The Importance of Dispensable Amino Acids for Maximal Growth in the Rat[1] (34826)

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Earlier (1) it was observed that over 1% of arginine, 0.6% of asparagine, and feeding of the diet as an agar gel containing about 50% of water were all necessary in order to obtain maximal weight gains of rats fed amino acid diets. The present work was initiated as an attempt to establish the requirements of the rat for the dispensable amino acids. Even though, under certain conditions, growth responses can be obtained with several of the dispensable amino acids, it was soon found that the requirement for one dispensable amino acid depended upon the level of several other amino acids, and, therefore, it was not feasible to determine requirements. We would, therefore, like only to report results which show combinations of dietary amino acids which allow maximum growth even when one or more of the dispensable amino acids is completely absent.

Experimental. Male, weanling, albino rats (50–60 g) were fed a basal diet containing 16% of crystalline amino acids with or without 2.47% of diammonium citrate (depending upon the experimental design) for 2 days to allow the rats to adjust to the animal laboratory. The rats were then separated into groups of six of equal average weight which were assigned at random to the experimental diets. The rats were housed individually in suspended galvanized cages with wire mesh bottoms in an animal laboratory maintained at about 28° and 50% relative humidity. Water and diets were provided *ad libitum*. The rats were fed and weighed daily for 5 days and at 7, 9, 11, and 14 days after the beginning of the experiment.

The basal amino acid mixture used in most of the experiments is shown in Table I. Changes in the proportions of the dispensable amino acids in the basal mixture for each experiment are indicated in the other tables. All amino acids used were L-isomers.[2]

The diets contained, in per cent: corn oil, 10; salt mixture, 5[3]; vitamin mixture, 0.5[4]; choline chloride, 0.2; amino acids, 9–26, as indicated in the tables; and dextrin · sucrose at a ratio of 2:1 to make to 100%. This diet was premixed dry in a Hobart diet mixer

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[2] Purchased from General Biochemicals Inc., Chagrin Falls, Ohio.

[3] The salt mixture contained (in per cent) $CaCO_3$, 29.29; $CaHPO_4 \cdot 2H_2O$, 0.43; KH_2PO_4, 34.31; NaCl, 25.06; $MgSO_4 \cdot 7H_2O$, 9.98; $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.623; $CuSO_4$ 0.156; $MnSO_4 \cdot H_2O$, 0.121; $ZnCl_2$, 0.020; KI, 0.0005; $(NH_4)_6MO_7O_{24} \cdot 4H_2O$, 0.0025; $Na_2SeO_3 \cdot 5H_2O$, 0.0015. The salt mix was prepared by and purchased from General Biochemicals, Inc. The salts at 5% of the diet provided in percentage of element; Ca, 0.592; P, 0.394; K, 0.493; Na, 0.493; Cl, 0.760; Mg, 0.049; Fe, 0.0049; Cu, 0.0019; Mn, 0.00195; Zn, 0.0004; I, 0.000019; Mo, 0.000005; Se, 0.0000025.

[4] Five-tenths per cent of the vitamin mixture in the diet provided the rats with 0.44% sucrose plus the following vitamins in mg/kg diet: thiamine·HCl, 5; riboflavin, 5; niacinamide, 25.0; Ca D-pantothenate, 20; pyridoxine HCl, 5; folic acid, 0.5; menadione, 0.5; *d*-biotin, 0.2; vitamin B_{12} (0.1 % in mannitol), 30; ascorbic acid, 50 (added to help prevent thiamine destruction); vitamin E acetate (25% in a mixture of gelatin, sugar, and starch), 400; vitamin A acetate and vitamin D_2 (325,000 USP units of A/g and 32,500 USP units of D_2/g in a mixture of gelatin, sugar, and starch), 12.31.

TABLE I. Composition of Basal Amino Acid Mixture.

