The Concerted Effects of Thyroid Function and Dietary Protein on Growth and Protein Metabolism in Mice at Different Growth Stages

Kunioki HAYASHI,1* Yukio AKIBA,1 Yuichirō TOMITA,2 and Tatsuro MATSUMOTO1

1Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai 980, Japan
2Department of Animal Science, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan
(Received August 5, 1983)

Summary The effects of thyroxine (T4) and thiouracil (TU) on growth and protein metabolism were examined in male mice given diets containing different levels of protein (casein) at two different growth stages (25 and 60 days old). Changes in protein metabolism were assessed from the expiratory 14CO2 from [U-14C]leucine injected, the liver nucleic acid contents and the rates of synthesis and degradation of liver protein estimated by single injection method using [6-14C]arginine. Each mouse, excluding the control group, received daily intraperitoneal injection of 10 μg of L-thyroxine sodium salt per 100 g b.w. (T4 group) or were given a diet containing 0.05% 2-thiouracil (TU group). In the 25-day-old mice, growth of the T4 group was accelerated at protein levels above 15% and that of the TU group was severely retarded at protein levels below 10%. On the other hand, in the 60-day-old mice, growth of the TU group tended to be accelerated at protein levels from 10% to 25%, while it was significantly retarded at the 5%-protein level. The expiratory 14CO2 increased when the growth was retarded, and decreased when growth was accelerated by T4 or TU in both age groups, but was not significant in either case. The nucleic acid content of the liver was increased by both T4 and TU when the dietary protein level was above 15%. The rate of protein synthesis was increased, but not significantly, by T4, while it was not affected by TU. The rate of protein degradation was increased, but not significantly, by TU, while it was not affected by T4 in the 25-day-old mice. In the 60-day-old mice, the rates of both liver-protein synthesis and degradation were significantly increased by TU, while they were not affected by T4. These results definitely indicate that the growth stage and

1 林 國興, 秋篠征夫, 2 富田裕一郎, 1 松本達郎
* Present address: Department of Animal Science, Faculty of Agriculture, Kagoshima University, Kagoshima 890, Japan, to whom reprint requests should be sent.

235
the dietary protein level change the effects of thyroid function on growth and protein metabolism of mice.

**Key Words** thyroxine, protein metabolism, dietary protein, growth stage

Thyroid hormone has been confirmed to be essential for growth of animals and plays an important role in protein metabolism (1, 2). However, little is known about the concerted effects of thyroid hormone and nutrients on growth and protein metabolism of mammals.

In previous papers (3, 4), we reported that the effect of thyroxine on growth of mice was influenced by dietary protein level and the growth stage. The present study was conducted to investigate in detail the influence of thyroid function on growth and protein metabolism of mice given diets different in protein levels at two different growth stages.

**MATERIALS AND METHODS**

*Animals.* Three experiments were conducted with male dd-strain mice. Prior to the experiment the animals were given a diet containing 20% casein (Table 1) *ad libitum* for 3 days. They were maintained in individual wire cages and kept under controlled light (13 h light and 11 h dark) and temperature (24°C) throughout the experiment. Diets and water were provided on free access.

*Experiments 1 and 2.* Twenty-five-day-old mice weighing on average 14g and 60-day-old mice weighing on average 27g were used in experiments 1 and 2, respectively. They were assigned to 3 treatment groups; control (C), thyroxine-injected (T₄) and thiouracil-fed (TU); and received diets with different protein levels for 8 days. L-Thyroxine was dissolved in physiological saline and intraperitoneally injected (10 µg/100 g body weight) every day for 8 days. Thiouracil was added to the diets to provide 0.5 g/kg.

At 2 h before killing by heart puncture, L-[U-¹⁴C]leucine (The Radiochemical Centre, Amersham, 324 mCi/mmol) was intraperitoneally injected at a dose of 4 µCi/100 g body weight for mice on 5, 15 and 35% protein diet. Expiratory ¹⁴CO₂ was trapped in ethanolamine-ethylene glycol monoethyl ether and the radioactivity was measured in the manner reported by Jeffay and Alvarez (5). The livers were removed and the DNA and RNA, and protein contents were determined by the method of Munro and Fleck (6) and Hartree (7), respectively.

