BODY WEIGHT CHANGE AND NITROGEN EFFICIENCIES IN GROWING AND ADULT RATS FED DIETS CONTAINING VARIOUS PROPORTIONS OF ESSENTIAL AMINO ACIDS TO TOTAL AMINO ACIDS

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Summary By using amino acid mixtures, a comparative study of nutritional effects of dietary essential amino acid proportions (EA %) has been made between growing and adult rats. Included were adult rats which were repleting from an 8-day protein depletion. It was shown that for growing rats at least 55 EA % was required to attain the maximum values of growth, nitrogen balance, nitrogen balance efficiency (nitrogen balance/nitrogen intake) and protein efficiency ratio (PER). The maximum biological value was found to be 94 at the 50 EA % level. For adult rats, at least 40 EA % was required to gain the maximum values of nitrogen balance and nitrogen balance efficiency under both maintenance and repletion. The biological values were found to be nearly 60 and 80 for maintenance and repletion, respectively.

The proportion of essential amino acids to total amino acids in diets is one of the important factors in the metabolism or physiological functions of animals. And it concurrently relates to what extent mankind should depend on animal protein or some essential amino acids as a future protein source. Although there have been several studies on the estimation of the appropriate level of dietary essential amino acids or of non-specific nitrogen including nonessential amino acids, few investigations have been made on their metabolic or physiological significance. In 1973 FAO/WHO presented suggested patterns of essential amino acid requirements expressed as mg per g of protein, equivalent to the E/T ratio, in which the proportion of total essential amino acids fell extraordinarily with

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The present study was undertaken to afford the fundamental data for estimating the optimal level of the dietary essential amino acid proportion in growing and adult rats. Measurements were made on body weight change, nitrogen efficiencies such as protein efficiency ratio (PER), biological value and nitrogen balance efficiency (nitrogen balance/nitrogen intake), liver and urine compositions, plasma enzyme activities, $^{15}$N-glycine recovery in urine, tissue free amino acid concentrations and food preference (2). In this paper, the results in body weight change and nitrogen efficiencies are presented.

**EXPERIMENTAL**

Throughout this study Wistar male albino rats, which were purchased from Clea Japan Co., Ltd., were used. A comparative investigation was made between growing rats, *i.e.* approximately 70 g, and adult ones, *i.e.* approximately 500 g, including those repleting from protein depletion. Experimental diets contained essential and nonessential amino acid mixtures at the 10% level in total, whose compositions simulated those of whole egg protein as indicated in the table of "The Amino Acid Composition of Foods in Japan" (3). The essential amino acid proportion in these mixtures was graded from 0 to 100%. This proportion will, hereafter, be briefly denoted as EA%.

The compositions of the mixtures are shown in Table 1, where arginine and histidine were allotted to the essential group even for adult rats, for the sake of convenience. Examples of diet composition are shown in Table 2. The E/T ratios for rats (+Arg·His) and humans (−Arg·His) are indicated at the bottom of each column. The proportion of essential amino acid in the amino acid mixture and the content of the amino acid mixtures in diets were adjusted by nitrogen con-

| Essential amino acid mixturea: |
|-------------------------------|
| l-Ile | 8.5 | l-Tyr | 6.2 |
| l-Leu | 13.7 | l-Thr | 7.5 |
| l-Lys·HCl | 14.2 | l-Trp | 2.6 |
| l-Met | 5.4 | l-Val | 10.6 |
| l-Cys·HCl | 5.1 | l-Arg·HCl | 12.5 |
| l-Phe | 8.3 | l-His·HCl·H2O | 5.6 |
| Total | | | 100.2 |

| Nonessential amino acid mixtureb: |
|-------------------------------|
| l-Ala | 13.4 | Gly | 7.7 |
| l-Asp | 21.8 | l-Pro | 9.6 |
| l-Glu | 30.3 | l-Ser | 17.2 |
| Total | | | 100.0 |

Nitrogen content: a 13.7%, b 12.2%.
Table 2. Composition of the main experimental diets (g/100 g diet).

|          | Essential amino acid percent (EA %) |
|----------|--------------------------------------|
|          | 0  | 20 | 40 | 60 | 80 | 100 |
| Essential amino acid mixture | 0.0 | 2.3 | 4.7 | 7.0 | 9.4 | 11.7 |
| Nonessential amino acid mixture | 13.1 | 10.5 | 7.9 | 5.2 | 2.6 | 0.0 |
| α Corn starch | 69.9 | 70.2 | 70.4 | 70.8 | 71.0 | 71.3 |
| Soybean oil | 9.9 |
| Cod liver oil | 0.1 |
| Salt mixture | 4.0 |
| Cellulose powder | 2.0 |
| Vitamin mixture | 0.85 |
| Choline chloride | 0.15 |

| E/T ratio | + Arg·Hisb | 0.0 | 1.4 | 2.7 | 4.1 | 5.4 | 6.8 |
|-----------|-------------|-----|-----|-----|-----|-----|-----|
| - Arg·Hisa | 0.0 | 1.1 | 2.3 | 3.4 | 4.6 | 5.7 |

