THE RELATIONSHIP BETWEEN WEIGHT GAIN AND FREE AMINO ACID CONCENTRATION OF PLASMA AND LIVER IN RATS FED A DIET SUPPLEMENTED WITH VARIOUS AMOUNTS OF LYSINE

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This study was conducted to determine the effect of feeding graded levels of dietary lysine on weight gain and free amino acid concentrations in blood plasma and liver of weanling rats. Animals were fed on diets containing 11.6% wheat gluten (equivalent in nitrogen to a 10% casein diet) supplemented with graded levels of L-lysine·HCl (0 to 10%) for 14 days. An outline of the results obtained follows:

1) Maximum weight gain was observed with the groups fed the lysine supplement in the 0.64 to 1.8% range. Growth declined with further increase of dietary lysine.

2) Blood and liver were sampled after decapitation 6 hr after the final feeding on the 14th day, and amino acid concentrations were determined. Plasma lysine concentrations rose rapidly as dietary lysine increased and reached the maximum level with the 1.8% lysine supplementation. With the large supplement diet, the lysine concentrations maintained a plateau. Plasma threonine levels were high when dietary lysine was low.

3) Similar responses of lysine and threonine concentrations in liver to the increase of dietary lysine was also observed, but the effect was markedly less than those of plasma. Most other amino acids in plasma and liver were nearly unchanged even when the dietary lysine was varied over a wide range.

Extensive studies (1-3) have been made on the relationship between weight gain and the concentration of free lysine in the blood plasma of animals fed

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a lysine-deficient diet supplemented with graded levels of lysine. MORRISON et al. (1) reported that plasma lysine concentrations of growing rats increased by elevating the lysine content in the diet and reached a maximum level at a lysine intake somewhat larger than that required for maximum growth. They also noted that plasma threonine levels showed a reciprocal relationship with those of free lysine. ZIMMERMAN and SCOTT (2) found that there was a definite relationship between plasma lysine level and the amino acid requirement of chicks.

It was demonstrated by previous studies (4, 5) that the addition of excess lysine to a low casein diet resulted in growth-depression, but the effect of this excess intake was slight as compared with other essential amino acids (4), and that when wheat gluten was used as protein source the addition of 5% lysine·HCl in the diet resulted in a better growth than the control (5).

Thus, in an attempt to investigate quantitatively the effect of lysine supplementation on the growth of rat, groups of young rats were fed a wheat gluten diet supplemented with lysine of graded amount in wide range, and their growth rate was compared among various groups, and the relationship between the weight gain and the lysine concentrations in plasma and liver was also studied with those rats.

EXPERIMENTAL

Male weanling rats of the Donryu strain weighing about 60 to 65g were randomly divided into 9 groups of 4 to 6 animals each, and were maintained individually in suspended cages at 25°C in a room with a 12-hr light-dark cycle. The composition of the basal lysine-deficient diet containing 11.6% wheat gluten (equivalent to the nitrogen content of 10% casein) is shown in Table 1. The lysine content in this basal lysine-deficient diet was 0.14% and this was confirmed by amino acid analysis as seen in Table 2. Experimental diets were prepared by the addition of graded levels (0, 0.1, 0.3, 0.6, 1.0, 2.0, 3.0, 5.0, and 10%) of L-lysine·HCl* to the basal diet at the expense of α-starch. Diet in paste form and water were fed ad libitum during the 14-day experimental period. The animals were weighed each morning just before feeding and the consumed food was measured in dry form.

At the end of the experimental period, each rat was offered its respective diet for 1 hr in the morning and fasted 6 hr. Animals were killed by decapitation and the blood was collected into a heparinized centrifuge tube. After centrifugation in the cold, samples of plasma from rats of each group were pooled and were deproteinized with equal volumes of 3% sulfosalicylic acid solution (6). The protein-free filtrate was diluted with equal volumes of pH 2.2 citrate buffer for amino acid analysis.

Each decapitated rat was perfused with ice-cold 0.9% NaCl solution until

* Tanabe Amino Acid Research Foundation, Osaka.
Table 1. Composition of basal wheat gluten diet.

| Ingredient                  | % of diet |
|-----------------------------|-----------|
| Wheat gluten<sup>a</sup>    | 11.6      |
| α-Potato starch             | 72.7      |
| Soybean oil                 | 5.0       |
| Salt mixture<sup>b</sup>    | 5.0       |
| Vitamin mixture<sup>b</sup> | 1.0       |
| Choline chloride            | 0.1       |
| NaHCO<sub>3</sub><sup>c</sup> | 0.0       |
| Cellulose powder            | 4.6       |
| Total                       | 100.0     |

