Influence of Addition of Water or "Nonessential" Nitrogen on Growth of Rats Fed Low Levels of Essential L-Amino Acids. (32488)

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A previous study in this laboratory(1) demonstrated the importance of "nonessential" amino acids (NEAA) in a purified diet containing a high level (15.7%) of essential L-amino acids (EAA). An amino acid (AA) diet patterned after the diet of Rechcigl *et al* (2), but supplemented with NEAA, promoted weight gain of rats equal to that obtained with a casein diet. However, the diet (equivalent to 15.9% protein) supplied the EAA at levels much greater than those suggested by the National Research Council (NRC) (3), or by Rama Rao *et al*(4). Ranhotra and Johnson(5) recently reported excellent growth (6 g/day) by feeding weanling rats a wet purified AA diet equivalent to only 10% protein. The present report deals with studies involving (a) the modification of the AA mixture previously used in an attempt to obtain maximal growth with the minimal levels of EAA; and (b) further determination of the effect of the addition of water, NEAA or other N sources to purified AA diets.

Materials and methods. Weanling male Holtzman rats, 45-50 g, were used in all experiments. The rats (6-8 per group) were housed in individual cages in a room maintained at 22 ± 1°C and were fed *ad libitum* casein or purified AA diets for 14 or 21 days. Food intakes and body weights were recorded twice weekly. Water was available at all times.

The composition of the AA mixtures is given in Table I. Diets A (14% casein), B and C and the constant non-nitrogenous ingredients of the other diets were the same as previously used(1) except that L-serine was omitted from diet C. The amino acid mixture used in diet B was patterned after mixture 2-L of Rechcigl *et al*(2). Diets AI, BI, and CI were diets A, B, and C propor-

TABLE I. Amino Acid Composition of the Diets.

Amino acid	Diet B*	BI	C	CI	D†	E
	%					
L-Arginine.HCl	2.12	1.61	2.12	1.39	.50	.65
L-Histidine.HCl.H_2O	.79	.60	.79	.52	.34	.30
L-Isoleucine (allo-free)	2.08	1.58	2.08	1.37	.55	.50
L-Leucine	1.93	1.46	1.93	1.27	.73	.80
L-Lysine.HCl	2.84	2.15	2.84	1.86	1.18	.90
L-Methionine	.50	.38	.50	.33	.54	.35
L-Cystine	.45	.34	.45	.29	.23	.25
L-Phenylalanine	1.09	.83	1.09	.72	.76	.90
L-Tyrosine	.86	.65	.86	.56	.23	.30
L-Threonine	1.16	.88	1.16	.76	.50	.50
L-Tryptophan	.23	.17	.23	.15	.15	.15
L-Valine	1.63	1.23	1.63	1.07	.55	.70
L-Alanine	—	—	.33	.22	—	—
DL-Alanine	—	—	—	—	.23	—
L-Asparagine	—	—	—	—	.40	—
L-Aspartic acid	—	—	.65	.43	.23	—
L-Glutamic acid	—	—	1.14	.75	3.29	6.10
Glycine	—	—	.33	.22	1.39	1.00
L-Proline	—	—	.39	.26	.23	—
DL-Serine	—	—	—	—	.23	—
Total	15.68	11.88	18.52	12.17	12.26	13.40
Protein equivalent (N × 6.25)	13.2	10.0	15.2	10.0	10.0	10.0

* Patterned after mixture 2-L, Rechcigl *et al*(2).
† Patterned after mixture CIII, Ranhotra and Johnson(5).

TABLE II. Effect of Addition of 50% Water on Growth Rate and Carcass Composition of Weanling Rats Fed 10% Protein Equivalent Casein or Purified Amino Acid Diets for 14 Days.*

