The Utilization of Carnosine in Rats Fed on a Histidine-Free Diet and Its Effect on the Levels of Tissue Histidine and Carnosine

Nanaya TAMAKI, Akimi FUNATSUKA, Shigeko FUJIMOTO,¹ and Takao HAMA¹,²

¹Laboratory of Nutritional Chemistry, Faculty of Nutrition, and ²Laboratory of Physiological Chemistry, Faculty of Pharmacy, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 673, Japan
(Received February 25, 1984)

Summary Carnosine can support the growth of rats fed on a histidine-free diet. Rats fed on the histidine-free diet lost weight rapidly for a few days, then remained at a relatively constant weight for 2 weeks at least. However, rats fed on a 0.90% carnosine diet, which contains histidine equimolar to that in a 20% casein diet, increased their weight at the same rate as rats fed on a 20% amino acid diet simulated with casein. On the other hand, the growth of rats fed on a 5% carnosine diet was about 70% compared with that of control rats fed on the 20% amino acid diet for a 2-week experimental period.

Carnosinase activity was not significantly affected in the kidney of rats fed on the histidine-free or the 5% carnosine diet. On the other hand, carnosinase activity in the small intestine of rats fed on the histidine-free diet was significantly increased. Histidine content of serum of rats fed on the histidine-free diet decreased to 1/3 of that of control rats, while that of rats fed on the 5% carnosine diet increased to about 14 times. Carnosine content of rat gastrocnemius muscle increased with carnosine content of diets, followed by an increase of histidine in the muscle. However, carnosinase activity of gastrocnemius muscle was not affected by carnosine in diets.

Key Words carnosine, histidine, β-alanine, carnosinase, gastrocnemius muscle, kidney, small intestine, serum

Carnosine (β-alanylhistidine) is a dipeptide ordinarily present in skeletal muscles of many species of animals. The content of carnosine in skeletal muscle is variable under various conditions. In particular, the peptide content increased in the muscle of rats fed on a histidine-excess diet (I), but markedly decreased by feeding a
Histidine is an essential amino acid for infants (4) and rats (5–7). Recently Cho et al. (8) proposed that histidine is indispensable for young men consuming a low-nitrogen diet. However, Ousterhout (9) found that chicks fed on a diet lacking histidine lost weight, lived longer and were stronger and more vigorous than chicks fed on any of the other amino acid-deficient diets. These results suggest that histidine is less indispensable than other essential amino acids because histidine is supplied from carnosine in the muscles or body proteins.

Carnosinase [aminoacyl-L-histidine hydrolase, EC 3.4.13.3] occurs in many tissues of wide variety of vertebrates. Carnosinase activities in kidney and nasal olfactory epithelium are 40 times higher than those in other tissues such as skeletal muscle, olfactory bulb and brain which contain high concentrations of carnosine (10). Carnosinase was purified from hog (11) and mouse (12) kidneys. Immunocytochemistry with the antiserum shows that carnosinase is localized in the proximal tubules of kidney (12). Fukuda and Kopple (13) proposed that dog kidney appeared to produce histidine from carnosine and that histidine production increased on carnosine infusion. In a previous paper (14) we showed that orally administered carnosine appeared in liver and blood.

Carnosine supports the growth of rats (15) and mice (16) on a histidine-deficient diet, however, the mechanisms involved are not clear. The present study deals with the influence of histidine deficiency, histidine supplementation and carnosine-feeding upon carnosine and histidine contents as well as carnosinase activity of rat organs.

MATERIALS AND METHODS

Chemicals. All chemicals used were of analytical grade and amino acids used were all L-form. They were purchased from Nakarai Chemicals, Ltd., Kyoto, unless otherwise stated. L-Carnosine was obtained from the Protein Research Foundation, Osaka, and o-phthaldialdehyde from Wako Pure Chemicals Industries Co., Osaka. Materials for the diets of animals were obtained from Oriental Yeast Ltd., Tokyo. The reagents for amino acid analysis were purchased from Merck.