*Experiment 3.* Male dd-strain mice of 25 and 60 days of age weighing on average 13 and 28g, respectively, were used in experiment 3. They were assigned to 3 groups of 10 animals each; C, T₄ and TU groups, and were given 15P diet (15% casein diet, Table 1) during the experimental period. Two days after the feeding, all mice were injected intraperitoneally with 2 µCi[6-¹⁴C]arginine (New England Nuclear, Boston, 23.1 mCi/mmol) in 0.1 ml physiological saline to measure the rates of synthesis and degradation of liver protein in the manner reported by Dallman.
Table 1. Composition of experimental diets.

| Ingredients          | 5  | 10 | 15 | 20 | 25 | 35 |
|----------------------|----|----|----|----|----|----|
| Casein               | 33 | 31 | 29 | 27 | 25 | 21 |
| Sucrose              | 46 | 43 | 40 | 37 | 34 | 28 |
| Salt mix. 1          | 4.0| Soybean oil | 6.4|
| Vitamin mix. 2       | 0.85| Cod liver oil | 1.6|
| Filter paper         | 3.0| Choline chloride | 0.15|

1 Salt mix. (%), CaCO₃, 29.29; CaHPO₄·2H₂O, 0.43; KH₂PO₄, 34.31; NaCl, 25.06; MgSO₄·H₂O, 9.98; Fe(C₆H₅O₇)·6H₂O, 0.623; CuSO₄·5H₂O, 0.156; MnSO₄·H₂O, 0.121; ZnCl₂, 0.02; KI, 0.0005; (NH₄)₆Mo₇O₂₄·4H₂O, 0.0025. 2 Vitamin mix. (%), thiamin HCl, 0.059; riboflavin, 0.059; calcium pantothenate, 0.235; pyridoxine HCl, 0.029; biotin, 0.001; folic acid, 0.002; menadione, 0.006; vitamin B-12, 0.0002; ascorbic acid, 0.588; nicotinic acid, 0.294; inositol, 1.176; lactose, 97.551.

and Manies (8). The labeled carbon of the [6-¹⁴C]arginine is less re-utilized than the other labeled amino acids in the liver because of the strong arginase activity in mammals.

Five mice of each group were killed by heart puncture 2 days after isotope injection, and the remaining mice were killed 6 days after the injection. The liver was removed and rinsed with physiological saline and stored at −20°C for later analysis. It was cut into small pieces with a knife, and a part thereof was homogenized with water in a glass homogenizer. The homogenate was hydrolyzed with 1 M NaOH, and the aliquots were used for the protein determination (7). Liver protein was precipitated by addition of trichloroacetic acid (TCA) in the manner reported by Yamashita and Ashida (9). Radioactivities of the precipitates were measured by the method of Kawakami and Shimura (10). The rates of liver protein synthesis and degradation were calculated through the equations reported by Millward (11).

RESULTS

Experiments 1 and 2

Body weight gain increased with increasing dietary protein level, attaining equilibrium at the 15% level in experiments 1 and 2 (Fig. 1). A significant stimulation of the body weight gain was observed in T₄ the group on diets with high protein content (35%) in experiment 1. On the other hand, severe decrease of body weight gain was found in the TU group on the diet with lower protein content (5 and 10%) in experiment 1 and was accompanied by reduced food intake (55–68% of the control). In experiment 2, body weight gain in mice given TU was significantly
Fig. 1. Body weight gain (mean ± SD) of mice given diets containing different levels of protein (casein) in experiment 1 (25–33 days of age) and experiment 2 (60–68 days of age). The mice in the thyroxine group (---●--) received daily an intraperitoneal injection of L-thyroxine-sodium salt (10 μg/100 g b.w), and the mice in the thiouracil group (---△--) were given diets containing 0.05% 2-thiouracil. Points represent the mean ± SD. *Significantly different from corresponding control group (---○--) (p < 0.05).

lowered on feeding lowest-level (5%) protein diet and it was increased, but not significantly, by feeding diets with protein levels above 10%. However, a significant difference (p < 0.01) in body weight gain was observed between the C and TU groups when two-way layout analysis of covariance was applied. No consistent change in body weight gain was observed in mice injected with T4.

Liver weight was significantly reduced by T4 injection with 35% dietary protein in experiments 1 and 2 and it was significantly increased by feeding TU with 35% dietary protein (Table 2). No significant differences in protein content of the livers were demonstrated among the treatments, excluding a significantly higher content in the TU groups on diet with 35% protein level. The DNA and RNA contents of the liver were increased by thyroxine injection in mice on diets with 15 or 35% protein levels in both experiments. Feeding thiouracil induced significant increases in the DNA content of mice on diet with 35% protein level in experiment 1 and in the RNA content of mice on diets with 15 and 35% protein levels in experiment 2.

