Lysine Requirements of Rats of Various Body Weights

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The lysine requirements of rats of various body weights were estimated using the feeding and isotope tests.

The regression equation obtained by the feeding test was \( Y = 1.03 - 0.58 \log X \). Where \( Y \) is lysine percentage of the diet and \( X \) is the mean of initial and final body weights (g) of rats achieving optimal growth gains during the feeding period.

The regression equation obtained by the isotope test was \( Y = 0.90 - 0.49 \log X \), where \( Y \) and \( X \) are lysine percentage in the diet and body weights (g) of rats achieving optimal growth gains at the injection time respectively.

Lysine is essential for growth and maintenance of rats. Since some plant proteins such as sesame oil meal and corn gluten are deficient in lysine, the lysine requirements of rats have been estimated using growth and feeding efficiency, nitrogen balance and other methods.\(^1\)\(^-\)\(^9\) The requirements of lysine from data easily citable, however, ranged from 0.05 to 1.00% of the diet as shown in Fig. 1.

From these data, it is suggested that firstly, the requirements of lysine shown at a dietary level decreased with increasing body weight and secondly, the requirements of lysine estimated on the basis of low lysine protein diets were higher than those estimated on the basis of amino acid diets. The latter suggestion may be partly due to the low availability of lysine in the feedstuffs. Thirdly, the lysine requirements obtained from the oxidation study\(^5\) or plasma free lysine levels were somewhat lower than those estimated from the average daily gain and feed efficiency data. It should be noted that whereas the growth study covered a period of longer than 7 days, studies of the oxidation rate of labeled lysine and the determination of plasma free lysine were conducted only during the last few days of this period, and the lysine requirements of rats might have been decreasing with age.

The objective of the following experiments was to estimate the lysine requirement of rats with various body weights using an oxidation method as an useful method for determination of amino acid requirement of both growing and adult animals.\(^5\)\(^,\)\(^12\)\(^-\)\(^15\)

EXPERIMENTAL

Expt. 1. To investigate the effect of different body weights on the lysine oxidation rate, male rats of the Wistar strain, weighing 40 g, were randomly divided into 2 groups of 4 rats each. These groups were individually self-fed a 12.5% amino acid diet (Table I) containing 0.68 or 0.34% lysine for 10 weeks. In the course of the feeding test, 3 rats from each dietary group were used to examine lysine oxidation rates. Each rat was injected with 0.51 µCi of L-lysine-U-\(^{14}\)C (330 mCi/m mol, The Radiochemical Centre, Amersham) in 0.1 ml of 0.9% saline solution intraperitoneally at 1:00 p.m.

To further examine the effect of different body weights on the degradation rate of nonessential amino acid, the rats fed the diet containing 0.68% lysine diet were injected with 0.50 µCi of glycine-U-\(^{14}\)C intraperitoneally at the intervals of the lysine oxidation study. Immediately following injection, each rat was placed in a respiratory chamber for 18 hr.\(^{15}\) Expired gases were trapped through 2 carbon dioxide traps consisting of 150 ml of KOH solution. Concentration of KOH was increased from 2 to 6 N with increasing body weight. The radioactivity in trapping solutions and in excreta for 18 hr was determined using a nonione-toluene scintillator and an ALOKA LSC 651 liquid scintillation spectrometer in the same way as described in the previous report.\(^{11}\)

Expt. 2. The second experiment was conducted to determine the period within which the lysine degrada-
FIG. 1. Lysine Requirements of Rats Which were Reported in the Literature.
Numbers in parentheses indicate number of literature cited.

Fig. 2. Percentage of Recovery of $^{14}$C in Respiratory CO$_2$ (R) and in Excreta (E) for 18 hr after Injection. The percentage of recovery of $^{14}$C in excreta was determined at 18 hr. Counting in trapping solutions and in excreta was conducted in the same way as described for Expt. 1.

Expt. 4. The fourth experiment was conducted to examine the lysine requirements of rats with various body weights of 40, 100, 170 and 300 g, respectively, using the oxidation and feeding tests. The rats tested were randomly divided into 6 groups of 4 rats each. Each group was randomly allotted to 6 experimental diets and individually self-fed for 7 days. At the end of the feeding test, the rats were injected with 0.51 µCi of L-lysine-$^{14}$C intraperitoneally at 1:00 p.m. Carbon dioxide and excreta were collected for 18 hr and treated as described for Expt. 1.

**RESULTS**

The rats fed the 0.68% lysine diet achieved optimal growth gains, and the rats on the 0.34% lysine diet gained slowly as expected, with average gains of 7 and 3 g respectively. Figure 2 shows the percentage of recovery of $^{14}$C in the respiratory CO$_2$ and in excreta for 18 hr plotted against body weight in Expt. 1.