Animals. Male albino rats (Sprague-Dawley strain, weighing 100 to 110 g) were housed in individual screen-bottom cages in a room maintained at 23 ± 1°C with 50% humidity under controlled lighting conditions (12 to 12 h light-dark cycle). The animals were fed on a commercial stock diet (Oriental Yeast Ltd.) and water ad libitum for 1 week before the experiment to acclimate them to the new environment. Acclimated rats showing progressive weight gain were selected and separated into groups. Body weight and food intake were determined daily. Four to six animals were assigned to each experimental subgroup. The animals were given the experimental diet for 2 weeks and killed by decapitation between 9 and 11 a.m. on day 15.

Histidine-free and carnosine diets. The compositions of basal and experimental diets are presented in Table 1. The amino acid mixture was simulated with casein. In

J. Nutr. Sci. Vitaminol.
Table 1. Composition of diets (%).

| Ingredients          | Control | Histidine-free | 0.90% Carnosine | 1.80% Carnosine | 5% Carnosine |
|----------------------|---------|----------------|-----------------|-----------------|--------------|
| Amino acid mixture   | 19.38   | 19.38          | 19.38           | 19.38           | 19.38        |
| Histidine            | 0.62    | 0              | 0               | 0               | 0            |
| Glycine              | 0       | 0.62           | 0               | 0               | 0            |
| Carnosine            | 0       | 0              | 0.90            | 1.80            | 5            |
| Sucrose + starch     | 58      | 58             | 57.72           | 56.82           | 53.62        |
| Soybean oil          | 6       | 6              | 6               | 6               | 6            |
| Salt mixture         | 6       | 6              | 6               | 6               | 6            |
| Vitamin mixture      | 2       | 2              | 2               | 2               | 2            |
| Cellulose powder     | 8       | 8              | 8               | 8               | 8            |

* Amino acid mixture in grams; Gly 2.0, Ala 3.2, Val 7.2, Leu 9.2, Ile 6.1, Pro 10.6, Phe 5.0, Tyr 6.3, Trp 1.2, Ser 6.3, Thr 4.9, Cys 0.39, Met 2.8, Arg·HCl 5.0, Lys·HCl 10.2, Asp 7.1, Glu 22.4. * Sucrose: corn starch = 2:1. * Salt mixture and vitamin mixture were purchased from Oriental Yeast Co., Ltd.

Table 2. Composition of diets (%).

| Ingredients          | Control | 5% Histidine |
|----------------------|---------|--------------|
| Casein               | 20      | 20           |
| Histidine            | 0       | 5            |
| Sucrose + starch     | 58      | 53           |
| Soybean oil          | 6       | 6            |
| Salt mixture         | 6       | 6            |
| Vitamin mixture      | 2       | 2            |
| Cellulose powder     | 8       | 8            |

* Sucrose: corn starch = 2:1. * Salt mixture and vitamin mixture were purchased from Oriental Yeast Co., Ltd.

The histidine-free diet, glycine was substituted for histidine. Three experimental diets contained varied amounts of carnosine, i.e. 0.90, 1.80 and 5%, in which amounts of histidine were equivalent, 2-fold and excess, respectively, compared with the 20% casein diet.

**Histidine-excess diet.** The compositions of histidine-excess and control diets are shown in Table 2. The 20% casein diet was used as the control, and the histidine-excess diet was composed of 20% casein diet containing 5% histidine.

**Analysis of histidine and carnosine.** The gastrocnemius muscle was homogenized with 80% ethanol and the amino acids and peptides were extracted as shown previously (17). The alcohol extract was evaporated to dryness in vacuo and the dried materials were dissolved in citrate buffer, pH 2.2, for amino acid analysis. The
quantitative determination of the amino acids and peptides was performed with an amino acid autoanalyzer (Hitachi KLA-3B type). The measurement of histidine in serum was based upon a fluorometric determination \((18)\). In this procedure, a fluorescent product formed from histidine and \(o\)-phthalaldehyde in alkali solution was determined with a fluorescence spectrometer (Hitachi 650-10S).