Nitrogen level: 1.6. a Purchased from Tanabe Amino Acid Research Foundation, Osaka, Japan. Their compositions are almost identical with A. E. Harper's (J. Nutr., 68, 405 (1959)). b Inclusive in essential amino acids as the E/T ratio for rats. a Exclusive from essential amino acids as that for human.

tent multiplied by 6.25. The protein-free diet, which was given to adult rats before the repletion period, was prepared by replacing the amino acid mixtures with α corn starch.

Experiment 1. In this experiment, body weight change, protein efficiency ratio (PER), nitrogen balance, nitrogen balance efficiency and biological value were determined in growing and adult rats fed on the 0, 20, 40, 60 and 100 EA% diets during the 28-day experimental period. The repletion test was also conducted in adult rats after 8 days of protein depletion. Each dietary group contained 6 animals. Nitrogen balance, nitrogen balance efficiency, and biological value were determined for 6 days from the 3rd to 8th day of the experiments, with the exclusion of direct influences of the diet change. However, these determinations were made for the first 8 days in the repletion group.

The diets were given ad libitum to the animals. However, in the case of estimating the above nutritive values for the maintenance group in adult rats, a matched group was selected and the diet intake was adjusted to 15 g per day, that is from 80 to 90% of the mean unrestricted diet intake. The variance in food intake would have much influence on the values of nitrogen efficiencies, especially the biological value, in this case.

Urine and feces were collected in the metabolism cage during the nitrogen balance test period, and their nitrogen was determined by the Kjeldahl method. Endogenous urinary nitrogen (EUN) per body weight and metabolic fecal nitrogen (MFN) per food intake, which were used for the calculation of biological value, were estimated from the nitrogen excretion of the protein-free diet group measured at the same time. The values were as follows: EUN 26.3 mg/100 g body weight
and MFN 87.8 mg/100 g diet for growing rats, and EUN 15.5 mg/100 g body weight and MFN 112 mg/100 g diet for adult rats. The calculation of the biological value was made according to the methods of MITCHELL (1923–24) and MITCHELL et al. (1936), which have been adopted as a standard by the National Research Council (1963) (4), FAO/WHO (1965) (5) and FAO (1970) (6).

**Experiment 2.** A further experiment was conducted on body weight gain and nitrogen efficiencies of growing rats at more precisely graded levels of dietary EA %, as it is considered important to estimate a more exact dietary EA % level to give their maximum or maintenance value in the growing stage.

The animals, 6 in some groups and 5 in the others, were fed on 0, 10, 20, 30, 40, 50, 55, 60, 65, 70, 80 and 100 EA % diets. Since, in Experiment 1, most of the growth curves were shown to be almost linear for 28 days, the experimental period was shortened to 14 days in this experiment. The nitrogen balance, nitrogen balance efficiency and biological value were measured as described in Experiment 1.

**RESULTS**

Body weight change of the animals in Experiment 1 is shown in Figs. 1 to 3. Figure 1 indicates that the maximum growth, approximately 4.5 g per day, was obtained in the 40 and 60 EA % groups in growing rats, where the increasing rates were almost linear. The growth rate of the 100 EA % group was about half as much as those of the 40 and 60 EA % groups, and there was a slight lag in growth during the first 8 days. The 20 EA % group almost maintained the initial body weight throughout the experimental period. The 0 EA % group showed a weight decrease, particularly at the initial stage, which was as much as that in the group...
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Fig. 2. Body weight change in adult rats under maintenance.

Fig. 3. Body weight change in adult rats under repletion.

undergoing protein depletion, which was conducted at the same time.

In adult rats under maintenance, the body weight, 496 g on average at first, slightly increased in the 20 to 60 EA% groups and was maintained in the 100 EA% group, as seen in Fig. 2. However, in the 0 EA% group it decreased to a great extent.

Figure 3 shows that in adult rats under repletion, the body weight loss during the 8-day protein depletion was completely recovered during the first 8 days, in the 20 to 60 EA% groups. It thereafter increased slightly, as much as under maintenance, as mentioned above. But, the 100 EA% group did not recover the weight loss until the termination of the experiment. The 0 EA% group continued losing as much weight as during the preceding protein depletion.

