STUDIES ON NATURAL AND RACEMIC AMINO ACIDS WITH RATS*

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We have previously reported that glycine, DL-phenylalanine, and L-proline inhibit the growth of young rats when added to an adequate diet at a 5 per cent level (1). Toxicity of amino acids has been reported by other workers (2–6). We have extended these studies to a number of other amino acids and have shown differences, in some cases, between the growth-inhibiting effects of natural isomers and racemic mixtures. In the case of aspartic acid a more thorough study has been made.

EXPERIMENTAL

In the preliminary experiments 21 day-old male albino rats weighing 40 ± 2 gm. were divided into groups of five each and fed the experimental diets *ad libitum*. The diets contained 20 per cent Labco casein and other ingredients, as previously reported (1). The amino acids were fed at a 2 or 5 per cent level of the diet.

Subsequently, the effects of feeding L- and DL-aspartic acids at a 5 per cent level were similarly studied on groups of ten rats each. After 2 weeks on this diet, the animals were kept in metabolism cages for 5 days and the urine collected under toluene. No food was offered in these cages, but the rats were transferred to other cages for feeding each morning and afternoon for 2 hour periods. We were thus able to collect uniform urine samples which were not contaminated with food or amino acids. These samples were analyzed for "free" and total amino acids by the method of Woodson *et al.* (7).

Results

Table I shows the average weight gain per rat for a 3 week period. Addition of L-leucine to the diet caused no inhibition of growth, whereas DL-leucine caused a slight inhibition at the 5 per cent level. Glutamic acid or lysine had no inhibitory effect, regardless of the form fed. L-Tryptophan inhibited growth slightly, while DL-tryptophan was more severe

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in effect. The DL form of aspartic acid was inhibitory, while the L form was not. Addition of 5 per cent L-tyrosine to the basal diet did not inhibit growth but caused large amounts of tyrosine to appear in the urine.[1] This urine appeared dark brown in color, indicating a state of alkaptonuria such as reported by Schweizer (8).

Table II shows the urinary content of "free" and total amino acids found for rats fed the basal diet and those fed the basal diet supplemented with 5 per cent L- or DL-aspartic acid. No significant differences from the controls are apparent, except for aspartic and glutamic acid excre-

TABLE I

Effect of Feeding L- *and* DL-*Amino Acids on 21 Day Weight Gain of Rats*

The basal diet which contained no added amino acids allowed an average gain per rat of 82 gm.

Amino acid*	L isomer		DL mixture	
	Per cent added	Average gain per rat	Per cent added	Average gain per rat
		gm.		*gm.*
Leucine	2	78.0	2	75.5
	5	86.0	5	63.0
Glutamic acid	2	78.0	2	82.0
	5	82.0	5	75.5
Lysine·HCl	5	78.0	5	78.0
Tryptophan	2	63.0	2	52.5
	5	42.0	5	35.6
Aspartic acid	2	78.0	2	84.0
	5	71.0	5	46.0
Tyrosine	5	76.0†		

* Merck.

† Analysis of the urine from this group for "free" and total amino acids showed no significant differences from the urine of rats on the basal diet, except for tyrosine. Excretion of this amino acid per rat per day was as follows: On basal diet, 110 γ (free); 350 γ (total). With added tyrosine, 3100 γ (free); 3650 γ (total)

tion. The rats fed DL-aspartic acid excreted 50 times more total aspartic acid and 20 times more total glutamic acid than the control group. The rats fed L-aspartic acid showed no deviation from normal.

α-Amino nitrogen was determined in these urines by the HNO_2 method of Peters and Van Slyke (9) and the copper method of Pope and Stevens (10) as employed by Albanese and Irby (11), and the results recorded in Table III. Values obtained by the Van Slyke procedure are much

[1] In previous tests not shown in Table I, DL-valine, DL-threonine, L-arginine hydrochloride, DL-isoleucine, and DL-serine at a 5 per cent level in the diet did not inhibit growth. DL-Methionine was markedly inhibitory and L-cystine caused death in 6 to 8 days at this level.

higher than those obtained by the copper method. The former procedure is known to respond to substances other than amino acids (12). This discrepancy between the amounts of α-amino nitrogen as determined by these two methods has been reported by Sauberlich and Baumann (13). The differences in our experiments are much greater than those reported by these authors.

TABLE II

Effect of Feeding L- *and* DL-*Aspartic Acids on Urinary Excretion of "Free" and Total Amino Acids by Rats*

Urine was collected for 20 hours per day for 5 days. The values are reported in mg. per gm. of urine solids. At the end of the first 2 weeks the control rats averaged 108 gm. and those receiving L-aspartic acid 106 gm. The rats receiving DL-aspartic acid averaged 75 gm.