**Estimation of carnosinase activity.** Assay of carnosinase activity was based upon the fluorometric determination of histidine in reaction with \(o\)-phthalaldehyde \((19)\). The gastrocnemius muscle, kidney, small intestine and brain were homogenized with a glass homogenizer in 10 volumes of \(50\,\text{mM Tris-HCl, pH 8.4}\). The precipitate was removed by centrifugation at \(12,000 \times g\) for 20 min.

0.1 ml of the supernatant and 0.05 ml of \(1\,\text{mM MnCl}_2\) were added to 0.25 ml of \(50\,\text{mM Tris-HCl buffer, pH 8.4}\). After incubation for 5 min at \(37^\circ C\), 0.1 ml of \(50\,\text{mM carnosine was added and the reaction mixture was incubated for 30 min at the same temperature. The reaction was terminated by the addition of 0.5 ml of 10\% (w/v) trichloroacetic acid. After standing for 30 min, the precipitate was removed by centrifugation. Free histidine in the supernatant was assayed by adding 0.1 ml of the supernatant to 4.0 ml of \(0.3\,\text{n NaOH and 0.4 ml of 1\% (w/v) o-phthalaldehyde in dimethylcellosolve. After vigorous stirring and incubating at room temperature for 15 min, 0.4 ml of 6\,\text{n HCl was added. Exactly 15 min later, the intensity of fluorescence was measured at 344 nm of excitation and 432 nm of emission. Carnosinase activity was expressed as } \mu \text{mol histidine formed per min per gram wet weight or per gram protein. Protein concentration was measured by the method of Lowry et al.}(20) \text{ with bovine serum albumin as a standard.}

**RESULTS**

**Body weight and food intake of rats fed on histidine-free and carnosine- and histidine-excess diets**

Figure 1 represents the average growth curve of each group. The body weight of control animals increased linearly under the experimental conditions, while that of the histidine-deficient animals decreased. However, there was no significant difference in weight gain or food consumption between the group on a diet containing 0.90\% of carnosine and the group on a diet containing 20\% amino acid mixture (Figs. 1 and 2). These results suggest that carnosine is utilized as effectively as histidine in the rat. On the other hand, the growth of 5\% carnosine-fed rats was obviously retarded. Also, the food intake was less in the 5\% carnosine-fed animals than in the 20\% amino acid mixture-fed animals.

The effect of histidine-excess diet on rat growth is presented in Fig. 3. The body weight of rats fed on a 20\% casein diet increased linearly and the weight gain was 23 g more than that of the 20\% amino acid mixture-fed animals for a two-week feeding period. The weight gain of rats fed on a diet containing 5\% histidine was 41 g less than that of control rats for the period (Fig. 3). The food intake was also less in histidine-excess animals, \(16.2 \pm 2.3 \text{ g/day/rat, than that in the control}

\[\text{J. Nutr. Sci. Vitaminol.}\]
Fig. 1. The average weight of the rats fed on histidine-free and carnosine diets. •, control (20% amino acids mixture) diet; ○, histidine-free diet; ▲, 0.90% carnosine diet; ■, 1.80% carnosine diet; ×, 5% carnosine diet.

Fig. 2. Diet intakes of experimental animals. •, control (20% amino acids mixture) diet; ○, histidine-free diet; ▲, 0.90% carnosine diet; ■, 1.80% carnosine diet; ×, 5% carnosine diet.

animals, 21.5 ± 3.0 g/day/rat.

Histidine and carnosine contents in serum of rats fed on histidine-free and carnosine- and histidine-excess diets

Acid-soluble histidine in serum of rats fed on a histidine-free diet decreased to about one-third of that of the control rats and recovered to control level by the addition of carnosine to the histidine-free diet (Table 3). Moreover, the level of free histidine in rat serum increased with the concentration of carnosine in the diets. In particular, the free histidine content of serum of rats fed on 5% carnosine-diet was 14-fold more than that of control rats. As expected, histidine in serum of rats fed on
546 N. TAMAKI et al.