Amino acid	% of diet[a]	Amino acid	% of diet[a]
Cystine	0.35	Alanine	0.35
Histidine·HCl·H_2O	0.40	Arginine·HCl	1.35
Isoleucine	0.65	Asparagine	0.60
Leucine	0.85	Aspartic acid	0.35
Lysine · HCl	1.35	Glutamic acid	4.50
Methionine	0.60	Glycine	1.40
Phenylalanine	0.85	Proline	1.0
Threonine	0.60	Serine	0.35
Tryptophan	0.17	Sodium acetate	1.29
Tyrosine	0.35		
Valine	0.65	Total	18.01

[a] Percentage of the dry weight of the diet.

then made into a gel by mixing the dry diet vigorously into an equal quantity of boiling 2% agar solution. When the diet had been mixed thoroughly, it was poured into trays with airtight covers and allowed to cool before the covers were replaced. All diets were refrigerated until used. The agar gel diet was then firm at room temperature and was cut into cubes and fed daily. Food intake was measured in some experiments by weighing the quantity of diet fed, determining the percentage of moisture in the diet, then collecting the leftover diet each day, drying it in a vacuum oven at 70°, and calculating the food eaten by difference.

Results. Experiment 1 was carried out to determine the effect of adding diammonium citrate, a nonspecific nitrogen source, while deleting three or more dispensable amino acids on weight gain. The results are shown in Table II. Normally, maximal weight gains in our laboratory are about 95–105 g/2 weeks for the weanling rat. In this first experiment weight gain of rats fed the basal diet was only 86 g/2 weeks. In later experiments (see Tables III–V), rats fed this basal diet grew at close to the maximum rate. The removal of aspartic acid, alanine, and serine and the addition of 2.47–4.94% diammonium citrate did not depress growth (see groups I-d and I-e, Table II).

Weight gain was essentially the same whether the diet contained amino acids or casein and was similar to that of rats fed stock diet. It is interesting that 4.94% diammonium citrate reduced the weight gain of rats fed the casein diet whereas inhibition did not regularly occur when that level was added to the amino acid diet.

In the second experiment (Table III) large quantities of several individual dispensable amino acids were tested to determine if any would inhibit growth. No marked inhibitions occurred with any of the amino acids tested at high levels; *i.e.*, with glutamic acid, glycine, proline, asparagine, or alanine. Slight depressions occurred, however, when the proline content of the diet was increased to 3% ($p = .07$; group II-i vs II-g) or when the alanine content was increased to 5% ($p > .10$; group II-m vs II-k). Deletion of aspartic acid, alanine, and serine also appeared to depress weight gain ($p = .085$; group II-b vs II-c).

Experiments III and IV were designed to study some of the interrelationships among arginine, proline, glutamic acid, and possibly,

TABLE II. Effect of Diammonium Citrate on Weight Gain of Rats Fed Amino Acid Diets.

Diet no.	Percentage of dispensable amino acids used in diets									Average weight gain (g/2 weeks)
	Arg·HCl	Asp·NH_2	Ser	Pro	Gly	Glu	Asp	Ala	$(NH_4)_2$cit	
I-a	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	85.8 ± 2.2[a]
I-b	15.0% casein plus 0.3% L-methionine									95.3 ± 4.6
I-c	Stock diet									97.0 ± 3.7
I-d	1.35	0.6	0	1.0	0.5	4.5	0	0	2.47	95.7 ± 5.3
I-e	1.35	0.6	0	1.0	0.5	4.5	0	0	4.94	96.4 ± 3.4
I-f	1.35	1.0	0	0	0	0	0	0	6.97	60.3 ± 3.0
I-g	15% casein plus 0.3% L-methionine plus 4.94% $(NH_4)_2$ citrate									75.6 ± 4.4

[a] Average of six rats ± standard error.

TABLE III. Effect of Large Quantities of Individual Amino Acid on Weight Gain of Rats Fed Amino Acid Diets.