Measurement	Diet AI Dry	Diet AI Wet	Diet CI Dry	Diet CI Wet	Diet D† Dry	Diet D† Wet
Avg wt gain, g/day	5.3 ± .1	6.0 ± 0.2	4.3 ± .1	4.7 ± .2	4.5 ± .3	5.8 ± .2
Food efficiency, g gain/g food	.43 ± .01	.46 ± .01	.38 ± .01	.41 ± .01	.37 ± .01	.49 ± .01
Gastrointestinal tract wt, % body wt	6.87 ± .11	7.52 ± .27	7.06 ± .25	7.12 ± .30	6.59 ± .22	7.42 ± .30
Stomach contents moisture, %	93.3 ± .7	82.0 ± 2.7	85.5 ± 2.5	89.7 ± 3.0	84.6 ± 1.3	86.0 ± 1.6
Avg carcass moisture, %	68.5 ± .5	67.5 ± .3	70.6 ± .4	69.6 ± .3	70.6 ± .3	69.0 ± .4
Avg carcass protein, % (N × 6.25)	16.2 ± .3	17.2 ± .3	16.9 ± .2	17.2 ± .4	16.3 ± .5	15.7 ± .3
Avg carcass fat, % on fresh wt basis	9.97 ± .30	10.36 ± .31	7.76 ± .28	9.36 ± .16	8.43 ± .40	10.14 ± .38
Protein/Fat Ratio	1.65 ± .09	1.66 ± .06	2.19 ± .11	1.83 ± .06	1.95 ± .14	1.56 ± .09

* Mean ± S.E. for 15 rats in groups AI & D and for 8 rats in group CI.
† Ranhotra and Johnson (5), Diet C III. The same non-nitrogenous ingredients were used in all diets in this study.

tionally reduced to supply an equivalent of 10% protein. Diet D was diet C III of Ranhotra and Johnson(5) and diet E supplied the EAA in amounts suggested by the NRC(3) with nonessential N supplied only by glycine and L-glutamic acid. All optically active AA were supplied as the NRC grade L-isomers (General Biochemicals, Inc., Chagrin Falls, Ohio). All additions to the diet were made at the expense of corn dextrin.

In some experiments, 50% water (w/v) was added in advance to diets AI, CI and D. In these studies the non-nitrogenous components of Ranhotra and Johnson(5) were used and the diets were fed daily in clean porcelain cups. All diets were refrigerated (4°C) in airtight containers until used. In a preliminary study we found that agar was not necessary in the wet diet to prevent diet separation and that moisture lost by evaporation from the wet diets was negligible. At the end of the experiments in which wet diets were fed, all rats were sacrificed and carcasses were analyzed according to the Miller-Bender procedure(6). The data were analyzed statistically by the Student's "t" test (7).

Results. Effect of water addition. The effects of addition of 50% water to diets AI, CI and D (10% protein equivalent), on the growth rates and carcass analyses of weanling rats are summarized in Table II. The rate of gain of rats fed the casein diet AI or AA diet D in the wet form was significantly greater ($P<0.01$) than that of rats fed the same diets in the dry form. Growth of rats fed diet CI was improved by the addition of water but the difference was not significant. Food efficiency (g gain/g food) was significantly greater when water was added to all diets ($P<0.05$ for diets AI and CI; $P<0.001$ for D).

Addition of water to any of the diets did not change the weights of the empty gastrointestinal tract, the amounts of carcass moisture and protein (N × 6.25), or the amount of moisture of the stomach contents. The carcass fat, expressed on a fresh-weight basis, was significantly higher ($P<0.01$) with the wet diets CI and D *vs* these diets fed dry. Therefore, the protein/fat ratio of the wet diets CI and D was significantly lower ($P<0.05$).

Addition of water to a variety of other diets containing different AA mixtures but the same non-nitrogenous basal ingredients did not improve growth. For example, weanling rats (7/group) fed the AA mixture M patterned after Salmon(8) or the AA mixture G of Sauberlich(9) for 14 days gained 3.9 ± 0.2 and 3.5 ± 0.2 g/day, respectively, when the diets were fed dry as compared to 4.0 ± 0.2 and 3.5 ± 0.2 g/day, respectively, when the diets were fed wet.

Dose response to variable protein equivalent levels. Fig 1 shows the weight gains of

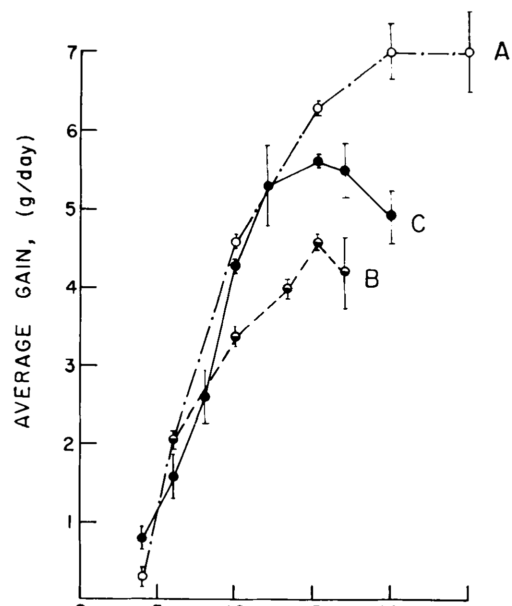

FIG. 1. Effect of the dietary protein equivalent level on average body weight gain (g/day) of weanling rats fed casein (A) or purified L-amino acid diets (B or C) for 21 days. Each point represents mean ± S. E. of at least 5 rats. Diet B contained only essential amino acids. Diet C contained essential and nonessential amino acids (see Table I).