Nitrogen intake, and nitrogen excretion in urine and feces for the last 4 days (from the 5 to 8th day) of the nitrogen balance test period are shown in Table 3. Nitrogen intake, to which the food intake is comparable in this experiment, was shown to be approximately proportional to body weight gain in growing rats and in adult rats under repletion. But, in the adult rats under maintenance it was almost the same in all the groups.

Urine nitrogen decreased linearly with increases in the dietary EA% level, except in the adult rats under repletion. Fecal nitrogen did not show any remarkable change, but had similar patterns to those of nitrogen intake.

The PER for growing rats in Experiment 1, was -2.1, 0.4, 3.2, 3.6 and 2.7;
Table 3. Nitrogen intake, and nitrogen excretion in urine and feces (mg/day).

|            | Growing                      | Adult                      |
|------------|------------------------------|----------------------------|
|            | EA % | 0   | 20  | 40  | 60  | 100 | 0  | 20  | 40  | 60  | 100 | 0  | 20  | 40  | 60  | 100 |
| N intake   |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| ±2         | 69   | 106 | 209 | 181 | 102 |     | 320 | 341 | 328 | 300 | 306 | 156 | 400 | 339 | 373 | 235 |
| ±4         | 71   | 70  | 57  | 38  | 38  |     | 367 | 311 | 288 | 243 | 221 | 202 | 270 | 185 | 221 | 140 |
| Urinary N  |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|            | 8    | 11  | 21  | 18  | 9   | 43  | 55  | 47  | 54  | 47  | 15  | 46  | 41  | 43  | 21  |
| ±0.2       | ±1.3 | ±1.5| ±0.8| ±0.6|     | ±3.7| ±4.4| ±4.7| ±3.4| ±1.9| ±1.0| ±3.5| ±3.9| ±3.8| ±1.1|
| Fecal N    |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| ±2         | 55   | 64  | 92  | 88  | 68  | 480 | 503 | 484 | 487 | 503 | 460 | 544 | 523 | 519 | 500 |
| Mean body  | ±1.0 | ±4.4| ±2.8| ±1.7| ±2.3| ±25 | ±18 | ±30 | ±31 | ±39 | ±15 | ±20 | ±32 | ±70 | ±13 |
| weight     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| during     |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| this period (g) | | 55   | 64  | 92  | 88  | 68  | 480 | 503 | 484 | 487 | 503 | 460 | 544 | 523 | 519 | 500 |
|            | ±1.0 | ±4.4| ±2.8| ±1.7| ±2.3| ±25 | ±18 | ±30 | ±31 | ±39 | ±15 | ±20 | ±32 | ±70 | ±13 |

* Mean values with standard errors. Values in the table each represent the mean of the nitrogen during 4 days in the later period of the nitrogen balance test for comparison during the same period corresponding to each other. Thus the direct effects of dietary changes were avoided.
nitrogen balance efficiency (%) was -10, 29, 63, 69 and 52; and the biological value was 29, 60, 87, 92 and 83 in the 0, 20, 40, 60 and 100 EA% groups, respectively. Since these values showed similar patterns to those obtained among the corresponding groups in Experiment 2, the comparative data on the body weight change, nitrogen balance efficiency and biological value between growing and adult rats are presented in Fig. 4 to 6 using the results in Experiments 1 and 2, for the adult and growing rats, respectively.

![Diagram showing body weight gain and PER in growing and adult rats.](image)

Figure 4. Body weight gain and PER in growing and adult rats. Vertical bars show the standard errors of the mean.

Figure 4 shows the results of body weight change and PER. In growing rats, the maximum body weight gain, approximately 5.5 g per day, was obtained at the 50 and 55 EA% levels, where the PER was at its maximum value of 4. In adult rats both maintenance and repletion were observed to be equally satisfactory at the 20 to 60 EA% levels. It should be noted that growing and adult rats nearly maintained their initial body weights at the 20 EA% level.