Diet no.	Percentage of dispensable amino acids used in diets							Average weight gain (g/2 weeks)
	Asp•NH_2	Ser	Pro	Gly	Glu	Asp	Ala	
II-a	0.6	0.35	1.0	1.4	4.5	0.35	0.35	98.0 ± 2.2[a]
II-b	0.6	0.35	1.0	1.4	4.5	0.35	0.35	100.6 ± 3.3
II-c	0.6	0	1.0	0.5	4.5	0	0	92.5 ± 2.4
II-d	0.6	0	1.0	0.5	6.0	0	0	98.3 ± 2.6
II-e	0.6	0	1.0	0.5	8.0	0	0	99.0 ± 4.4
II-f	0.6	0	1.0	2.0	4.5	0	0	100.3 ± 2.5
II-g	0.6	0	1.0	3.0	4.5	0	0	97.6 ± 1.3
II-h	0.6	0	2.0	0.5	4.5	0	0	97.1 ± 2.2
II-i	0.6	0	3.0	0.5	4.5	0	0	91.7 ± 2.6
II-j	2.0	0	1.0	0.5	4.5	0	0	95.8 ± 2.1
II-k	3.0	0	1.0	0.5	4.5	0	0	96.0 ± 2.5
II-l	0.6	0	1.0	0.5	4.5	0	3.0	97.8 ± 2.3
II-m	0.6	0	1.0	0.5	4.5	0	5.0	92.1 ± 2.5

[a] Average of six rats ± standard error. All diets contained 1.35% Arg • HCl and all but group II-a contained 2.47% ammonium citrate.

asparagine and glycine. Weight gains decreased markedly (Table IV) when all of the dispensable amino acids except arginine and asparagine (*i.e.*, no proline or glutamic acid) were omitted from the diet (see group III-d). However, growth was maximal when either proline or glutamic acid was left out completely if the other, plus other dispensable amino acids, were added at high enough levels (see group III-1 and IV-i).

In groups III-e, III-f, and III-g 0.6% arginine•HCl was sufficient for maximum growth, and 0.2% was not sufficient even when asparagine, proline, and glutamic acid were all fed at high levels (group III-f). According to the results of experiment III (Table IV), asparagine may be required for maximal growth (compare groups III-a and III-b to group III-i); however, no response was seen to graded levels of asparagine from

TABLE IV. Effect of Arginine, Proline, Asparagine, and Glutamic Acid on Weight Gain of Rats Fed Amino Acid Diets.

Diet no.	Percentage of dispensable amino acids used in diets										Average weight gain, g/2 weeks
	Arg• HCl	Asp• NH_2	Ser	Pro	Gly	Glu	Asp	Ala	Glu• NH_2	$(NH_4)_2$cit	
III-a	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	0	96.0 ± 2.5[a]
III-b	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	2.47	92.2 ± 1.7
III-c	18% casein plus 0.3% L-methionine										98.4 ± 1.8
III-d	2.0	3.0	0	0	0	0	0	0	0	5.0	66.8 ± 2.4
III-e	0.6	1.0	0.35	2.0	1.4	5.5	0.35	0.35	0	1.65	95.8 ± 3.3
III-f	0.2	2.0	0.35	2.0	1.4	8.0	0.35	0.35	0	2.0	79.0 ± 3.1
III-g	0.2	1.0	0.35	2.0	1.4	5.5	0.35	0.35	0	2.47	76.4 ± 1.9
III-h	1.35	0	0.35	1.0	1.4	0	0	0	4.5	0.73	86.5 ± 1.6
III-i	1.35	0	0.35	1.5	1.4	5.2	0.35	0.35	0	2.47	85.0 ± 1.9
III-j	2.0	1.0	0.35	2.0	2.0	0	0.35	0.35	0	1.96	88.2 ± 2.5
III-k	1.35	1.0	0.35	0	1.4	8.0	0	0	0	0.82	92.2 ± 0.7
III-l	2.0	2.0	0.35	0	2.0	8.0	0.35	0.35	0	2.47	99.0 ± 2.1

[a] Average of six rats ± standard error.

TABLE V. Effect of Arginine, Asparagine, and Glutamine on Weight Gain of Rats Fed Amino Acid Diets.