Fig. 3. The average weight of the rats fed on a histidine-excess diet. ○, control (20% casein) diet; △, 20% casein + 5% histidine diet.

Table 3. Effect of histidine-free and carnosine- and histidine-excess diets on histidine and carnosine contents of rat serum.

| Diet                  | Histidine (μmol/ml serum) | Carnosine     |
|-----------------------|---------------------------|---------------|
| Experiment 1          |                           |               |
| Control               | 0.166 ± 0.005             | nil           |
| Histidine-free        | 0.055 ± 0.008**           | nil           |
| 0.90% Carnosine       | 0.132 ± 0.008             | nil           |
| 1.80% Carnosine       | 0.251 ± 0.035             | nil           |
| 5% Carnosine          | 2.278 ± 0.387**           | 0.237 ± 0.073 |
| Experiment 2          |                           |               |
| Control               | 0.181 ± 0.017             | nil           |
| 5% Histidine          | 9.605 ± 1.253**           | nil           |

Each value is the mean ± SE in four to six separate experiments. **p < 0.01 compared to control.

5% histidine-diet increased 50-fold more than that in serum of control rats.

Carnosine was detected neither in sera of control rats nor in sera of rats fed on 0.90% carnosine-, 1.80% carnosine- and 5% histidine-diets. However, carnosine appeared in sera of rats fed on 5% carnosine, the content being much lower than that of histidine (Table 3).

Effect of histidine-free and carnosine- and histidine-excess diets on carnosine and histidine contents of rat gastrocnemius muscle

Both carnosine and histidine in the gastrocnemius muscle of rats fed on histidine-free diet decreased, as shown in previous papers (1, 3) (Table 4). On the other hand, when carnosine, which was in an amount equivalent to that of histidine

J. Nutr. Sci. Vitaminol.
Table 4. Effect of histidine-free and carnosine- and histidine-excess diets on carnosine and histidine contents of rat gastrocnemius muscle.

| Diet               | Carnosine (µmol/g wet tissue) | Histidine (µmol/g wet tissue) |
|--------------------|-------------------------------|-------------------------------|
| Experiment 1       |                               |                               |
| Control            | 6.552 ± 0.707                 | 0.164 ± 0.005                 |
| Histidine-free     | 2.094 ± 0.199**               | 0.067 ± 0.004**               |
| 0.90% Carnosine    | 7.592 ± 0.564                 | 0.176 ± 0.019                 |
| 1.80% Carnosine    | 12.063 ± 1.032*               | 0.297 ± 0.039*                |
| 5% Carnosine       | 14.316 ± 1.131**              | 1.161 ± 0.122**               |
| Experiment 2       |                               |                               |
| Control            | 6.341 ± 0.778                 | 0.181 ± 0.019                 |
| 5% Histidine       | 17.451 ± 0.856**              | 9.852 ± 0.302**               |

Each value is the mean ± SE in four to six separate experiments. *p < 0.05 compared to control. **p < 0.01 compared to control.

In 20% amino acid mixture-diet, was added to the histidine-free diet, carnosine as well as histidine in rat gastrocnemius muscle was maintained at the control levels (Table 4). Moreover, the levels of carnosine and histidine in rat gastrocnemius muscle increased with carnosine concentration in diet. Comparison was also made between the 20% casein diet and the histidine-excess diet groups for carnosine and histidine contents of rat gastrocnemius muscle. Carnosine increased approximately 3-fold and histidine 54-fold over the levels in control rat (Table 4). The rate of increase of histidine in the muscle was similar to that in serum with the histidine-excess diet.

Effect of histidine-free, histidine-excess and carnosine-supplemented diets on carnosinase activity of kidney, small intestine, gastrocnemius muscle and brain of rats

It is well known that carnosinase activity is present in many tissues of vertebrates. A high level of carnosinase activity was found in kidney, the existence of multiple forms (12, 19, 21).

The effect of the diets on carnosinase activity of rat kidney and small intestine is shown in Table 5. Carnosinase activity in the small intestine was significantly increased by histidine-free and -excess diet feeding, whereas that in the kidney was not affected by any of the diet given. Carnosinase activity in the small intestine was not affected by 5% carnosine-diet feeding.