J. Nutr. Sci. Vitaminol.
Table 2. Effect of thyroxine and thiouracil on liver weight and contents of protein, DNA and RNA in the liver of mice given diets containing different levels of protein (casein) at 35 (Experiment 1) and 68 (Experiment 2) days of age.

| Group       | 5       | 15      | 35      | 15      | 35      |
|-------------|---------|---------|---------|---------|---------|
| Control*    | 5.25 ± 0.39** | 5.43 ± 0.55 | 5.77 ± 0.13 | 4.87 ± 0.77 | 6.10 |
| Thyroxine   | 4.89 ± 0.40 | 5.28 ± 0.15b | 4.43 ± 0.46 | 4.89 ± 0.47 | 6.42 ± 0.59b |
| Thiouracil  | 5.48 ± 0.77 | 5.77 ± 0.15b | 4.43 ± 0.46 | 4.89 ± 0.47 | 6.42 ± 0.59b |

| Protein (mg) | 214 ± 18 | 260 ± 21a | 208 ± 41 | 245 ± 18 | 306 ± 21 |
|-------------|----------|----------|----------|----------|----------|
| Control     | 141 ± 18 | 192 ± 23 | 130 ± 17 | 175 ± 23 | 235 ± 30 |
| Thyroxine   | 203 ± 13 | 248 ± 21a | 205 ± 10 | 236 ± 16 | 273 ± 23 |
| Thiouracil  | 224 ± 12 | 290 ± 19b | 253 ± 13 | 236 ± 16 | 273 ± 23 |

| DNA (mg)    | 3.0 ± 0.2a | 3.4 ± 0.2b | 3.0 ± 0.3 | 3.4 ± 0.2b | 3.0 ± 0.3 |
|-------------|------------|------------|------------|------------|------------|
| Control     | 2.8 ± 0.3 | 3.9 ± 0.3b | 4.7 ± 0.3b | 4.9 ± 0.3 | 4.7 ± 0.3b |
| Thyroxine   | 2.8 ± 0.3 | 3.9 ± 0.3b | 4.7 ± 0.3b | 4.9 ± 0.3 | 4.7 ± 0.3b |
| Thiouracil  | 2.7       | 3.0 ± 0.5b | 4.0 ± 0.5b | 4.6 ± 0.4 | 4.0 ± 0.5b |

| RNA (mg)    | 4.6 ± 0.1 | 4.4 ± 0.4b | 5.5 ± 0.3b | 5.0 ± 0.3 | 5.5 ± 0.3b |
|-------------|-----------|------------|------------|----------|------------|
| Control     | 3.2 ± 0.4 | 3.6 ± 0.4b | 4.2 ± 0.5b | 4.6 ± 0.4 | 4.2 ± 0.5b |
| Thyroxine   | 2.7       | 3.0 ± 0.5b | 4.0 ± 0.5b | 4.6 ± 0.4 | 4.0 ± 0.5b |
| Thiouracil  | 2.7       | 3.0 ± 0.5b | 4.0 ± 0.5b | 4.6 ± 0.4 | 4.0 ± 0.5b |

* Each mouse in the thyroxine group received daily an intraperitoneal injection of L-thyroxine-sodium salt (100 μg/100 g b.w.), and the mice in the thiouracil group were given diets containing 0.05% 2-thiouracil. ** Mean ± SD. Means with different superscripts are significantly different (p<0.05).
Fig. 2. Expiration of $^{14}C\text{O}_2$ for 2 h after injection of L-[U-$^{14}$C]leucine into mice given diets containing different levels of protein (casein) at 33 (experiment 1) and 68 (experiment 2) days of age. The experimental conditions are the same as described in the legend to Fig. 1. Points represent the mean ± SD. * Significantly different from corresponding control group ($p<0.05$).

Expiratory $^{14}C\text{O}_2$ was increased proportionately with increasing the dietary protein levels (Fig. 2). In experiment 1, the expiratory $^{14}C\text{O}_2$ was reduced in the T$_4$ groups on diet with 35% protein and was increased in the TU groups on diets with 5 and 15% protein levels. In experiment 2, injection of thyroxine reduced, while feeding TU increased, the expiratory $^{14}C\text{O}_2$ at the lowest dietary protein level (5%).