Diet no.	Percentage of dispensable amino acids used in diets										Average weight gain, g/2 weeks
	Arg·HCl	Asp·NH_2	Ser	Pro	Gly	Glu	Asp	Ala	Glu·NH_2	$(NH_4)_2$cit	
IV-a	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	0	93.2 ± 2.4[a]
IV-b	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	2.47	102.0 ± 2.8
IV-c	1.35	0.2	0.35	1.0	1.4	4.5	0.35	0.35	0	0	95.2 ± 2.9
IV-d	1.35	0.3	0.35	1.0	1.4	4.5	0.35	0.35	0	0	92.6 ± 2.5
IV-e	1.35	0.4	0.35	1.0	1.4	4.5	0.35	0.35	0	0	93.5 ± 3.9
IV-f	0.3	2.0	0.35	2.0	1.4	8.0	0.35	0.35	0	2.00	93.3 ± 1.2
IV-g	0.4	2.0	0.35	2.0	1.4	8.0	0.35	0.35	0	2.00	95.0 ± 2.1
IV-h	0.5	2.0	0.35	2.0	1.4	8.0	0.35	0.35	0	2.00	93.8 ± 2.7
IV-i	2.0	2.0	0.35	2.0	2.0	0	0.35	0.35	0	1.96	98.1 ± 2.6
IV-j	2.0	2.0	0.35	2.0	2.0	0.5	0.35	0.35	0	1.96	92.3 ± 1.8
IV-k	2.0	2.0	0.35	2.0	2.0	1.0	0.35	0.35	0	1.96	94.8 ± 2.6
IV-l	2.0	2.0	0.35	2.0	2.0	2.0	0.35	0.35	0	1.96	95.8 ± 3.2
IV-m	2.0	2.0	0.35	2.0	2.0	4.0	0.35	0.35	0	1.96	95.9 ± 2.5
IV-n	1.35	0	0.35	1.0	1.4	0	0.35	0.35	8.0	0.73	92.8 ± 1.7
IV-o[b]	0.68	0.3	0.18	0.5	0.7	2.3	0.18	0.18	0	0	45.5 ± 4.4
IV-p[b]	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	0	36.9 ± 1.9
IV-q[c]	1.35	0.6	0.35	1.0	1.4	4.5	0.35	0.35	0	0	55.8 ± 4.3

[a] Average of six rats ± standard error.

[b] Diets o and p contained one-half as much of each amino acid as the basal except for those listed in the table.

[c] Diet q contained two-thirds as much of each amino acid as the basal diet (cys-cys and tyr were the same as the basal) except for those listed in the table.

0.3–0.6% (see groups IV-a, c, d, and e).

In experiment IV (Table V) no response to graded levels of arginine hydrochloride from 0.3–0.5% was observed (groups IV-f, g, and h). Group IV-i shows that maximal weight gain can be obtained without any glutamic acid in the diet.

When the indispensable amino acids were decreased to ½ the usual concentration, weight gain was markedly depressed (group IV-p) but when the dispensable amino acids were also decreased by ½, weight gain was somewhat improved (group IV-o).

Discussion. The results taken together indicate that no requirement for specific dispensable amino acids can be established for maximal growth of the rat since any one of them can be omitted from certain diets without retarding growth. This is in contrast to the statement of Birnbaum *et al.* (4) that "No amino acid is 'nonessential' for maximum growth." However, if two or more of the glutamic–proline–arginine group of amino acids are omitted, growth is drastically reduced.

Apparently, the rat cannot synthesize both arginine and proline fast enough from glutamic acid to maintain maximal weight gain. Each can spare the other, apparently because of the common intermediate, glutamic acid semialdehyde. The limiting step is apparently not in the synthesis of glutamic acid but between glutamic acid and ornithine since maximal growth rates were obtaned without glutamic acid in the diet. In an earlier study (1) over 1% of arginine was necessary for maximal growth. Others have reported 0.8% arginine (5) or 0.5% arginine (6) as the requirement for the weanling rat. If all other amino acids are present in excess, it would appear from the present study that the minimal requirement must be near 0.3% (see group IV-f and III-f). King (16) has also found that arginine appears to contribute greatly to the metabolic demands for nitrogen. Many subtle relationships exist among amino acids which influence the need for one or a group of amino acids. An increase in the ammonia load can increase the need for arginine (7). The adverse effect of an excess of lysine in the diet can be alleviated by additional arginine (8).

