EFFECT OF HYDROCHLORIDE-FREE AMINO ACID MIXTURE ON GROWTH AND FOOD INTAKE IN RATS

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The effect of hydrochloride-free, casein-simulated amino acid mixture on growth and food intake in young rats was studied. Weight gain of rats fed a hydrochloride-free amino acid diet ad libitum was greater than that of rats fed an amino acid diet containing basic amino acids in hydrochloride form due to an increase in food intake. A hydrochloride-free amino acid diet was, however, still considerably inferior to a casein diet in supporting growth. Addition of sodium bicarbonate to the amino acid diet containing amino acid hydrochloride had no effect on rat growth.

There have been many reports on the comparison of nutritional effect of purified amino acid mixtures with that of intact protein. In an exact comparative study, strict similarity not only in the amino acid pattern of diet but also in the other dietary components is required first of all. In earlier studies, however, amino acid mixtures used did not simulate to the intact protein, or the amino acid diet contained much larger amounts of chloride than the protein diet because the basic amino acids were supplied in hydrochloride form. The excess dietary chloride supplied as amino acid hydrochloride was found to depress the growth rate and food intake of chicks (1, 2) and rats (3). Additional study showed that acidosis was produced by supplement of dietary chloride in excess (4, 5).

In our previous paper (6), the slower growth rate and lower food intake of rats fed amino acid diet with 3.2% nitrogen content compared with those of rats fed isonitrogenous casein diet were reported. The amino acid diet used was similar to the casein diet in amino acid pattern but not in chloride content because the amino acid diet contained lysine, arginine and histidine in the hydrochloride form. The possibility exists, therefore, that the difference in the chloride content may be one of the factors causing the slower growth rate of rats fed the amino

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acid diet. The present study was designed to compare the nutritional effect of a hydrochloride-free, casein-simulated amino acid mixture with that of intact casein, as well as to clarify the effect of dietary excess chloride on rat growth with purified amino acid diets.

MATERIALS AND METHODS

Animals and diets. Male rats of the Wistar strain were used in all experiments. They were individually housed in wire cages in an air conditioned room (23 ± 1°C) with a 12-hr light-dark cycle.

The compositions of the casein diet (diet C) and the amino acid diet containing basic amino acids in hydrochloride form (diet B) were identical to those previously used (6). The amino acid mixture was patterned after the amino acid composition of casein. The amino acid composition of the hydrochloride-free amino acid diet (diet F) is shown in Table 1 along with that of diet B. With the exception that half the amount of glutamic acid in diet B was replaced by glutamine.

### Table 1. Composition of amino acid mixture in diets F and B.

| Component                  | Diet F | Diet B |
|----------------------------|--------|--------|
| L-Arginine·HCl             | —      | 0.95   |
| L-Arginine·L-Glutamate     | 1.46   | —      |
| L-Histidine                | 0.69   | —      |
| L-Histidine·HCl·H2O        | —      | 0.93   |
| L-Isoleucine               | 1.03   | 1.03   |
| L-Leucine                  | 1.87   | 1.87   |
| L-Lysine·HCl               | —      | 2.00   |
| L-Lysine·L-Aspartate       | 1.19   | —      |
| L-Lysine·L-Glutamate       | 1.99   | —      |
| L-Methionine               | 0.63   | 0.63   |
| L-Cystine                  | 0.08   | 0.08   |
| L-Phenylalanine            | 1.04   | 1.04   |
| L-Tyrosine                 | 1.10   | 1.10   |
| L-Threonine                | 0.87   | 0.87   |
| L-Tryptophan               | 0.22   | 0.22   |
| L-Valine                   | 1.28   | 1.28   |
| L-Alanine                  | 0.81   | 0.81   |
| L-Aspartic acid            | —      | 0.56   |
| L-Asparagine               | 0.85   | 0.85   |
| L-Glutamic acid            | 0.57   | 4.44   |
| L-Glutamine                | 2.22   | —      |
| Glycine                    | 0.37   | 0.37   |
| L-Proline                  | 2.11   | 2.11   |
| L-Serine                   | 1.11   | 1.11   |
| Diammonium citrate         | 1.13   | 2.81   |
| Total                      | 22.62  | 25.06  |
HYDROCHLORIDE-FREE AMINO ACID MIXTURE