**Experiment 3**

No significant differences in body weight gain were observed among the treatments. However, TU increased liver weight significantly in 60-day-old mice, while in the 25-day-old mice, TU had no significant effect. T$_4$ had no significant effect on liver weight in either age group. The rate of liver protein synthesis was increased, but not significantly, by T$_4$ while it was not affected by TU. The rate of protein degradation was increased, but not significantly, by TU, while it was not

*J. Nutr. Sci. Vitaminol.*
Table 3. Effect of thyroxine and thiouracil on body weight gain, liver weight and rates of liver protein synthesis ($K_s$) and degradation ($K_d$).

| Group        | Body weight gain (g/8 days) | Liver weight (g/100 g b.w.) | $K_s$ (%/day) | $K_d$ (%/day) |
|--------------|-----------------------------|-----------------------------|---------------|---------------|
| 25-day-old   |                             |                             |               |               |
| Control*     | 6.1 ± 0.9**                 | 7.15 ± 0.51                 | 17.3 ± 3.2    | 12.2 ± 3.6    |
| Thyroxine    | 6.8 ± 0.5                   | 6.57 ± 0.38                 | 21.2 ± 5.8    | 13.1 ± 4.2    |
| Thiouracil   | 6.1 ± 0.6                   | 7.39 ± 0.46                 | 18.1 ± 2.7    | 15.4 ± 3.2    |
| 60-day-old   |                             |                             |               |               |
| Control      | 2.0 ± 0.4                   | 6.78 ± 0.22a                | 16.4 ± 3.7a   | 15.7 ± 4.6a   |
| Thyroxine    | 2.0 ± 0.4                   | 6.45 ± 0.33a                | 12.9 ± 7.6a   | 14.2 ± 5.9a   |
| Thiouracil   | 1.7 ± 0.5                   | 7.96 ± 0.50b                | 25.1 ± 3.6b   | 26.2 ± 3.4b   |

* Each mouse in the thyroxine group received daily an intraperitoneal injection of L-thyroxine-sodium salt (10 µg/100 g b.w.) and the mice in the thiouracil group were given diets containing 0.05% 2-thiouracil. ** Mean ± SD. Means with different superscripts are significantly different from each other ($p < 0.05$).

affected by T₄ in the 25-day-old mice. In the 60-day-old mice, the rates of both liver protein synthesis and degradation were significantly increased by TU, while they were not affected by T₄ (Table 3).

DISCUSSION

The present study demonstrated that the effects of exogenous thyroxine and thiouracil on growth and protein metabolism of intact mice were affected by growth stage of mice and dietary protein level. Exogenous thyroxine stimulated the growth of 25-day-old mice when the level of dietary protein given was 35%. This result confirm our previous findings that growth and protein retention of young mice was accelerated by thyroxine when a diet with 20% protein was given (3, 4). On the other hand, in the 60-day-old mice, thyroxine had little effect on growth. The effect of thiouracil on the growth of mice was also affected by the growth stage and dietary protein. When diets with protein level below 10% were given to mice, growth was strongly inhibited by thiouracil in 25-day-old mice, which is associated with a decrease in food intake. However, it is unlikely that the growth retarded by thiouracil could be accounted for by reduced food intake because the decrease of food intake per unit body weight was small. These results suggest that the growth of mice is accelerated by thyroxine within physiological levels in the early stage of growth and by thiouracil in the late stage of growth, when adequate dietary protein is available.

Growth in animals is accomplished by either an increase in the rate of tissue protein synthesis or a reduction in the rate of protein degradation (12), and both synthesis and degradation play almost equivalent roles in the regulation of protein
deposition (growth) in animals (13). It has also been suggested that in smaller animals, protein synthesis in liver contributes the greater part to whole-body protein synthesis, but in muscle a smaller part (14).

The rate of $^{14}$CO$_2$ expiration from $[^{14}$C]leucine has been demonstrated to be negatively correlated to the rate of protein synthesis in muscle (15-17). Similarly in this experiment $^{14}$CO$_2$ expiration had a trend toward decreasing with acceleration of growth rate and vice versa.

In the present study, rates of protein synthesis and degradation in the liver were determined with the aid of $^{14}$C-labeled amino acid. The most recent studies on technics for estimating rates of protein synthesis and degradation are based on constant infusion of tracer. Constant infusion technics are superior to single injection technics when the time-period required for measurement is so long that re-entry of label from catabolism of protein becomes significant. However, in most previous studies adopting the constant infusion method, diurnal variation has not been considered. Diurnal variation in protein turnover has been shown to be significant, and measurements of daily protein synthesis and degradation by the constant infusion method may be erroneous (18). The single injection method is technically easier than the constant infusion method which usually requires prolonged cannulation of experimental animals and multiple sampling of labeled products (19).

