: 9% protein.
[2] SE of mean.

TABLE 3

Effect of leucine on the urinary tryptophan-niacin metabolites in adult rats [1]

Period	Diet	Diet intake/ day/rat	Wt gain/ 10 days/ rat	Avg for 3 days							
				Intake			Excretion				
				Nitrogen	Niacin	Trypto-phan	Nitrogen	Quinolinic acid	Niacin		N′-methyl-nicotinamide
									Total	Free	
		g	*g*	*mg*	*μg*	*mg*	*mg*	*μg*	*μg*	*μg*	*μg*
1	Basal diet A [2] (casein)	16.2	11.0	800.4	740.6	53.5	49.0 ± 2.2 [3]	56.0 ± 9.4	43.7 ± 5.5	22.4 ± 5.7	61.2 ± 8.2
2	Basal diet + 1.5% L-leucine	10.4	8.0	588.4	474.7	34.2	117.1 ± 9.3	66.1 ± 24.1	111.3 ± 23.7	53.1 ± 18.6	353.9 ± 113.3
3	Basal diet A	20.2	3.0	997.5	906.6	66.7	167.8 ± 12.8	56.7 ± 12.0	84.7 ± 9.1	47.4 ± 7.9	184.2 ± 8.1

[1] Six rats in each group: 9% protein.
[2] Contains 1 mg/100 g niacin.
[3] SE of mean.

TABLE 4

Urinary tryptophan-niacin metabolites in adult rats fed jowar and wheat diets [1]

Period	Diet	Diet intake/ day/rat	Wt gain/ 10 days/ rat	Avg for 3 days					
				Intake		Excretion			
				Niacin	Trypto-phan	Quinolinic acid	Niacin		N′-methyl-nicotinamide
							Total	Free	
		g	*g*	*mg*	*mg*	*μg*	*μg*	*μg*	*μg*
1	Jowar basal diet B	19.4	11.8	1.2	37.2	159.5 ± 55.9 [2]	233.2 ± 80.0	32.3 ± 5.4	91.2 ± 19.5
2	Wheat basal diet C	19.9	15.5	1.3	62.0	169.7 ± 37.4	178.2 ± 53.8	39.8 ± 0.5	63.1 ± 10.8

[1] Six rats in each group: 9% protein.
[2] SE of mean.

The N′-methylnicotinamide excretion increased to an equal and significant extent when niacin ($P < 0.05$) or tryptophan ($P < 0.01$) was added to the basal diet. An increase in the excretion of this metabolite was also observed when leucine was added to the basal diet or to the basal diet containing added niacin or tryptophan. These increases were not highly significant perhaps due to wide scatter in the individual values.

One observation was that addition of 0.2% isoleucine to the basal diet containing leucine significantly counteracted the increased excretion of quinolinic acid ($P < 0.05$) and N′-methylnicotinamide ($P < 0.001$) caused by leucine.

The differences observed in the excretion of total niacin among the different groups were not statistically significant. Free niacin excretion was not effected by the different additions to the basal diet. Leucine also did not affect nitrogen excretion in the young rats. This observation appears to correlate with the observed lack of growth retardation in young rats fed the diet containing leucine is the present study.

Experiment 2. Figures for urinary excretion of tryptophan-niacin metabolites in adult rats in experiment 2 are shown in table 3. There was an increase in the urinary excretion of nitrogen, quinolinic acid, total and free niacin and N′-methylnicotinamide accompanied by decreased food intake when leucine was incorporated into the diet at a level of 1.5%. These increases were highly significant when adjustments were made for differences in food intake and the results were analyzed according to analysis of covariance. The observed increase in nitrogen excretion on leucine feeding in adult rats was in contrast with the behavior of the young rats in which nitrogen excretion was not affected by leucine feeding. The results obtained in the adult rats with respect to nitrogen excretion agree with the observations of Deshpande et al. (18) and Kumta et al. (19), who demonstrated that an amino acid imbalance caused a decrease in nitrogen retention in adult rats. After withdrawal of leucine, urinary excretion of the different metabolites decreased, but the values did not return to the original level (i.e., first period). The persistence of a higher level of excretion of the metabolites even after leucine withdrawal might be partly due to a marked increase in food intake after leucine withdrawal. On the other hand, the higher level of excretion might indicate that the effect of leucine was not reversed within the short period of 8 days after its withdrawal. Metabolic alterations caused by leucine feeding would thus appear to extend beyond the period of leucine feeding.

Experiment 3. Urinary excretion levels of quinolinic acid, total and free niacin and N′-methylnicotinamide in adult rats fed jowar and wheat diets are shown in table 4. The intake of tryptophan was significantly lower with jowar diet than the wheat diet, whereas the intake of niacin was of the same order with the 2 diets. A higher level of excretion of N′-methylnicotinamide and total niacin with the jowar diet than with the wheat diet was not statistically significant.