in diet F, both the amino acid mixtures were similar in amino acid pattern. In the amino acid mixture of diet F, arginine hydrochloride was replaced by arginine glutamate, histidine hydrochloride monohydrate by histidine, and lysine hydrochloride by lysine aspartate and lysine glutamate. Both the amino acid mixtures of diets B and F were made isonitrogenous with casein of diet C by diammonium citrate. Diet D was prepared by addition of sodium bicarbonate at 2% level into diet B.

The diets contained: (in %) casein, 23.3 in diet C or amino acid mixture, 25.1 in diets B and D or 22.6 in diet F; corn starch, 40.0; corn oil, 8.0; cellulose, 2.0; salt mixture (6), 4.0; sodium bicarbonate, 2.0 only in diet D; vitamin mixture in lactose (6), 0.85; choline chloride, 0.15; and sucrose to make each diet 100%. Retinyl acetate, ergocalciferol and all-rac.-α-tocopherol were included at concentrations of 2,000 IU, 200 IU and 10 mg per 100 g of diet, respectively. All diets contained 3.2% nitrogen. The calculated amounts of dietary chloride were 18.0 mEq/100 g of diets C and F, and 37.7 mEq/100 g of diets B and D. The sodium contents were 17.1 mEq/100 g of diets C, B and F, and 41.0 mEq/100 g of diet D. The pH values of diets C, B, D and F were 6.2, 4.4, 6.7 and 6.8 in the state of 10% suspension, respectively.

Ad libitum feeding experiment. Rats weighing 70 to 90 g were separated into four groups of eight rats each. Diets and water were supplied ad libitum for 14 days. Weight gain and food intake were recorded daily through the experimental period.

Space-pair-feeding experiment. Amino acid diets B and F were space-pair-fed according to the method previously described (6). Prior to the regular feeding period, the rats were trained for 7 days to consume the diet in a short period twice daily. Rats weighing 80 to 100 g were separated into two groups of 12 rats each. One group of rats was space-fed diet B twice daily (9:00 AM to 10:00 AM, 5:00 PM to 6:00 PM) for 14 days. The other group was space-fed the same amounts of diet F twice daily with those of diet B consumed. Diets in which corn starch was replaced by α-corn starch were kneaded into dumplings with an equal weight of distilled water and the food intake was calculated on the dried basis every meal. Weight gain was recorded daily through the 14-day experimental period.

Statistical analysis. Data were treated statistically using Student's t-test (7).

RESULTS

Ad libitum feeding experiment

Weight gain and food intake of rats fed the experimental diets for 14 days are shown in Table 2. Maximum weight gain was achieved with casein diet C. A slower growth rate and lower food intake were observed with amino acid diet B containing arginine, histidine and lysine in hydrochloride form. Addition of sodium bicarbonate to diet B to neutralize the hydrochlorides of the basic amino
Table 2. Weight gain and food intake of rats fed experimental diets ad libitum for 14 days.

| Diet                                      | Weight gain\(^a\) | Food intake\(^b\) |
|-------------------------------------------|--------------------|-------------------|
| Casein diet (diet C)                      | g/14 days          | g/14 days         |
| Amino acid diet, contg. HCl (diet B)      | 83±2.7\(^b\)ed    | 185±5.2\(^f\)fg   |
| Diet B+NaHCO\(_3\) (diet D)              | 52±2.7\(^b\)       | 143±5.1\(^f\)h     |
| Amino acid diet, HCl-free (diet F)        | 51±3.0\(^e\)       | 141±5.1\(^f\)i     |
| Amino acid diet, HCl-free (diet F)        | 59±2.7\(^d\)       | 157±3.6\(^ghi\)    |

\(^a\) Mean \pm SEM of eight rats.
\(^b, c, d, e, f, g\) Significantly different \(p < 0.001\).
\(^h, i\) Significantly different \(p < 0.05\).