The level of carnosinase activity in gastrocnemius muscle of rats fed on control diet was low and was one-hundredth that of kidney, and it was not affected by carnosine-added and histidine-excess diet feeding (Table 6). Carnosinase activity in whole brain was 0.238 ± 0.009 µmol/min/wet tissue (g) (n = 4) and was not affected by histidine-free, -excess and carnosine-supplemented diets (data not shown).
Table 5. Effect of histidine-free and carnosine- and histidine-excess diets on carnosinase activity of rat kidney and small intestine.

| Diet            | Kidney (μmol/min/g wet tissue) | Small intestine (μmol/min/g wet tissue) |
|-----------------|--------------------------------|----------------------------------------|
|                 | (μmol/min/g protein)           |                                        |
| Experiment 1    |                                |                                        |
| Control         | 10.29 ± 1.40                   | 2.04 ± 0.27                            |
| Histidine-free  | 8.24 ± 0.53                    | 3.94 ± 0.45**                          |
| 0.90% Carnosine | 11.47 ± 0.87                   | 1.92 ± 0.29                            |
| 1.80% Carnosine | 10.21 ± 1.37                   | 2.35 ± 0.56                            |
| 5% Carnosine    | 11.44 ± 0.70                   | 2.38 ± 0.23                            |
| Experiment 2    |                                |                                        |
| Control         | 9.04 ± 0.72                    | 2.14 ± 0.32                            |
| 5% Histidine    | 8.25 ± 0.63                    | 4.67 ± 0.37**                          |

The carnosinase activity was determined by estimating the amounts of histidine formed as described in MATERIALS AND METHODS. **p < 0.01 compared to control.

Table 6. Effect of histidine-free and -excess diets on carnosinase activity of rat gastrocnemius muscle.

| Diet            | μmol/min/g wet tissue | μmol/min/g protein |
|-----------------|-----------------------|--------------------|
| Experiment 1    |                       |                    |
| Control         | 0.128 ± 0.005         | 3.04 ± 0.21        |
| Histidine-free  | 0.115 ± 0.007         | 4.28 ± 0.22*       |
| 5% Carnosine    | 0.123 ± 0.009         | 3.15 ± 0.29        |
| Experiment 2    |                       |                    |
| Control         | 0.139 ± 0.013         | 3.12 ± 0.23        |
| 5% Histidine    | 0.138 ± 0.019         | 3.45 ± 0.37        |

The enzyme activities were estimated as described in MATERIALS AND METHODS. *p < 0.05 compared to control.

DISCUSSION

It is well known that histidine is an essential amino acid for rats (5–7). The histidine requirement of the growing rat was reported to be 0.2–0.4% of the total diet at the 10–15% protein level (22, 23). In the present study, amino acid mixture simulated with 20% casein was provided as the protein source of the control diet. Therefore, a level of 0.62% histidine was presented in the control diet and the administration of the diet caused a progressive increase in weight (Fig. 1).

Carnosine, at a level equimolar to that of histidine in the 20% casein diet, was
able to support the growth of rats fed on histidine-free diet (Fig. 1) and normal histidine levels in serum were maintained (Table 3). In a previous paper (14), we found that in rat intestine, carnosine was not actively transported in vitro using an everted sac method, but that the concentration of $\beta$-alanine and histidine on the serosal side increased clearly after incubating the intestinal sac with carnosine. Therefore, carnosine might be hydrolyzed during or after passage through the membrane. As expected, the content of free histidine in the serum of rats fed on 0.90% carnosine diet was maintained at the control level (Table 3). Moreover, we were able to find a high concentration of histidine in the serum of rats fed on 5% carnosine-supplemented diet (Table 3). Carnosinase activity and wet weight of intestine of rats fed on 0.90% casein-diet were $1.92 \pm 0.29 \mu\text{mol/min/wet tissue (g)}$ and $6.03 \pm 0.27 \text{g}$, respectively. Therefore, if a rat consumed 20 g of 0.90% carnosine-diet, the animal could digest carnosine and transform it to histidine and $\beta$-alanine in the intestine within 70 min.