In the present study, the single injection method using $[^{14}$C]guanidino-arginine was adopted. The use of $[^{14}$C]guanidino-arginine minimizes the underestimation of rates of liver protein synthesis and degradation by decreasing the reincorporation of the labeled amino acid molecule released by protein degradation which therefore is extremely useful to estimate relative protein turnover rates. Arginine is cleaved by the enzyme arginase. The $^{14}$C-labeled guanidino carbon atom is then eliminated primarily as urea, while the remaining unlabeled portion of the molecule, ornithine, is ultimately reconverted to arginine via the urea cycle. However, since recycling of the $^{14}$C isotope cannot be prevented completely, the turnover rate would be still underestimated (8, 20, 21).

Thyroxine administration induced, in mice in the early stage of growth, increases of liver DNA and RNA contents which are associated with an increase, but not of significance ($p < 0.3$), of the rate of protein synthesis (Tables 2 and 3), and expired $^{14}$CO$_2$ from $[^{14}$C]leucine injected was significantly decreased by thyroxine when a diet with 35% protein was given (Fig. 2). Degradation rate of the protein was not affected by thyroxine in the early stage of growth. These results suggest that thyroxine stimulates cell proliferation and RNA synthesis in liver irrespective of growth stage which, in the early stage of growth, is accompanied by increases in both body weight gain and rate of protein synthesis on the higher protein diet. Hence it seems likely that in the early stage of growth, growth stimulation by thyroxine is in part dependent upon stimulated protein synthesis but not upon degradation, and in the late stage of growth the initiation or elongation step in liver protein synthesis can not be accelerated by thyroxine.
In the early stage of growth, thiouracil administration increased the rate of protein degradation, but it did not affect the rate of synthesis, as shown in increases of the rate of protein degradation in the liver ($p < 0.2$) (Table 3) and expired $^{14}$CO$_2$ (Fig. 2), indicating that a decrease in growth rate of mice on a low-protein diet could be attributable to stimulated protein degradation. On the other hand, in the late stage of growth, rates of both protein synthesis and degradation were greatly increased by thiouracil administration though nucleic acids contents were lower in the thiouracil group than the thyroxine group. This indicates that RNA activity was higher in the thiouracil group than the thyroxine group.

It is well known that the rate of protein synthesis is markedly inhibited by dietary protein restriction while that of degradation is only slightly suppressed by protein restriction (22–25). Therefore, if the rate of protein degradation is increased by thyroxine and thiouracil, growth might be severely retarded by dietary protein deprivation. Although, in this experiment, the rates of liver protein synthesis and degradation were determined in mice on 15% protein diet, growth response (Fig. 1) suggests that, for instance, in the early stage of growth, the rate of protein synthesis is increased further by thyroxine in mice fed on a high-protein diet. On the other hand, on a low-protein diet, the rate of protein synthesis seems to be reduced by thiouracil.

The reasons for the differential influence of thyroid status induced by dietary protein level or growth stage of mice are not well understood in this study. Further studies will be necessary to elucidate the interrelationships among growth stage of animals, dietary protein level and thyroid status.

REFERENCES

1) Manchester, K. L. (1970): Sites of Hormonal Regulation of Protein Metabolism, ed. by Munro, H. N., Vol. 4, pp. 229–297, Academic Press, New York and London.

2) Young, V. R., and Pluskal, M. G. (1977): Mode of action of anabolic agents, with special reference to steroids and skeletal muscle: A summary review, in Proceedings of the Second International Symposium on Protein Metabolism and Nutrition, Centre for Agricultural Publishing and Documentation, Wageningen, pp. 17–28.

3) Hayashi, K., Tomita, Y., and Yamada, A. (1975): Effect of changes in thyroid function on protein metabolism of mice at different growth stages. *J. Jpn. Soc. Food Nutr.*, 28, 195–199.

4) Hayashi, K., and Tomita, Y. (1979): The effect of thyroid hormone, growth stage and dietary protein level on the growth and the liver arginase in the mice. *J. Jpn. Soc. Food Nutr.*, 32, 29–34.

5) Jeffay, H., and Alvarez, J. (1961): Liquid scintillation counting of carbon-14. Use of ethanolamine-ethylene glycol monoethy ether-toluene. *Anal. Chem.*, 33, 612–615.