DISCUSSION

The foregoing results indicate that feeding excess leucine affected the urinary excretion of niacin-tryptophan metabolites in rats. Inclusion of leucine at a level of 1.5% in the basal ration caused a significant increase in the excretion of N′-methylnicotinamide in adult rats and to a less significant extent in young rats. Quinolinic acid excretion was increased to a significant extent in both young and adult rats. Niacin (total and free) excretion was increased significantly in adult rats but not in young rats. Belavady et al. (2) observed that in human subjects leucine feeding markedly increased the excretion of quinolinic acid and reduced the excretion of 6-pyridone of N′-methylnicotinamide. Rosen and Perlzweig (8) have reported an increased excretion of N′-methylnicotinamide in rats by gelatin-induced amino acid imbalance. Truswell et al. (20), however, did not observe any effect on the excretion of N′-methylnicotinamide in female rats on the addition of L-leucine to the diet. However, the level of leucine used by these authors was possibly too low to show any demonstrable effect. A somewhat reduced excretion of N′-methylnicotinamide in rats maintained with a 9% casein diet to which threonine was added

has also been reported by Pearson and Phornphiboul.[3] Pearson and Song [4] have confirmed this and showed that in addition to N′-methylnicotinamide, the excretion of 3-hydroxy anthranilic acid, kynurenine and quinolinic acid was less in the group fed threonine.

These results suggest that dietary amino acid excess could cause a disturbance in the tryptophan-niacin metabolism. A comparison of our results with those of Pearson and co-workers [5] indicate that the qualitative nature of such a disturbance depends upon the amino acid which is fed in excess. These differences could be due possibly to the differences in the biochemical changes brought about by excess of threonine and leucine. Even in the case of a particular amino acid fed in excess, the metabolites affected appear to be influenced both qualitatively and quantitatively by the age of the animals. The differences may be related to the differences in the relative needs of tryptophan for growth and maintenance of young and adult rats.

Addition of isoleucine to the diet overcame the increase in the excretion of quinolinic acid and N′-methylnicotinamide in leucine fed young rats. Harper and coworkers (21, 22) have observed an antagonistic relationship between leucine and isoleucine with respect to the growth of rats. The present observation may be an instance of true antagonism. However, Gopalan and Srikantia (1) failed to observe an antagonistic effect of isoleucine on leucine in human subjects.

Concerning the mechanism underlying the observed effect of excess leucine on tryptophan niacin metabolites, there are 2 possibilities: one a generalized effect and the other specific interference in the chain of reactions of tryptophan-niacin metabolism. The generalized effect might be mediated through increased diversion of dietary tryptophan through the niacin pathway following a failure on the part of the tissue to utilize imbalanced amino acids for protein synthesis. Increased nitrogen excretion in adult rats fed leucine suggest an impaired utilization of amino acids. But other observations as lack of growth retardation in young rats fed leucine, and the absence of uniform increase in the various metabolites on leucine feeding, do not support this mechanism. Experimental evidence for the impaired utilization of tryptophan in threonine-induced imbalance is contradictory (23, 24). Further studies are necessary to evaluate the effect of amino acid imbalance on tryptophan utilization.

Some of the specific effects of excess leucine that might explain the present observation in rats and those of Belavady et al. (2) in humans are: (a) inhibition of conversion of quinolinic acid to niacin or more appropriately to niacin ribonucleotide (25, 26); (b) a block in the incorporation of niacin into nicotinamide nucleotide; (c) an increased breakdown of nicotinamide nucleotides; and (d) a decreased oxidation of N′-methylnicotinamide to the 6-pyridone of N′-methylnicotinamide. It has been shown now in humans that in vitro synthesis of nicotinamide nucleotide by erythrocytes is significantly reduced with leucine feeding.[6] The effects, if any, of excess leucine on other loci of tryptophan-niacin metabolism are under investigation.

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LITERATURE CITED

1. Gopalan, C., and S. G. Srikantia 1960 Leucine and pellagra. Lancet, *1:* 954.
2. Belavady, B., S. G. Srikantia and C. Gopalan 1963 The effect of the oral administration of leucine on the metabolism of tryptophan. Biochem. J., *87:* 652.
3. Hundley, J. M. 1954 In The Vitamins, Chemistry, Physiolgy, Pathology, vol. 2. Academic Press Inc., New York, p. 578.
4. Krehl, W. A., L. M. Henderson, J. De La Huerga and C. A. Elvehjem 1946 Relation of amino acid imbalance to niacin-tryptophane deficiency in growing rats. J. Biol. Chem., *166:* 531.