Amino acid	Supplement to basal diet					
	None		5 per cent L-aspartic acid		5 per cent DL-aspartic acid	
	Free	Total	Free	Total	Free	Total
Arginine	0.48	0.64	0.43	0.56	0.35	0.43
Aspartic acid	0.24	2.42	0.09	2.18	101.00	104.00
Glutamic "	0.68	2.54	0.85	1.80	35.50	36.80
Histidine	0.09	0.27	0.09	0.22	0.16	0.26
Isoleucine	0.20	0.95	0.22	0.87	0.11	0.45
Leucine	0.05	0.77	0.04	0.64	0.05	0.53
Lysine	0.22	1.52	0.04	1.09	0.34	1.28
Methionine	0.26	0.32	0.26	0.28	0.16	0.24
Phenylalanine	0.18	1.04	0.03	0.87	0.17	0.74
Proline	0.65	1.95	0.54	2.00	0.54	2.30
Threonine	0.41	1.28	0.33	1.16	0.30	0.86
Tryptophan	0.31	0.33	0.25	0.25	0.19	0.20
Tyrosine	0.16	0.51	0.05	0.44	0.15	0.43
Valine	0.05	0.74	0.04	0.74	0.05	0.47
Total volume, *cc.*	165.0		198.0		218.0	
" solids, *gm.*	8.55		11.0		10.2	

Although the results obtained by the two methods did not agree, comparable results were obtained on the urines from rats on the basal diet and from rats on the basal diet supplemented with L-aspartic acid when the same method was employed. Thus, these two urines appear to be similar according to these two chemical assays as well as microbiologically, as was shown in Table II. The higher values obtained on the unhydrolyzed urine of rats which received DL-aspartic acid are probably due to the presence of D-aspartic acid in the urine.

Both of these methods yield higher values for α-amino nitrogen than

those calculated from microbiological determinations of the amino acids.[2] This is in agreement with Sauberlich and Baumann (13), who have thoroughly discussed the possibilities for this discrepancy.

Heparinized blood samples from animals in each group were pooled and the proteins precipitated from the plasma as described by Hier and Bergeim (14). These were analyzed for aspartic and glutamic acids and the results expressed in Table IV.

TABLE III

α-Amino Nitrogen Excreted by Rats after Feeding L- *and* DL-*Aspartic Acids*

The values are reported in mg. of α-amino nitrogen per gm. of urine solids before and after hydrolysis.

Method	Supplement to basal diet					
	None		5 per cent L-aspartic acid		5 per cent DL-aspartic acid	
	Before	After	Before	After	Before	After
Van Slyke HNO_2	24.4	91.5	26.5	99.5	39.3	88.2
Copper	4.25	40.5	7.0	40.3	23.3	56.0

TABLE IV

Effect of Feeding L- *and* DL-*Aspartic Acids on "Free" Aspartic and Glutamic Acid Level of Rat Plasma*

The values are reported as micrograms per cc. of plasma.

Amino acid	Supplement to basal diet		
	None	5 per cent L-aspartic acid	5 per cent DL-aspartic acid
Aspartic acid	0	0	109
Glutamic "	45	37	47

The amount of aspartic acid found in the plasma from normal rats and those receiving L-aspartic acid was nil. This agrees with the results reported by Henderson *et al.* (15). However, an appreciable amount of aspartic acid was found in the plasma of rats receiving DL-aspartic acid in the food. Christensen *et al.* (16) have reported that the feeding of L-aspartic acid does not elevate the plasma aspartic acid, but these workers did not feed the DL mixture.

The quantity of glutamic acid present in these plasma samples was not influenced by the presence of L- or DL-aspartic acid in the food.

[2] This is also true for human urine. Unpublished data.

DISCUSSION

As we have previously reported (17), the DL mixtures of some amino acids are more inhibitory to growth than the natural isomers. This emphasizes the importance of the correct balance of amino acids in the diet of the rat and suggests caution in the use of large doses of certain amino acids, particularly the unnatural forms or racemic mixtures. The greater degree of inhibition caused by the racemic forms is in agreement with the observation of Howe *et al.* (18) that racemic mixtures of amino acids are less well tolerated than the natural forms when given intravenously.

Although Sullivan *et al.* (2), Martin (5), and Schweizer (8) have reported L-tyrosine to be toxic to the rat in high levels, we have not found this under the conditions of our experiments. Neither have we produced various pathological lesions such as those reported by these authors. These differences in results between laboratories are probably due to a difference in basal diets.

We did not determine the proportions of D- and L-aspartic acids present in the plasma and urine in these experiments since the organism employed (*Leuconostoc mesenteroides* P-60) utilizes both forms. However, the rats fed L-aspartic acid showed no increase in blood or urinary level of this amino acid, while the rats fed DL-aspartic acid showed an increase. It is probable, therefore, that the increase was due to unmetabolized D-aspartic acid.

It is difficult to explain the fact that the urinary level of glutamic acid was higher in the animals fed DL-aspartic acid than in that of the other groups, despite the fact that there was no increase in the plasma level. It is possible that the glutamic acid exists in a microbiologically unavailable form in the plasma.

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SUMMARY

Rats have been fed 2 or 5 per cent of various amino acids in the natural or racemic form, replacing an equivalent amount of sucrose in a complete casein diet.

The DL mixtures of leucine, tryptophan, and aspartic acid prevented growth to a greater extent than the natural isomers. The possible significance of these findings is discussed.

The amino acid pattern of urine from rats on the basal diet has been

compared with that from rats receiving L- or DL-aspartic acid in the diet and some differences noted.

Chemical determinations of α-amino nitrogen in these urines by two methods have yielded different values, both of which are significantly higher than can be accounted for microbiologically.

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