Expt. 3. To assess the effect of time after meals on the percentage of recovery of $^{14}$C, 16 rats weighing 200 to 220 g were divided into 2 groups of 8 rats each, and were trained to consume the 0.34 or 0.68% lysine diet from 10:00 a.m. to noon. On the 8th day, 3 rats were injected at 4:00 a.m. (before the meal) and 3 of the residual 5 rats were injected at 2:00 p.m. (after the meal) with 0.85 µCi of l-lysine-$^{14}$C. The time course of percentage of recovery of $^{14}$C in the respiratory CO$_2$ was determined 0.5, 1, 1.5, 3, 4, 6, 8 and 18 hr after injection. The percentage of recovery of $^{14}$C in excreta was determined at 18 hr. Counting in trapping solutions and in excreta was conducted in the same way as described for Expt. 1.

Regression equations were $Y = -22.9 + 17.4 \log X$ (body weight g) for percentage of recovery of $^{14}$C in respiratory CO$_2$ on 0.68% lysine diet and $Y = 0.40 + 0.80 \log X$ for that in excreta on both diets.
The percentage of recovery of $^{14}$C in excreta increased very slowly throughout the experiment, partly due to accumulated isotopes from repeated injections ($Y=0.40+0.80 \log X$; where $Y=$ percentage of recovery of $^{14}$C and $X=$ body weight g). In the rats on the 0.68% lysine diet, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ increased with increasing body weight ($Y=-22.9+17.4 \log X$). At a level of 0.34% lysine, the amount of lysine oxidised to CO$_2$ was low and relatively constant with increasing body weight to about 150 g. Above this body weight, the amount of lysine oxidised increased with increasing body weight as observed for the 0.68% lysine diet.

In contrast to the lysine oxidation rate, when glycine-U-$^{14}$C was injected, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ and in excreta increased very slowly with increasing body weight, perhaps because of the residual effect of $^{14}$C accumulated in the body by the repeated injections (Fig. 3). The regression equations obtained by the least squares method were $Y=13.7+2.8 \log X$ for the percentage of recovery of $^{14}$C in respiratory CO$_2$ and $Y=2.7+3.8 \log X$ for that in excreta.

Expt. 2 is shown in Fig. 4. When the dietary lysine level was decreased from 0.80 to 0.40%, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ decreased to one half of that for the 0.80% lysine diet within 2 days and remained almost constant until the 7th day. When the dietary lysine level returned to 0.80% again, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ was restored to the same level as observed for the 0.8% lysine diet on the 7th day. On the other hand, the percentage of recovery of $^{14}$C in excreta remained almost constant and increased slowly in the later part of this experiment, regardless of the dietary switching.

The time course of the percentage of recovery of $^{14}$C in the respiratory CO$_2$ of rats injected before or after meals is summarized in Fig. 5. When the lysine level stood at 0.34% in the diet, no significant difference was found in the time course of the percentage of recovery of $^{14}$C of rats for both groups. On the other hand, the percentage of recovery of $^{14}$C in excreta was significantly higher than that in rats before meals of 0.68% lysine over an 8-hr-period. In comparisons of 18 hr, no significant difference was found for either group.

Figure 6 shows the effects of feeding graded levels of supplemental lysine on the body
FIG. 5. Time Course of Percentage of Recovery of \(^{14}\text{C}\) in Respiratory CO\(_2\) of Rats Fed 0.68\% or 0.34\% Lysine Diet at 10:00 to 12:00 and Injected with 0.85 \(\mu\)Ci of L-Lysine-U-\(^{14}\text{C}\) at 4:00 or 14:00.

- fed 0.68\% lysine diet and injected after meal;
- fed 0.68\% lysine diet and injected before meal;
- fed 0.34\% lysine diet and injected after meal;
- fed 0.34\% lysine diet and injected before meal.

FIG. 6. Percentage of Recovery of \(^{14}\text{C}\) in Respiratory CO\(_2\) and Daily Gain of Body Weight of Rats Weighing 80 to 140 g Fed Diets with Graded Levels of Lysine. Each point is the mean of 4 rats.

Weight gain and on the percentage of recovery of \(^{14}\text{C}\) in the respiratory CO\(_2\) in rats weighing about 100 g. One broken-line regression was matched with the relationship between the level of lysine in the diet and body weight gain (below) and the other between the former and the percentage of recovery of \(^{14}\text{C}\) in the expired CO\(_2\) (upper). It was concluded that the smallest amount of lysine that resulted in a maximum body weight gain was 0.45\% of the diet.