<sup>a</sup> Hi-Pro (Vital Gluten), Shin-shin Food Industry Co., Ltd., Tokyo; the protein content on the basis of Kjeldahl N × 5.70 was 77.0%.
<sup>b</sup> Harper, A. E., *J. Nutr.*, 68, 405 (1959); these mixtures were purchased from Tanabe Amino Acid Research Foundation, Osaka. The diet also contained 0.07 ml of Chocora A (Eizai Co., Ltd., Tokyo) per 100 g as a source of vitamins A and D.
<sup>c</sup> When the experimental diet containing L-lysine·HCl was prepared, sufficient amount of sodium bicarbonate was included as a replacement of cellulose powder to neutralize the hydrochloride of lysine·HCl.

Table 2. Amino acid composition of the wheat gluten and the content in the basal diet.

The wheat gluten contained 13.2% N. It was hydrolyzed in 6 N HCl at 121°C in an autoclave for 18 hr and the amino acid content of the hydrolyzates was determined chromatographically by an amino acid analyzer.

| Amino acid      | g/16 g N | % in basal diet |
|-----------------|----------|-----------------|
| Lysine          | 1.43     | 0.14            |
| Histidine       | 1.68     | 0.16            |
| Arginine        | 3.20     | 0.31            |
| Threonine       | 2.38     | 0.23            |
| Valine          | 4.72     | 0.45            |
| Methionine      | 1.38     | 0.13            |
| Isoleucine      | 4.25     | 0.41            |
| Leucine         | 5.34     | 0.51            |
| Tyrosine        | 2.62     | 0.25            |
| Phenylalanine   | 5.40     | 0.52            |
| Aspartic acid   | 4.21     | 0.40            |
| Serine          | 3.38     | 0.32            |
| Glutamic acid   | 33.5     | 3.21            |
| Proline         | 11.8     | 1.13            |
| Glycine         | 3.06     | 0.29            |
| Alanine         | 2.64     | 0.25            |
the liver was cleared of all blood. The liver was quickly removed, weighed, and then deproteinized in glass homogenizer with a 5-fold amount of 3% sulfosalicylic acid solution. An aliquot of deproteinized filtrate of each group was pooled, and diluted with equal volumes of the pH 2.2 buffer for amino acid analysis.

Amino acids were determined by ion-exchange chromatography using a JEOL Model JLC-5AH amino acid analyzer.* A standard amino acid mixture was included in each automatic run of 6 to 12 samples. Serine, glutamine and asparaginase were not separated by this chromatography, and therefore this fraction was reported as serine (5).

RESULTS

Average weight gain and feed efficiency (gain/feed ratio) of rats fed graded levels of dietary lysine are shown in Table 3. The weight gain increased rapidly in response to supplemented lysine and reached a maximum at 0.64% of dietary lysine (0.6% lysine·HCl addition); the maximum weight gain levels remained constant until 1.79% lysine content (2.0% lysine·HCl addition), and then declined gradually as the dietary lysine level increased, and at 8.4% lysine level (10% lysine·HCl addition) only a slight gain occurred. The values of feed consumption and gain/feed ratio followed a pattern similar to those for weight gain.

The plasma and liver amino acid concentrations are summarized in Table 4 and 5. The lowest and almost constant plasma lysine concentrations were ob-

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* Japan Electron Optics Laboratory Co., Ltd., Tokyo.
Table 4. Plasma free amino acid concentrations of rats fed graded levels of lysine for 14 days.

| Dietary lysine content (%) | Plasma free amino acid concentrations (\(\mu\)mole/100 ml) |
|---------------------------|----------------------------------------------------------|
|                           | Lys  His  Arg  Thr  Val  Met  Ile  Leu  Tyr  Phe  Asp  Ser  Glu  Pro  Gly  Ala |
| 0.14 (0.0)\(^b\)          | 20     10   62    17    3    8    12    4    5    3   140    37    10    78    51 |
| 0.22 (0.1)                | 24     12   42    21    1    4    8    2    2    1   143    47    10    70    45 |
| 0.39 (0.3)                | 46     11   14    13    20   2    6    8    4    4   nd\(^c\)  139   53    10    62    51 |
| 0.64 (0.6)                | 58     10   15    10    14   2    7    8    4    7   1   136   44    10    62    54 |
| 0.97 (1.0)                | 70     12   16    7     27   2    5    8    5    3   2   133   37    10    74    48 |
| 1.79 (2.0)                | 87     14   14    5     16   2    5    8    3    4   nd\(^c\)  131   40    11    70    50 |
| 2.62 (3.0)                | 85     8    13    5     9    2    4    10   4    4   nd\(^c\)  110   30    10    58    48 |
| 4.28 (5.0)                | 80     12   11    5     11   1    8    12   4    5   1   124   34    10    42    71 |
| 8.40 (10.0)               | 80     10   12    8     8    1    5    8    2    4   2   119   36    8     58    59 |

\(^a\) Values represent samples pooled from groups with 4 animals each, except for the 4.28 and 8.40% lysine content groups which consisted of 6 animals each.