6) Munro, H. N., and Fleck, A. (1969): Analysis of tissues and body fluids for nitrogenous constituents, in Mammalian Protein Metabolism, Vol. 3, ed. by Munro, H. N., Academic Press, New York and London, pp. 481–483.

7) Hartree, E. F. (1972): Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.*, 48, 422–427.
8) Dallman, P. R., and Manies, E. C. (1973): Protein deficiency: Turnover of protein and reutilization of amino acid in cell fractions of rat liver. *J. Nutr.*, **103**, 257–266.

9) Yamashita, K., and Ashida, K. (1960): Fate of threonine and leucine in rats fed threonine-deficient diets. *J. Nutr.*, **97**, 367–374.

10) Kawakami, M., and Shimura, K. (1973): A new scintillator in liquid scintillation counting and simplified method of sample preparation for determination of tritium or carbon-14. *Radioisotopes*, **23**, 81–87.

11) Millward, D. J. (1970): Protein turnover in skeletal muscle. I. The measurement of rates of synthesis and catabolism of skeletal muscle protein using $[^{14}C]Na_2CO_3$ to label protein. *Clin. Sci.*, **39**, 577–590.

12) James, W. P. T. (1972): Protein synthesis and amino acid catabolism in protein-calorie malnutrition. *Proc. Nutr. Soc.*, **31**, 225–231.

13) Mayer, R. J., Burgess, R. J., and Russell, S. M. (1980): Factors controlling intracellular breakdown of proteins, in *Protein Deposition in Animals*, ed. by Buttery, P. J., and Lindsay, D. B., Butterworths, London, pp. 21–49.

14) Garlick, P. J. (1980): Assessment of protein metabolism in the intact animal, in *Protein Deposition in Animals*, ed. by Buttery P. J., and Lindsay, D. B., Butterworths, London, pp. 51–67.

15) Sketcher, R. D., Fern, E. B., and James, W. P. T. (1974): The adaptation in muscle oxidation of leucine to dietary protein and energy intake. *Br. J. Nutr.*, **31**, 333–342.

16) Tanaka, H., Yamaguchi, M., and Kametaka, M. (1975): Metabolism of leucine and alanine in growing rats fed the diets with various protein to energy ratios. *Agric. Biol. Chem.*, **39**, 507–514.

17) Lindsay, D. B., and Buttery, P. J. (1980): Metabolism in muscle, in *Protein Deposition in Animals*, Butterworths, London, pp. 125–146.

18) Obled, C., Arnal, M., and Fauconneau, G. (1975): Feeding schedule and *in vivo* protein synthesis in various tissues of the growing rat. *Ann. Biol. Biochem. Biophys.*, **15**, 73–93.

19) Buckley, W. T., and Marquardt, R. R. (1981): Estimation of whole body protein synthesis in rats by single injection of L-$[^{1-14}C]$leucine or DL-$[^{1-14}C]$lysine. *J. Nutr.*, **111**, 763–771.

20) Arias, I. M., Doyle, D., and Schimke, R. T. (1969): Studies on synthesis and degradation of proteins of the endoplasmic reticulum of rat liver. *J. Biol. Chem.*, **244**: 3303–3315.

21) Swick, R. W. (1958): Measurement of protein turnover in rat liver. *J. Biol. Chem.*, **231**, 751–764.

22) Millward, D. J. (1970): Protein turnover in skeletal muscle. II. The effect of starvation and a protein-free diet on the synthesis and catabolism of skeletal muscle proteins in comparison to liver. *Clin. Sci.*, **39**, 591–603.

23) Funabiki, R., Watanabe, Y., Nishizawa, N., and Hareyama, S. (1976): Quantitative aspects of myofibrillar protein turnover in transient state on dietary protein depletion and repletion revealed by urinary excretion of N-methylhistidine. *Biochim. Biophys. Acta*, **451**, 143–150.

24) Rikimaru, T., Yamamoto, S., Maeda, K., and Inoue, G. (1980): Effects of protein deficiency on muscle myofibrillar protein turnover in adult rats. *J. Nutr. Sci. Vitaminol.*, **26**, 39–57.

25) Smith, C. K., Duschlag, R. P., and Layman, D. K. (1982): Response of skeletal muscle protein synthesis and breakdown to levels of dietary protein and fat during growth in weanling rats. *J. Nutr.*, **112**, 255–262.