When less than the lysine requirement is fed, it is utilized most efficiently for synthesis of body protein. If it exceeds the requirement level, the spare lysine is oxidized to CO\(_2\). Thus the breaking point where the oxidation rate begins to increase may be a point where the lysine requirement is met. From these results a broken-line regression matched with the relationship between the level in the diet and the percentage of recovery of \(^{14}\text{C}\) in the respiratory CO\(_2\). The lysine requirement was found to be 0.37\% of the diet.

To provide a proper basis for the comparison of lysine levels required for each stage of growth (40, 170 and 300 g), a broken-line regression was matched with the data by plotting the body weight gain and the percentage of recovery of \(^{14}\text{C}\) in the respiratory CO\(_2\) against the lysine level in the diet as shown in Fig. 6. When the initial body weight of rats was greater than 300 g, no clear-cut breaking point was observed in the regression line of weight gain. The regression equation obtained was \(Y=1.03 - 0.58 \log X\). Where \(Y\) is lysine percentage of the diet and \(X\) is the mean

FIG. 7. Lysine Requirements of Rats of Various Body Weights Obtained by the Growth Test (---) and Oxidation Test (○).

Regression equations were \(Y=0.90 - 0.49 \log X\) for lysine requirements by the feeding test and \(Y=1.03 - 0.58 \log X\) for those by the oxidation test.
of initial and final body weights (g) of rats achieving optimal growth gains during the feeding period as shown in Fig. 7. The lysine requirements of rats of various body weights are shown as the regression equation, $Y = 0.90 - 0.49 \log X$, by the oxidation method, where $Y$ and $X$ are lysine percentage in the diet and body weights (g) of rats achieving optimal growth gains at the injection time respectively. As shown in Figs. 6 and 7, the requirements obtained by the feeding test were higher than those by the oxidation test.

**DISCUSSION**

The activity of production of CO$_2$ from lysine in growing rats did not adapt to the variation of dietary protein level within one week$^{18}$ in contrast to that from other amino acids. In contrast to the above observation, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ reached constant levels within 2 days after the change of dietary lysine levels as shown in Fig. 4.

Injection time (before or after meals) also did not affect the percentage of recovery of $^{14}$C in the respiratory CO$_2$ for 18 hr. These observations may be permissible on the basis of the experimental conditions used, especially the fact that the period of adaptation to the variation of dietary lysine level and of collection of CO$_2$ was sufficient to estimate the lysine requirement by the oxidation test.

At the 0.68% dietary lysine level, the percentage of recovery of $^{14}$C in the respiratory CO$_2$ increased with increasing body weight, but that of glycine-$^{14}$C remained rather constant. This observation suggests that glycine was nonspecifically utilized at all stages of body weight. At the 0.34% of dietary lysine level, a sudden increase of the percentage of recovery of $^{14}$C in the respiratory CO$_2$ means that the dietary supply of lysine exceeded the amount needed for the body protein synthesis of rats weighing about 160 g.

As mentioned in the results and in our other reports,$^{15-16}$ the concept of the oxidation test is as follows. When an amino acid is limited or deficient in a diet, a major portion of this amino acid will be used for body protein synthesis and little will be oxidized to CO$_2$. When dietary supply exceeds the animal's needs for body protein synthesis, increased use of the carbon skeleton for alternative processes such as lipogenesis, gluconeogenesis, excretion in excreta or increased oxidation to CO$_2$ can be expected. The above concept has been applied to the plasma concentration.$^{5,16-19}$

There is considerable information available regarding the essential amino acid requirements of rats, but findings are rather variable as shown in Fig. 1. This may be because of differences mainly in body weights of animals and the parameters used. The strain of animals and the physiological or nutritional status may also affect the requirements of amino acids. However, when the range of comparison of the requirements within rats of the same strain fed sufficient nutrients—amino acids except the given amino acid, fat, vitamins, minerals and energy—to gain optimal growth is defined, the above 3 factors may not be so closely concerned as thought.

The parameters used in our experiments were obtained by the classical feeding test and the new oxidation test. As Stockland et al.$^5$ suggested, the requirement estimated by the oxidation test was higher than that by the feeding test. This difference may be partly due to the expression of body weight. By the oxidation test, the estimation was completed within a day. On the other hand, the feeding test continued at least for 1 week with a gain of body weight. When the means of initial and final body weights of rats were substituted for initial body weights, the regression line obtained by the feeding test is nearly identical to that obtained by the oxidation test. The requirements obtained in our experiments were almost in the middle of those of other workers in Fig. 1.

**REFERENCES**

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