Amino Acid Feeding Studies: Effects of Various Nonessential Nitrogen Sources and of Added Water (34022)

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The utilization of nonessential nitrogen (NEN) and factors influencing growth of rats fed amino acid-containing diets have been investigated for years (1) but are still not fully understood. Results appear to depend in a complex way on the balance of amino acids in the diet and even in some cases on whether or not water has been added (2,3).

In planning a study of the utilization of amide nitrogen (4) we decided to feed rats a fixed level of essential amino acids and of calories and to determine the level of NEN which would contribute most efficiently to nitrogen storage. In the present report the same approach was used to compare the utilization of the nitrogen of glutamic acid, aspartic acid, a mixture of four other nonessential amino acids and of diammonium citrate (DAC) and to reinvestigate the effect of adding water.

Experimental Methods. Young male rats approximately 4 weeks old were given a nitrogen-free diet for 4 days,[1] divided into groups and either fed weighed amounts of food daily for 21 days or killed for initial controls.

Drinking water was available *ad libitum.* Food intake was restricted to the amount that would be readily consumed in 24 hr by all rats, so that variations in calorie intake would not be a factor in the response. The composition of the diets fed is given in Table I. The essential amino acid mixture was patterned after the amino acid requirements of the growing rat as given by Ranhotra and Johnson (3) and Rama Rao and co-workers (6). However, amino acids per 100 g of diet were increased approximately 13% so that the amounts in the daily food allotment would be enough to satisfy requirements. Except for two groups (Table II), 0.5 ml of water /g of food was mixed into all diets just before they were given to the rats. If any allotted food was scattered, it was collected, dried, allowed to equilibrate to room moisture, weighed, and subsequent food allotment was proportionately increased. Details of the analyses of diets and carcasses have been described elsewhere (4).

TABLE I. Composition of Diets Fed.

Amino acids	(%)	Other ingredients	(%)
L-Arginine HCl	0.56	NEN[a] source	0–9.00
L-Cystine	0.38	Cornstarch[b]	79.66–70.66
L-Histidine $HCl \cdot H_2O$	0.38	Salt mixture[c]	4.00
		Celluflour[d]	4.00
L-Isoleucine	0.62	A & D concentrate[e]	0.05
L-Leucine	0.79	Vitamin mix[f]	1.00
L-Lysine HCl	1.27	Corn oil (Mazola)	5.00
L-Methionine	0.18		
L-Phenylalanine	0.47		
L-Threonine	0.56		
L-Tryptophan	0.12		
L-Tyrosine	0.34		
L-Valine	0.62		

[a] NEN = nonessential nitrogen.
[b] Adjusted as necessary.
[c] Jones and Foster (5).
[d] Chicago Dietetic Supply House, Chicago.
[e] Percomorph oil, Mead Johnson Co., Evansville, Indiana.
[f] For composition, see (4).

Results. In preliminary trials it was found that the amount of food that all rats consumed in 24 hr decreased as the amounts of aspartic and glutamic acid increased, and decreased even further with increasing amounts of diammonium citrate (unpublished results). Food intake was also low when no source of NEN was present in the diet. To insure the same calorie intake for all groups each rat was restricted to 7 g of food daily.

[1] The components of the nitrogen-free diet were the same as those given in Table I but with additional cornstarch replacing the amino acids.

TABLE II. Utilization of Nonessential Nitrogen (NEN) by Young Rats.

	NEN					Nitrogen		
Source[a]	% in diet	Intake (mg/day)	No. of rats /group	Water added	Wt gain (g)	Intake[b] (g)	Gain[b] (g)	Gain (% of intake)
—	—	0	12	+	24	1.30	0.65 ± 0.023[c]	49.9 ± 1.78
Glu	4.50	30	15	—	38	1.92	1.14 ± 0.026	59.3 ± 1.34
Glu	4.50	30	20	+	38	1.92	1.23 ± 0.020	64.0 ± 1.02
Asp	4.07	30	10	+	38	1.92	1.26 ± 0.027	65.5 ± 1.44
NE	2.88	30	15	+	41	1.94	1.29 ± 0.026	66.8 ± 1.34
DAC	3.46	30	15	+	38	1.96	1.04 ± 0.038	53.0 ± 1.96
Glu	9.00	60	12	—	41	2.53	1.34 ± 0.048	52.9 ± 1.85
Glu	9.00	60	21	+	41	2.54	1.38 ± 0.026	54.4 ± 1.04

[a] Abbrev.: Glu = glutamic acid; Asp = aspartic acid; NE = nonessential amino acid mixture: equal parts by weight of L-alanine, glycine, L-proline, and L-serine; and DAC = diammonium citrate.

[b] Totals for 21 days; food intakes for the various groups were 146.5–147.0 g.

[c] SE of mean.

This amount contained 60 mg of essential amino acid nitrogen (EAAN). When 30 or 60 mg of NEN from glutamic acid was added, nitrogen gains as percentage of nitrogen intake increased from 49.9 (no NEN) to 64.0 (30 mg of NEN/day) and then decreased to 54.4% (60 mg of NEN/day). Differences between each successive set of values were highly significant ($p<0.01$).