rats fed dry diets A, B or C modified to supply different protein equivalent levels ($N \times 6.25$).

There was no significant difference in the weight gain of rats fed diets A and C at the 15% protein equivalent level. Both of these 15% protein equivalent diets provided a significantly greater gain ($P<0.01$) over the diets fed at 10% or at lower protein equivalent levels. Growth of rats fed diet A at the 20% protein equivalent level was significantly ($P<0.01$) greater than that of rats fed diet C at the 20% level. However, increasing the casein or AA in diets A and C, respectively, to provide a protein equivalent level greater than 15% did not significantly improve the growth rate (Fig. 1). The slight reduction in weight gain of rats fed diet C at the 20% protein equivalent level as compared to the 15% level was not statistically significant ($P>0.05$). As observed in our earlier study(1), rats fed both the EAA and NEAA (diet C) at the 15% protein equivalent level grew at a significantly ($P<0.05$) faster rate than rats receiving only the EAA (diet B).

Effect of addition of nonessential amino acids to 10% protein equivalent diets. The results of proportionately reducing the protein equivalence and supplementing diets A, B and C are given in Table III. Reducing the protein equivalence of diets A and C to 10% (diets AI and CI), resulted in a significantly slower ($P<0.001$) rate of gain and a decrease in food efficiency ($P<0.001$), although these diets still supplied the EAA at the level equal to or higher than the levels suggested by the NRC(3) or by Rama Rao *et al*(4). Adding glycine or glutamic acid individually to diet BI did not significantly improve growth rate or food efficiency, but a combination of the two brought the weight gain and food efficiency back to the level obtained with diet B. Adding a combination of glycine and glutamic acid or a mixture of NEAA to diet CI to raise the protein equivalent back to 15% significantly improved growth ($P<0.01$) and food efficiency ($P<0.001$); however, a diet made isonitrogenous by the addition of diammonium citrate (DAC) gave no better results than diet CI. Urea significantly ($P<0.05$) improved growth of rats fed diet CI but did not significantly improve the food efficiency. The addition of 0.5% asparagine slightly improved growth of rats fed diet CI but the difference was not significant. The addition of aspartic acid, alanine, glycine, serine, glutamic acid

TABLE III. Effect of Feeding Low Levels of Essential L-Amino Acids (EAA) and Various "Nonessential" N Sources on Growth of Weanling Rats.

Source of dietary N	Protein equivalence (N × 6.25) (% diet)	No. rats	Avg wt gain (g/day*)	Food efficiency (g gain/g food*)
Diet A (14% casein)	12.5	25	6.0 ± .3	.54 ± .01
Diet AI	10.0	34	4.9 ± .2	.41 ± .01
Diet B (EAA)	13.2	18	3.9 ± .2	.42 ± .01
Diet BI	10.0	18	3.4 ± .2	.38 ± .01
" + glycine 0.33% + L-glutamic acid 1.14%	11.1	7	4.1 ± .2	.44 ± .02
" + glycine 0.33%	10.4	12	3.7 ± .1	.41 ± .01
" + L-glutamic acid 1.14%	10.7	13	3.8 ± .2	.41 ± .01
Diet C (EAA + NEAA)	15.2	30	5.4 ± .2	.52 ± .01
Diet CI	10.0	37	4.1 ± .2	.39 ± .01
" + glycine 1.0% + L-glutamic acid 6.7%	15.2	23	5.0 ± .2	.44 ± .01
" + glycine 4.45%	15.2	7	4.1 ± .1	.36 ± .01
" + L-glutamic acid 8.74%	15.2	7	4.5 ± .2	.38 ± .02
" + NEAA mixture†	15.2	8	5.4 ± .2	.47 ± .01
" + urea 1.846%	15.2	7	4.8 ± .2	.40 ± .01
" + diammonium citrate 7.62%	15.2	9	4.1 ± .2	.35 ± .02
" + L-asparagine 0.5%	10.7	8	4.6 ± .2	.39 ± .01
Diet E	10.0	6	3.8 ± .4	.39 ± .01

* Mean ± S.E. after 21 days.
† Mixture contained the five NEAA in the same amounts as present in diet C, Table I.

or proline individually in the same amount found in the complete mixture (diet C) did not give a growth response (data not presented).