\(^b\) Figures in parentheses indicate the amount (\%) of lysine-HCl supplemented in lysine-deficient diet.

\(^c\) None detectable.

In this experiment, the maximum weight gain and gain/feed ratio were observed when dietary lysine content was 0.14% (basal lysine-deficient diet) to 0.22% (0.1% lysine-HCl addition), then the concentration rose rapidly with increasing level of dietary lysine (2.0% lysine-HCl addition), but did not result in any further increase thereafter. On the other hand, the plasma threonine concentration was exceptionally high when the dietary lysine was low, but the plasma threonine concentration decreased sharply when the dietary lysine levels were 1.0% or more. Similar responses were also observed for liver free lysine and threonine concentrations, but the ranges of the changes were markedly less than those of plasma. Most other amino acid concentrations of plasma and liver were either unaffected or only slightly changed over the entire range of the dietary lysine levels, except that the serine tended to be lower with the increase of dietary lysine.

**DISCUSSION**

In this experiment, the maximum weight gain and gain/feed ratio were observed at a dietary lysine level of 0.64%. This value for lysine requirement is in fair agreement with those reported by McLAUGHLAN and ILLMAN (7) (0.65%) and by BROOKES et al. (8) (0.65%), and is close to that reported by STOCKLAND et al. (3) (0.60%), but is somewhat lower than the values of 1.0, 0.9, 0.84 and 0.8% reported by WILLIAMS et al. (9), RAMA RAO et al. (10), BRESSANI and MERTZ (11), and MORRISON et al (1), respectively.

The results of weight response also indicate that a great growth-depression occurs in rats fed a high-lysine diet, as well as in animals receiving lysine-deficient diets. The shape of the response curve differed from those obtained previously (4).
Table 5. Liver free amino acid concentrations of rats fed graded levels of lysine for 14 days.

| Dietary lysine content (%) | Liver weight (g) | Liver free amino acid concentrations (∝μmole/g wet liver)
|---------------------------|------------------|-------------------------------------------------|
|                           | 2.3 ± 0.1**      | Lys  | His  | Thr  | Val  | Met  | Ile  | Leu  | Tyr  | Phe  | Asp  | Ser  | Glu  | Pro  | Gly  | Ala  |
| 0.14 (0.0)b               | 2.00             | 1.16 | 4.44 | 1.12 | 0.45 | 0.52 | 1.48 | 0.55 | 0.62 | 2.72 | 7.00 | 2.30 | 0.35 | 3.84 | 4.07 |
| 0.22 (0.1)                | 2.35             | 1.39 | 5.76 | 1.34 | 0.52 | 0.60 | 1.66 | 0.61 | 0.72 | 2.98 | 7.19 | 3.50 | 0.58 | 4.16 | 5.31 |
| 0.39 (0.3)                | 3.60             | 1.20 | 5.26 | 1.16 | 0.47 | 0.50 | 1.39 | 0.50 | 0.61 | 3.75 | 6.35 | 5.72 | 0.48 | 4.69 | 5.23 |
| 0.64 (0.6)                | 3.12             | 1.58 | 1.20 | 1.56 | 0.60 | 0.67 | 1.88 | 0.68 | 0.78 | 3.10 | 8.44 | 4.08 | 0.66 | 4.80 | 6.05 |
| 0.97 (1.0)                | 3.75             | 1.10 | 1.20 | 1.46 | 0.49 | 0.56 | 1.61 | 0.47 | 0.66 | 2.24 | 8.14 | 2.59 | 0.34 | 4.00 | 6.09 |
| 1.79 (2.0)                | 3.6 ± 0.1        | 2.40 | 1.13 | 0.70 | 1.13 | 0.47 | 0.54 | 1.48 | 0.60 | 0.62 | 2.61 | 5.16 | 2.48 | 0.28 | 3.32 | 5.48 |
| 2.62 (3.0)                | 3.7 ± 0.2        | 2.95 | 1.25 | 0.90 | 0.70 | 0.55 | 0.75 | 1.12 | 0.42 | 0.49 | 2.44 | 5.58 | 1.50 | 0.36 | 3.00 | 3.22 |
| 4.28 (5.0)                | 2.9 ± 0.2**      | 3.74 | 1.23 | 2.17 | 1.04 | 0.53 | 0.80 | 1.96 | 0.74 | 0.86 | 3.00 | 5.60 | 4.70 | 0.66 | 5.67 | 5.21 |
| 8.40 (10.0)               | 2.6 ± 0.1**      | 3.27 | 1.16 | 1.90 | 1.37 | 0.62 | 0.62 | 1.30 | 0.68 | 0.77 | 2.39 | 5.78 | 3.24 | 0.66 | 4.71 | 5.49 |

*a* Values represent samples pooled from groups with 4 animals each, except for the 4.28 and 8.40% lysine content groups consisted of 6 animals each.