Because the highest utilization of dietary nitrogen occurred when 30 mg of NEN were present in the diets, this amount was used for most of the comparisons. However, one comparison was made between groups fed diets containing 60 mg of NEN with and without added water.

Weight gains of the various groups of rats restricted to 7 g of food /day and given 60 mg of EAAN and either 30 or 60 mg of NEN varied only from 38 to 41 g (Table II). Utilization of dietary nitrogen for body nitrogen gain was significantly improved ($p<0.01$) when water was added to the diet containing 30 mg of NEN from glutamic acid, however. On the other hand, there was not a significant difference between the groups fed 60 mg of NEN with and without water added.

There were no significant differences between the groups fed 30 mg of NEN from glutamic acid, aspartic acid, or a mixture of 4 nonessential amino acids. However, the utilization of DAC nitrogen as compared with an equal quantity of α-amino nitrogen was significantly lower.

Discussion. Hepburn *et al.* (7) reported increased rates of growth as dietary glutamic acid was increased from 0 to 5.66%. They suggested that under certain conditions a requirement for glutamic acid may exist and that glutamic acid is superior to other sources of nonspecific nitrogen in purified amino acid diets. They found that a level of DAC providing 0.539 g of nitrogen/100 g of food was only 15% as effective as glutamic acid fed at an isonitrogenous level. In the present study, glutamic acid nitrogen was no better utilized than an equal quantity of aspartic acid nitrogen or of a mixture (NE) of equal quantities of L-alanine, glycine, L-proline, and L-serine (Table II). At the level fed (0.428 g of nitrogen /100 g of food) the nitrogen of DAC was 85% as well utilized as that of the diet containing an equal quantity of glutamic acid nitrogen. Comparisons between the present results and those of Hepburn *et al.* are difficult because their diets contained some D-amino acids. It should be pointed out, however, that Rose *et al.* (8), whose diets also contained some D-amino acids, found that amounts of DAC and glutamic acid providing 0.574 g of nitrogen /100 g of food were equally effective in promoting weight gains in young rats.

Adkins *et al.* (2) reported that the addition of water to various amino acid-containing diets sometimes did and sometimes did not improve weight gains and food efficiencies of young rats and suggested that the response is related to the balance of the amino acids in the diets. The response obtained in the present study does not appear to be related to the balance of the essential amino acids. The essential amino acid intake was not altered but the response was eliminated when the amount of glutamic acid was doubled. This finding may or may not be related to the overall poorer utilization of dietary nitrogen under these conditions at the higher intake level.

Ratios of essential to nonessential nitrogen do not appear to be involved. The response to added water was obtained when the ratio was 2:1 and not obtained when the ratio was 1:1. In the report of Adkins *et al.* the ratio was about 1:1 for the diet in which the response was obtained and 6.5:1 in the diet where there was no response. It is apparent that the solution of the problem will require many comparisons. It is of course possible that the different responses in the various studies are due to different factors. The rats of the present study, restricted in calorie intake as well as NEN, did not respond with increased weight gains—only with increased nitrogen gains when water was added. However, the fact that a response to added water (either increased weight gains, increased protein efficiency ratios, or increased protein gains) has been obtained not only with diets containing amino acids but also with diets in which type of protein and carbohydrate were varied (9) would suggest that some common factor is involved.

Meanwhile, in our laboratory, because feeding low-fat amino acid-containing diets with added water results in less scattering and therefore less labor in recovering scattered food, we have in most instances adopted the procedure of feeding such diets wet.

Summary. Young male rats fed equal, restricted amounts of diets containing low but presumably adequate levels of essential L-amino acids made equally efficient use of the nitrogen of glutamic acid, aspartic acid or a mixture of 4 other nonessential amino acids for nitrogen gains. The nitrogen of diammonium citrate was only 85% as well utilized as that of the other compounds. At the same restricted calorie intake, nitrogen was more efficiently used when water was added to a diet containing low levels of essential amino acid nitrogen and glutamic acid nitrogen. The response to added water was abolished when the amount of glutamic acid nitrogen was doubled.

1. Greenstein, J. P. and Winitz, M., "Chemistry of the Amino Acids," John Wiley & Sons, Inc. New York Vol. 1. New York (1961).

2. Adkins, J. S., Wertz, J. M., Boffman, R. H., and Hove, E. L., Proc. Soc. Exptl. Biol. Med. **126**, 500 (1967).

3. Ranhotra, G. S. and Johnson, B. C., Proc. Soc. Exptl. Biol. Med. **118**, 1197 (1965).

4. Womack, M. and Wilson, J. E., Jr., J. Food Sci., in press.

5. Jones, J. H. and Foster, C., J. Nutr. **24**, 245 (1942).

6. Rama Rao, P. B., Metta, V. C., and Johnson, B. C., J. Nutr. **69**, 387 (1959).

7. Hepburn, F. N., Calhoun, W. K., and Bradley, W. B., J. Nutr. **72**, 163 (1960).

8. Rose, W. C., Smith, L. C., Womack, M., and Shane, M., J. Biol. Chem. **181**, 307 (1949).

9. Keane, K. W., Smutko, C. J., Krieger, C. H., and Denton, A. E., J. Nutr. **81**, 87 (1963).

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