The rate of gain of rats fed diet E, based on NRC amino acid requirements, was similar to that of rats fed diet CI.

Discussion. The results reported here indicate that the response to added water when growth and food efficiency are used as the criteria, is related to the balance of the AA in the diet. Diet D gave a greater response than diets AI or CI and the AA mixtures patterned after others(8,9) gave no significant growth response. The non-nitrogenous ingredients of all diets were the same. Rogers and Harper(10) observed that the beneficial effects of feeding diets in agar-gel form was greatest when the diet was inadequate in some respect. These workers suggest that one factor responsible for increased food intake and weight gain when diets were fed in gel form is that the gel prevents the severe osmotic effect; *i.e.,* water is prevented from being drawn into the stomach and thus activating the stretch receptors so that food intake is reduced. In a preliminary study, and in the study of Ranhotra and Johnson(5), the growth rate was not significantly improved in rats fed wet diets with agar compared to those fed wet diets without agar. In the present study, in contrast to the finding of Rogers and Harper(10), food intake was essentially the same whether the diets were fed wet or dry, but the addition of water significantly improved the food efficiency.

Results of the carcass analyses indicate that the increased weight gain of rats fed diets with 50% added water was not water accretion. This finding is in agreement with the work of Ranhotra and Johnson(5) and Keane *et al*(11).

Keane *et al*(11) showed that the addition of 20% water to purified diets containing 6 to 12% protein resulted in an increased growth rate and a higher protein efficiency ratio (PER) in rats. They later showed(12) that the increased PER was not due to increased protein consumption. Ranhotra and Johnson(5) reported a gain of over 6 g/day by feeding weanling rats a purified AA diet equivalent to 10% protein. These workers added water to all diets. Rogers and Harper (10) have also reported a beneficial response to the addition of water. The reason for the increase in growth rate when water is added to the diet has not been explained but has been discussed(5,10-12).

The addition of a combination of glycine and glutamic acid to increase the protein equivalent of diet C from 10 to 15% was as effective as adding a mixture of six NEAA but addition of DAC was not as effective. In agreement with the observations of Rose *et al*(13), urea also provided the extra nitrogen needed for good growth of weanling rats. Breuer *et al*(14) and Rogers and Harper (10) reported that addition of asparagine to their AA diet increased the weight gain of rats. In our studies when 0.5% asparagine was added to the 10% AA diet CI, growth was improved but the difference was not significant. Ranhotra and Johnson(5) also reported only a small insignificant response to asparagine.

All of the purified AA diets fed in the dry form at the 10% protein equivalent level failed to support maximal growth, although the EAA were supplied at the recommended levels(3,4) and NEAA were included in the diet. The addition of water to diet D (10% protein equivalent) significantly improved growth over the dry diet. The results indicate that water in some manner improved the utilization of AA. The factors influencing the response to added water need further investigation.

Summary. Male weanling rats were fed casein or purified amino acid (AA) diets equivalent to 10% protein (N $\times$ 6.25) to determine the effect of the addition of 50% water, nonessential amino acids (NEAA) or other N sources on growth. The daily weight gain of rats fed the wet casein diet (6.0 g) or the wet AA diet of Ranhotra and Johnson (5) (5.8 g) was significantly greater than that of rats fed comparable dry diets (5.3 and 4.5 g, respectively). However, the addition of water to a variety of other diets containing different AA mixtures but the same nonnitrogenous ingredients did not significantly improve growth. The carcass fat was significantly higher with the wet AA diets than with the dry AA diets. The data indicate that the response to added water is related to the balance of the AA in the diet. Rats fed the dry casein or AA diet at the 15% protein equivalent level had a significantly faster rate of gain and improved food efficiency over those fed the diets reduced to 10% protein equivalent, even though the essential AA were supplied in adequate amounts. The addition of a combination of glycine and glutamic acid, urea, or a mixture of NEAA significantly improved growth, but diammonium citrate or other NEAA added singly did not give a significant response.

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