On the other hand, it has recently been proposed that intestinal cells can transport carnosine efficiently across their brush-border membrane vesicles (24–26). In this paper, carnosine was detected in serum of rats fed on 5% carnosine-supplemented diet, but at a level much lower than that of histidine (Table 3). Nutzenadel and Scriver (25) proposed that rat intestine transports carnosine with a High-$K_m$ (5–10 mM) system which differs from that for $\alpha$-amino acids and other dipeptides. Carnosinase activity was present in many tissues of vertebrate and was most active in kidney (11, 21, 27). Most of the carnosine crossing the intestinal membrane could be easily hydrolyzed to the constituent amino acids in kidney. Therefore, we found no carnosine in the blood of rats fed on 0.90% and 1.80% carnosine-diets (Table 3).

Assuming the body weight of a rat fed on histidine-free diet is 200 g and that 45% of the total weight represents skeletal muscle, the carnosine content of the rat can be calculated as 550 $\mu\text{mol}$ from Table 4. Therefore, if the level of histidine of a diet required to maintain body weight is 0.2% (23) and the diet intake is 20 g per day, the rat can provide sufficient histidine from endogenous carnosine for two days. However, rats fed on histidine-free diet rapidly lost weight for 4 days and thereafter maintained a constant weight (Fig. 1). Therefore, endogenous carnosine might be important in providing histidine in the latter period.

These results suggest that dietary carnosine as well as skeletal muscle carnosine might be efficiently utilized to produce histidine in vivo.

The carnosine content of gastrocnemius muscle of rats fed on carnosine diet increased with its concentration in the diet (Table 4). The histidine content of the muscle also increased with carnosine concentration in diet. We were not able to detect carnosine in sera of rats on 0.90% and 1.80% carnosine diets (Table 3). Carnosinase activity in muscle was much lower than that in kidney or small intestine. Thus the increase of carnosine content in gastrocnemius muscle of rats fed on carnosine diet might not result in the reduction of carnosinase activity but in induction of carnosine synthesis caused by the presence of a high concentration of...
histidine in the muscle.

We are grateful to Miss N. Shigemichi, Mr. F. Fujino and Miss Y. Kojima for their excellent technical assistance.

REFERENCES

1) Tamaki, N., Tsunemori, F., Wakabayashi, M., and Hama, T. (1977): Effect of histidine-free and -excess diets on anserine and carnosine contents in rat gastrocnemius muscle. J. Nutr. Sci. Vitaminol., 23, 331–340.

2) Quinn, M. R., and Fisher, H. (1977): Effect of dietary histidine on olfaction, and rat brain and muscle concentration of histidine-containing dipeptides. J. Neurochem., 29, 717–728.

3) Tamaki, N., Morioka, S., Ikeda, T., Harada, M., and Hama, T. (1980): Biosynthesis and degradation of carnosine and turnover rate of its constituent amino acids in rats. J. Nutr. Sci. Vitaminol., 26, 127–139.

4) Snyderman, S. E., Holt, L. E., Norton, P. M., Smellie, F., and Boyer, A. (1957): Valine and histidine requirement of the normal infant. Fed. Proc., 16, 399.

5) Ackroyd, H., and Hopkins, F. G. (1916): Feeding experiments with deficiencies in the amino acid supply: Arginine and histidine as possible precursors of purines. Biochem. J., 10, 551–576.

6) Rose, W. C., and Cox, G. J. (1924): The relation of arginine and histidine to growth. J. Biol. Chem., 61, 747–773.

7) Benditt, E. P., Woolridge, R. L, Steffee, G. H., and Franzier, L. E. (1950): Amino acid utilization. IV. The minimum requirements of the indispensable amino acids for maintenance of the adult well-nourished male albino rat. J. Nutr., 40, 335–350.