Acids (diet D) had no effect on the growth rate and food intake and a similar result was also obtained when the experiment was repeated. On the other hand, rats fed diet F containing hydrochloride-free amino acid mixture showed weight gain somewhat greater than that of rats fed diet B. The difference in weight gain was not significant \(p > 0.05\), but was always observed in repeated experiments. The difference in food intake between two dietary groups (diets B and F) was statistically significant \(p < 0.05\). The results mentioned above indicate that weight gain and food intake are influenced by excess dietary chloride rather than by dietary sodium-to-chloride ratio.

With respect to the comparison of diet F with casein diet C, rats fed diet F were still considerably inferior to those fed diet C \(p < 0.01\) both in weight gain and food intake despite of the equal level of dietary chloride. The differences between both the dietary groups, therefore, can not be connected with dietary chloride level.

![Fig. 1. Changes in weight gain of rats space-pair-fed diet B (---○---) and diet F (---●---) for 14 days. Each point represents the average of eight rats. Diet B; Amino acid diet containing basic amino acids in hydrochloride form. Diet F; Amino acid diet containing amino acids in hydrochloride-free form.](image-url)
Space-pair-feeding experiment

The experiment was conducted to clarify the cause of the deleterious effect of excess chloride on rat growth. Weight gain of rats space-pair-fed the experimental amino acid diets is shown in Fig. 1. Average weight gain on diet F (56 g/14 days) was the same as that on diet B. Average food intake in space-pair-feeding experiment was 141 g/14 days and was almost the same with that of rats fed diet B ad libitum (143 g/14 days). This result indicates that the difference in growth rate between two amino acid groups in ad libitum feeding experiment is due to the difference in food intake of the two groups.

DISCUSSION

The excess chloride supplied by the crystalline form of amino acids has been reported to lower the weight gain of experimental animals (1-3). The deleterious effect could be counteracted by addition of extra sodium and potassium in conjunction with metabolizable anions such as bicarbonate and acetate. BREUER et al. (3) demonstrated that growth rate and food intake increased significantly when an amino acid diet containing arginine, histidine and lysine in hydrochloride form was supplemented with levels of 2 or 4% sodium bicarbonate. However, reports on the efficiency of sodium supplementation to high chloride diet are inconsistent. In the study of WOMACK and WILSON (8), addition of sodium bicarbonate to neutralize the dietary amino acid hydrochlorides had no effect on nitrogen gain of rats. In our experiments, the amino acid dietary groups were the same both in growth rate and in food intake whether the hydrochlorides in the amino acid diet were neutralized with sodium bicarbonate or not, supporting the results of WOMACK and WILSON. Feeding of an amino acid mixture without hydrochloride (diet F) resulted in an increase in growth rate (Table 2). This improvement was demonstrated to be due to the increase in food intake by space-pair-feeding experiment (Fig. 1). Although diet F contained some glutamine in place of glutamic acid, dietary glutamine had no effect on rat growth (6, 9). In our previous paper (10) the difference in voluntary food intake between the casein and amino acid groups was indicated to be mainly due to the stomach distension after feeding. In a preliminary experiment, however, the stomach of rats force-fed both amino acid diets B and F was found to distend to a similar degree. Therefore, the increase in food intake of rats fed diet F ad libitum seems to be not connected with stomach distension but with others such as taste of diet.

With respect to the comparison of nutritional effect of amino acid mixtures with intact protein, many investigators have suggested that the excess chloride in diet is one of the major causes of the inferiority of amino acid diets in supporting growth (1, 3). However, there has been very little comparative study using a purified amino acid mixture without amino acid hydrochloride. In our experiments reported here, the amino acid mixture used was patterned after the amino acid composition of casein and contained no hydrochloride. The result showed
that amino acid diet F had still considerably inferior effect on casein diet C in weight gain and food intake although the removal of hydrochloride from amino acid mixture was effective in improvement of rat growth (Table 2). The stomach of rats force-fed diet F was found to distend much more than that of rats fed diet C in a preliminary experiment. It was therefore apparent that the depressed growth rate and food intake of rats fed diet F was attributed to the stomach distension after feeding due to the lower molecularity of amino acids as previously discussed (10).

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