*b* Figures in parentheses indicate the amount (%) of lysine-HCl supplemented in lysine-deficient diet.

*c* Mean ± SE: different from 0.64% lysine content group at **P < 0.01.
in rats fed by 10% casein diet containing graded levels of methionine and glycine singly.

Many studies on animals (1–3, 7, 12–15) and men (16–18) have demonstrated that plasma amino acid concentrations can be utilized as response criteria for estimating the amino acid requirements. ZIMMERMAN and SCOTT (2) reported that plasma lysine of chicks does not accumulate when the dietary lysine is less than that required for maximum weight gain, at which point the plasma lysine level rises linearly. In this study, however, it was observed that the plasma lysine level at low dietary lysine content remained flat only within the narrow range from 0.14 to 0.22% which level was significantly lower than that of maximum growth. The response curve of plasma lysine in the present experiment showed a sigmoidal shape, and was rather similar to those observed by MORRISON et al. (1) and STOCKLAND et al. (3) when rats were fed graded dietary lysine ad libitum for a long-term period, whereas MCLAUGHLAN and ILLMAN (7) observed that the plasma lysine levels elevated almost linearly with increase of dietary lysine in a short-term study of rats. It may be suggested that the shape of the response curve is appreciably influenced by the feeding method and the experimental period. MORRISON et al. (1) reported that plasma lysine concentration reached a maximum at about the 1.0% dietary lysine level, whereas maximum growth was obtained at the 0.8% dietary lysine level. Our results indicated that the maximum weight gain was obtained at the 0.64% dietary lysine level, much lower than the approximately 1.8% level at maximum plasma lysine concentration. Thus the plateau point of plasma lysine concentration was not necessarily consistent with the maximum growth point. YOUNG et al. (18) recently indicated that in men plasma lysine levels do not appear to be a response criterion to assess the human maintenance requirement for this amino acid.

The present authors' findings on the inverse relationship between plasma lysine and threonine levels confirm those of other investigators (1, 19). Threonine has also been shown to increase when tryptophan intake is low in men (16). MORRISON et al. (1) suggested that plasma lysine/threonine ratio may be useful as a very sensitive indicator of lysine nutrition, and that the ratio approaches unity when the dietary lysine level is adequate for growth. As shown in Fig. 1, lysine/threonine ratio plotted against dietary lysine level increased almost linearly as the lysine in the diet was increased, and above approximately 1.8% of dietary lysine maintained a plateau. This bending point corresponded to the upper limit of the maximum gain, like that of the plasma lysine concentration. Threonine concentration in liver of rats receiving lysine-deficient diet also increased. This phenomenon may be related to the increase of nonutilizable threonine for protein synthesis in tissues and the decrease of liver threonine dehydrase of rats fed lysine-deficient diet.

The data of ZIMMERMAN and SCOTT (2), and OHNO and TASAKI (20) in hens indicate that other amino acid concentrations in plasma can also undergo various
alterations in birds fed diets containing graded levels of lysine, whereas the results of this study in rats indicate that other amino acid concentrations in plasma and liver showed no marked and consistent changes, except serine. This difference might be related, in part, to the difference in species and dietary conditions.

Even when dietary lysine level was high above 1.8% further elevation of the lysine in plasma and liver did not occur. This may be due to an induction of lysine catabolyzing enzyme in liver, with resulting increased degradation of the excessive lysine intake tending to maintain a constant plasma lysine level. Indeed, with the use of radioactively labeled lysine, BROOKES et al. (8) recently found that the oxidation of lysine does not increase when dietary lysine intake is low but elevates linearly with greater lysine intake.

The response of liver free lysine concentration for the graded level of dietary lysine was considerably less than that of the plasma. It seems improbable, therefore, that the liver lysine level can be utilized as a criterion of nutritional adequacy. In this connection, the report of PAWLAK and PION (12), who observed in rats that the free lysine level of muscle was even more sensitive for lysine intake than plasma lysine level, is of interest.

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