Figure 5 shows the nitrogen balance and nitrogen balance efficiency. In growing rats, nitrogen balance was at its maximum value at the 55 EA% level, where nitrogen balance efficiency was nearly at its maximum. The nitrogen balance efficiency did not decrease as much as was expected from observing nitrogen balance above the 60 EA% level. In adult rats a negative nitrogen balance and a lower nitrogen retention were observed at the 20 EA% level, even though body weight change indicated that satisfactory maintenance and repletion were occurring, for the maintenance and repletion groups respectively. Thus, at least 40 EA% was required in the amino acid mixtures to gain the maximum nitrogen balance and nitrogen balance efficiency. During repletion these two values remained almost constant in the 40 to 100 EA% groups of adult rats in contrast
with their gradual decrease in the case of growing rats. During maintenance, the 40 and 60 EA% groups also remained constant, but decreased considerably at the 100 EA% level. The constancy of nitrogen balance efficiency at above 40 EA% in the repletion group may imply the more effective retention of essential amino acids in the metabolic pool of nitrogen during the process of repletion.
As shown in Fig. 6, the biological value for growing rats increased greatly with the increase of dietary EA%. The maximum value was shown to be 94 at the 50 EA% level. The value remained at nearly 90 up to the 60 EA% level, but gradually decreased to 80 at the 100 EA% level. The maximum values for adult rats were nearly 60 under maintenance and nearly 80 under repletion, where both values were attained at the 40 EA% level. The value for repletion thereafter remained unchanged, whereas that for maintenance decreased considerably at the 100 EA% level.

Since the true digestibility of the amino acid mixtures was calculated to be over 95% in every dietary group in these experiments, the net protein utilization (NPU) was not presented here because it can be represented by the biological value obtained in the experiments.

From these facts it was concluded that at least 55 EA% is required in dietary amino acid mixtures, for growing rats to gain maximum values of growth, nitrogen balance and nitrogen efficiencies, and at least 40 EA% is required for adult rats including those repleting from protein depletion.

**DISCUSSION**

Several studies have been made particularly in an attempt to estimate the appropriate proportion of total essential amino acids in dietary protein. A dilution or replacement of dietary proteins with nonspecific nitrogen, especially with non-essential amino acids, has often been used for these estimations. The assessment of nutritional effects has usually been made by use of body weight gain, nitrogen balance and various other indices of nutritional efficiencies.

By using whole egg, milk and beef as a basal protein, SCRIMSHAW and his colleagues (7-9) demonstrated that in young human subjects about a 2.1 E/T ratio, equivalent to 34 EA%, was almost sufficient to gain satisfactory results.

For growing rats, DANIEL et al. (10) demonstrated that a dilution of cow's milk protein with glutamic acid decreased nutritional effects such as growth and PER. Using essential and nonessential amino acid mixtures, STUCKI et al.(11) and YOUNG et al. (12) indicated from results of body weight gain and PER that about a 1.0 I/D ratio (the indispensable to dispensable amino acid ratio), equivalent to 50 EA%, was satisfactory. On the other hand, in the chick assay, STUCKI et al. (13) showed a greater requirement for the essential amino acid proportion, as high as a 2.0 I/D ratio or approximately 65 EA%.

These papers have presented useful data on the approximate requirements of dietary EA% for humans and animals. However, there still remains a problem in the relationship between the requirements and their growth rates which can arise from differences in age and species. Moreover, metabolic changes which may have occurred are not well known.

This study, which compared nutritional effects of the dietary essential amino
acid proportions between growing and adult rats, dealt extensively with the measurement of the biological value. For growing rats, it is well known that the 10% level of dietary protein, that has no limiting essential amino acids, is sufficient to allow maximum growth and nitrogen retention. Adult rats are supposed to require only a 5% level of the same kind of protein in diets under maintenance, but at least a 10% level under repletion. Since the amino acid composition of the essential and nonessential amino acid mixtures of this experiment simulated that of whole egg protein, the 10% level of total amino acids in diets is considered to be reasonable for comparative studies on the nutritional effects at a definite level of amino acid intake between growing and adult rats.

In this study, at least 55 EA%, which is equivalent to the value of egg or milk protein, was required to gain the maximum values of growth, PER, nitrogen balance and nitrogen balance efficiency for growing rats. In addition, the biological value was shown to be 94, nearly equal to the value of whole egg protein. These results generally agreed with those of STUCKI et al. (11) and YOUNG et al. (12).

In adult rats, a lower requirement, i.e. 40 EA%, was indicated by the nitrogen balance and nitrogen balance efficiency under both maintenance and repletion. This proportion of total essential amino acids is, on the average, approximately equal to that of vegetable proteins apart from their essential amino acid patterns. On the other hand, the biological values were estimated to be nearly 60 and 80 for maintenance and repletion, respectively.

It is probable that the dietary EA% requirements and biological value for maintenance would be a little higher than the above values if the dietary amino acid level is taken at 5%. Moreover, if repletion is conducted after a much longer period of protein depletion, the required level of dietary EA% and the biological value would give values a little different to those obtained in this experiment. The retained nitrogen after the 8-day protein depletion, as in this experiment, probably represents in some part the so-called labile or reserve protein which might contain much of the nitrogen pool other than essential amino acids. On any case, the dietary EA% requirements and the biological value of the amino acid mixtures when estimated for the repleting adult rats were less than for growing rats under these experimental conditions.

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