8) Cho, E. S., Anderson, H. L., Wixom, R. L., Hanson, K. C., and Krause, G. F. (1984): Long term effects of low histidine intake on men. J. Nutr., 114, 369–384.

9) Ousterhout, L. E. (1960): Survival time and biochemical changes in chicks fed diets lacking different essential amino acids. J. Nutr., 70, 226–234.

10) Harding, J., and Margolis, F. L. (1976): Denervation in the primary olfactory pathway of mice. III. Effect on enzymes of carnosine metabolism. Brain Res., 110, 351–360.

11) Lenny, J. F. (1976): Specificity and distribution of mammalian carnosinase. Biochim. Biophys. Acta, 429, 214–219.

12) Margolis, F. L., Grillo, M., Grannot-Reisfeld, N., and Farbman, A. I. (1983): Purification, characterization and immunocytochemical localization of mouse kidney carnosinase. Biochim. Biophys. Acta, 744, 237–248.

13) Fukuda, S., and Kopple, J. D. (1979): Evidence that dog kidney is an endogenous source of histidine. Am. J. Physiol., 237, E1-E5.

14) Hama, T., Tamaki, N., Miyamoto, F., Kita, M., and Tsunemori, F. (1976): Intestinal absorption of β-alanine, anserine and carnosine in rats. J. Nutr. Sci. Vitaminol., 22, 147–157.

15) du Vigneaud, V., Sifferd, R. H., and Irving, G. W., Jr. (1937): The utilization of l-carnosine by animals on a histidine deficient diet. J. Biol. Chem., 117, 589–597.

16) Olejer, V., Fisher, H., and Margolis, F. L. (1982): The histidine requirement of two strains of mice with genetic differences in level of carnosinase activity. Nutr. Rep. Inter., 26, 879–885.
17) Aonuma, S., Hama, T., Tamaki, N., and Okumura, H. (1969): Orotate as a β-alanine donor for anserine and carnosine biosynthesis, and effects of actinomycin D and azauracil on their pathway. *J. Biochem.*, 66, 123–132.

18) Gerber, D. A. (1970): Determination of histidine in serum with o-phthaldialdehyde. *Anal. Biochem.*, 34, 500–504.

19) Murphey, W. H., Patchen, L., and Lindmark, D. G. (1972): Carnosinase: A fluorometric assay and demonstration of two electrophoretic forms in human tissue extracts. * Clin. Chim. Acta*, 42, 309–314.

20) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

21) Margolis, F. L., Grillo, M., Brown, C., Williams, T. H., Pitcher, R. G., and Elgar, G. J. (1979): Enzymatic and immunological evidence for two forms of carnosinase in the mouse. *Biochim. Biophys. Acta*, 570, 311–323.

22) Rose, W. C., Oesterling, M. J., and Womack, M. (1948): Comparative growth on diets containing ten and nineteen amino acids, with further observations upon the role of glutamic and aspartic acids. *J. Biol. Chem.*, 176, 753–762.

23) Rao, R. P. B., Metta, C. V., and Johnson, C. B. (1959): The amino acid composition and the nutritive value of proteins. I. Essential amino acid requirements of the growing rat. *J. Nutr.*, 69, 387–391.

24) Mattews, D. M., Addison, J. M., and Burston, D. (1974): Evidence for active transport of the dipeptide carnosine (β-alanyl-l-histidine) by hamster jejunum in *vitro*. *Clin. Sci. Mol. Med.*, 46, 693–705.

25) Nutzenadel, W., and Scriver, C. R. (1976): Uptake and metabolism of β-alanine and L-carnosine by rat tissues in *vitro*: role in nutrition. *Am. J. Physiol.*, 230, 643–651.

26) Ganapathy, V., and Leibach, F. H. (1983): Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-border membrane vesicles from the rabbit. Studies with L-carnosine and glycyl-L-proline. *J. Biol. Chem.*, 258, 14189–14192.

27) Wolos, A., and Swidowicz, K. (1979): Kidney and liver carnosinase activity and carnosine level in goose. *Comp. Biochem. Physiol.*, 62B, 515–519.