Although ammonia (either ammonium citrate or ammonium acetate) will improve the growth rate of rats fed only the indispensable amino acids (9), there does not appear to be any benefit from an additional ammonia source if total dispensable amino acid nitrogen is not low. Although all combinations were not tested (the possible number of trials required in order to study each dispensable amino acid at several levels in combination with all of the others is staggering), it would appear that if the necessary quantities of the carbon chains of the indispensable amino acids are added, then the rest of the nitrogen needed can come from almost any source. It would be interesting to test this hypothesis since, in the present study, total nitrogen was always in excess of actual need. Stucki and Harper (10) found graded responses to increments of dispensable amino acids; but found the indispensable to dispensable amino acid ratio not too critical when minimal quantities of dispensable amino acids were provided.

Rose and co-workers (11) found that a single source of dispensable nitrogen was inferior to a mixture of dispensable amino acids and others (6, 12, 13) have reported the need for a mixture of dispensable amino acids in order to achieve maximal weight gains.

The relationships among the dispensable amino acids that determine the need for asparagine are not clear. Breuer and co-workers (13) as well as Rogers and Harper (1) showed a clear-cut need for asparagine, yet others (6, 14) have not been able to show a need for asparagine. In the present study (see groups III-a, b, and i, Table IV), asparagine clearly stimulated growth in one experiment, and yet, in another experiment (group IV-a, Table V) near maximal weight gains were obtained without asparagine; and, in the latter experiment no improvement was obtained by raising asparagine from 0.3 to 0.6%.

Hepburn and co-workers (15) have shown that glutamine increases growth and have traced part of the benefit to glutamine counteracting the adverse effect of high glycine in their diets. We did not note any special effect of glutamine with our particular mixture of amino acids.

An interesting point is that a dispensable amino acid mixture which will support maximal growth when adequate levels of the indispensable amino acids are present (group IV-a) will depress growth when included in the same amount in a diet with lower levels of indispensable amino acids (see groups IV-o and IV-p, Table V). This situation resembles an amino acid imbalance, and the growth depression is corrected by raising the levels of all of the indispensable amino acids (group IV-g). It would be interesting to know if only one indispensable amino acid might have improved the growth.

Summary. Various quantities of dispensable amino acids have been fed to rats in an attempt to determine what combination is best for maximal growth. The quantity of one dispensable amino acid that was necessary for maximal growth has been shown to depend upon the quantity of one or more other dispensable amino acids. In fact, individual groups of rats which were fed diets that were devoid of one of the following: glutamic acid, proline, aspartic acid, and asparagine, alanine, serine, or glutamine and glutamic acid, grew maximally. Thus, no minimal requirements could be established for the above amino acids. However, a minimal requirement for arginine was found to be about 0.3% of the diet even when proline and glutamic acid were both added in excess.

1. Rogers, Q. R., and Harper, A. E., J. Nutr. **87**, 267 (1965).

2. Harper, A. E., J. Nutr. **67**, 109 (1959).

3. Block, R. J., and Bolling, D., "The Amino Acid Composition of Proteins and Foods." Thomas, Springfield, Illinois (1951).

4. Birnbaum, S. M., Winitz, M., and Greenstein, J. P., Arch. Biochem. Biophys. **72**, 428 (1957).

5. Hepburn, F. N., and Bradley, W. B., J. Nutr. **84**, 305 (1964).

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

7. DuRuisseau, J. P., Greenstein, J. P., Winitz, M.,

and Birnbaum, S. M., Arch. Biochem. Biophys. **64**, 355 (1956).

8. Jones, J. D., Wolters, R., and Burnett, P. C., J. Nutr. **89**, 171 (1966).

9. Lardy, H. A., and Feldott, G., J. Biol. Chem. **186**, 85 (1950).

10. Stucki, W. P., and Harper, A. E., J. Nutr. **78**, 278 (1962).

11. Rose, W. C., Oesterling, M. J., and Womack, M., J. Biol. Chem. **176**, 753 (1948).

12. Sauberlich, H. E., J. Nutr. **74**, 298 (1961).

13. Breuer, L. H., Jr., Pond, W. G., Warner, R. G., and Loosli, J. K., J. Nutr. **82**, 499 (1964).

14. Salmon, W. D., J. Nutr. **82**, 76 (1964).

15. Hepburn, F. N., and Bradley, W. B., J. Nutr. **94**, 504 (1968).

16. King, K. W., Fed. Proc. **22**, 1115 (1963).

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