[3] Pearson, W. N., and B. Phornphiboul 1961 Niacin-tryptophan interrelationships in niacin deficiency induced by amino acid imbalance. Federation Proc., *20:* 7 (abstract).
[4] Pearson, W. N., and C. S. Song 1963 Amino acid imbalance and excretion of tryptophan metabolites in the rat. In Nutrition, Proceedings of the 6th International Congress, Edinburgh. E. and S. Livingstone Ltd., Edinburgh, 1964, p. 482 (abstract).
[5] See footnotes 3 and 4.
[6] Unpublished observations by the authors.

5. Singal, S. A., V. P. Sydenstricker and J. M. LittleJohn 1947 The effect of some amino acids on the growth and nicotinic acid storage of rats on low casein diets. J. Biol. Chem., *171:* 203.
6. Singal, S. A., V. P. Sydenstricker and J. M. LittleJohn 1948 Further studies on the effect of some amino acids on the growth and nicotinic acid storage of rats on low casein diets. J. Biol. Chem., *176:* 1063.
7. Hankes, L. V., L. M. Henderson, W. L. Brickson and C. A. Elvehjem 1948 Effect of amino acids on the growth of rats on niacin-tryptophan-deficient rations. J. Biol. Chem., *174:* 873.
8. Rosen, F., and W. A. Perlzweig 1949 The effect of gelatin on the transformation of tryptophan to niacin in rats on low casein diets. J. Biol. Chem., *177:* 163.
9. Henderson, L. M., O. J. Koeppe and H. H. Zimmerman 1953 Niacin tryptophan deficiency resulting from amino acid imbalance in non-casein diets. J. Biol. Chem., *201:* 697.
10. Koeppe, O. J., and L. M. Henderson 1955 Niacin-tryptophan deficiency resulting from imbalances in amino acid diets. J. Nutrition, *55:* 23.
11. Lyman, R. L., and C. A. Elvehjem 1951 Further studies on amino acid imbalance produced by gelatin in rats on niacin-tryptophan low rations. J. Nutrition, *45:* 101.
12. Sauberlich, H. E., and W. D. Salmon 1955 Amino acid imbalance as related to tryptophan requirement of the rat. J. Biol. Chem., *214:* 463.
13. Friedemann, T. E., and E. I. Frazier 1950 The determination of nicotinic acid. Arch. Biochem. Biophys., *26:* 361.
14. Swaminathan, M. 1939 The urinary excretion of nicotinic acid. Indian J. Med. Res., *27:* 417.
15. Carpenter, K. J., and E. Kodicek 1950 The fluorimetric estimation of N′-methylnicotinamide and its differentiation from coenzyme 1. Biochem. J., *46:* 421.
16. Henderson, L. M. 1949 Quinolinic acid metabolism. II. Replacement of nicotinic acid for the growth of the rat and neurospora. J. Biol. Chem., *181:* 677.
17. Barton-Wright, E. C. 1946 The microbiological assay of amino acids. I. The assay of tryptophan, leucine, isoleucine, valine, cystine, methionine, lysine, phenylalanine, histidine, arginine and threonine. Analyst, *71:* 267.
18. Deshpande, P. D., A. E. Harper and C. A. Elvehjem 1958 Amino acid imbalance and nitrogen retention. J. Biol. Chem., *230:* 335.
19. Kumta, U. S., A. E. Harper and C. A. Elvehjem 1958 Amino acid imbalance and nitrogen retention in adult rats. J. Biol. Chem., *233:* 1505.
20. Truswell, A. S., G. A. Goldsmith and W. N. Pearson 1963 Leucine and pellagra. Lancet, *1:* 778.
21. Harper, A. E., D. A. Benton and C. A. Elvehjem 1955 L-Leucine, an isoleucine antagonist in the rat. Arch. Biochem. Biophys., *57:* 1.
22. Benton, D. A., A. E. Harper, H. E. Spivey and C. A. Elvehjem 1956 Leucine, isoleucine and valine relationships in the rat. Arch. Biochem., Biophys., *60:* 147.
23. Wilson, R. G., J. S. Wortham, D. A. Benton and L. M. Henderson 1962 Effect of threonine-induced amino acid imbalance on the distribution of isotope from DL-tryptophan-5-C^{14}. J. Nutrition, *77:* 142.
24. Florentino, R. F., and W. N. Pearson 1962 Effect of threonine-induced amino acid imbalance on the excretion of tryptophan metabolites by the rat. J. Nutrition, *78:* 101.
25. Nishizuka, Y., and O. Hayaishi 1963 Studies on the biosynthesis of nicotinamide adenine dinucleotide. I. Enzymic synthesis of niacin ribonucleotides from 3-hydroxyanthranilic acid in mammalian tissues. J. Biol. Chem., *238:* 3369.
26. Gholson, R. K., I. Ueda, N. Ogasawara and L. M. Henderson 1964 The enzymatic conversion of quinolinate to nicotinic acid mononucleotide in mammalian liver. J. Biol. Chem., *239:* 1208.