Effects of Glucose and Amino Acid Depletions on Protein Synthetic Parameters in Liver and Skeletal Muscle of Rats during Parenteral Nutrition

Takeo KIKUCHI, Hiroh OKAMOTO, Kazuo CHIKU, and Yasuo NATORI

1 Research Laboratories, Morishita Pharmaceutical Co., Ltd., Yasu-machi, Yasu-gun, Shiga 520-23, Japan
2 Department of Nutritional Chemistry, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima 770, Japan
(Received June 27, 1986)

Summary Rats were nourished by intravenous infusion of four different experimental solutions: total parenteral nutrition (TPN) solution (group I); glucose-depleted TPN solution (group II); amino acid-depleted TPN solution (group III); and glucose- and amino acid-depleted TPN solution (group IV). All except group I animals lost body weight and showed negative nitrogen balance during a 7-day experimental period; glucose-depleted groups II and IV generally suffered more severely than amino acid-depleted group III. However, the concentration of plasma albumin in group III was significantly lower than that in the other groups. The weights of liver and gastrocnemius muscles after 7 days of infusion with different nutrient compositions were fairly well correlated with the RNA/DNA ratios in these tissues. Infusion of nutritionally deficient solutions caused progressive disaggregation of polysomes in both liver and gastrocnemius muscles, indicating variable degrees of impairment of protein synthesis in these tissues. The changes in polysomal profiles were rapid and sensitive; the polysomal disaggregation was evident within one day of infusion with deficient solutions. The determination of polysomal profiles in various tissues may be useful in optimizing the composition of TPN solutions.

Key Words total parenteral nutrition, protein synthesis, polysomal profile, liver, skeletal muscle

The technique of total parenteral nutrition (TPN) has been successfully applied to animals and human patients (1–3). However, many unanswered questions remain concerning the optimal composition of amino acid solutions and the amounts of...
different nutrients required for various conditions and ages. The nutritional status of animals and patients during TPN has been assessed mostly on the basis of such parameters as body weight changes, nitrogen balance, plasma protein concentrations and immunological indices. Very few attempts have been made to study intracellular biochemical events as a means to evaluate the nutritional status of particular organs.

Protein synthesis in mammalian cells is carried out on polysomes where one strand of messenger RNA (mRNA) is associated with several ribosomes, each one carrying a peptide chain. There is growing evidence that the nutritional state dictates the number of ribosomes associated with the mRNA strand and, as a consequence, prescribes the rate of protein synthesis (for review see Refs. 4 and 5).

The present study was designed to investigate the usefulness of "polysomal profiles" as a parameter of protein synthetic activities in the liver and skeletal muscle of rats during parenteral nutrition. We found that the polysomal profiles readily changed in response to nutrient deficiencies caused by depletion of glucose and/or amino acids from a complete infusion mixture.

MATERIALS AND METHODS

Animal preparation and infusion procedure. Male Wistar rats (Japan Clea Co., Tokyo), each weighing 150–160 g, were housed in individual cages in a controlled environment (temperature 23 ± 1°C, and light from 0700 to 1900 h) and fed a commercial diet (CE-II®, Japan Clea Co., Tokyo) before initiation of infusion. When body weight reached about 200 g (180–220 g), animals were divided into four body-weight-matched groups of 8–12 animals and prepared for continuous infusion by using techniques similar to that described by Steiger et al. (2).

Experimental design. During the overnight surgical recovery period, a TPN solution was infused intravenously at a rate of 30 ml/day/rat, and the rate was doubled on and after the next day. Three days following surgery, which is designated as 0 day of the experiment, infusion of the experimental solutions was initiated. The composition of the four experimental solutions is shown in Table 1: i.e., TPN solution (group I), glucose-depleted TPN solution (group II), amino acid-depleted TPN solution (group III), and glucose- and amino acid-depleted TPN solution (group IV). Infusion of experimental solutions continued for 7 consecutive days.

Sample collection and analytical methods. The rats' body weight was recorded daily. Total urine was collected each day and analyzed for output of nitrogen (6) from which the nitrogen balance was calculated. Urinary creatinine was also determined by the method of Bonsnes and Taussky (7).

On day 7 of the test period, blood was collected by cardiac puncture, and
Table 1. Composition of intravenous infusates.

|                  | Group I | Group II | Group III | Group IV |
|------------------|---------|----------|-----------|----------|
| Glucose (g/liter)| 206     | —        | 206       | —        |
| Amino acids (g/liter) | 33   | 33       | —         | —        |
| Na⁺ (mEq/liter)  | 77      | 77       | 77        | 77       |
| K⁺ (mEq/liter)   | 25      | 25       | 25        | 25       |
| Cl⁻ (mEq/liter)  | 72      | 72       | 72        | 72       |
| Mg²⁺ (mEq/liter) | 5       | 5        | 5         | 5        |
| Ca²⁺ (mEq/liter) | 6.7     | 6.7      | 6.7       | 6.7      |
| Vitamins         | B₁, B₂, B₆, B₁₂, choline, nicotinic acid, pantothenic acid |
| Trace elements   | Zn, Fe, Cu, I |
| Total volume (ml/rat/day) | 60  | 60       | 60        | 60       |
| Total energy (kcal/rat/day) | 64  | 8.8      | 55        | 0        |

¹ Group I, TPN solution; group II, glucose-depleted TPN solution; group III, amino acid-depleted TPN solution; group IV, glucose- and amino acid-depleted TPN solution.
² Group I and group II solutions contained the following amino acids in one liter: L-Ile (1.74 g), L-Leu (3.88 g), L-Lys·HCl (3.41 g), L-Met (1.09 g), L-Phe (2.90 g), L-Thr (2.02 g), L-Trp (0.40 g), L-Val (1.40 g), L-Ala (1.92 g), L-Arg·HCl (2.96 g), L-Asp (1.81 g), L-Cys·HCl (0.45 g), L-Glu (2.02 g), L-His·HCl (2.51 g), L-Pro (1.02 g), L-Ser (0.68 g), L-Tyr (0.11 g), and Gly (3.32 g).

plasma was analyzed for glucose (8), urea nitrogen (9), total protein (10) and albumin (11). Liver and gastrocnemius muscles were also excised for the determinations of DNA (12), RNA (12) and protein (13) contents.

Results are expressed as means ± SEM. All data were subjected to analysis of variance and, when appropriate, differences between group means were tested for significance with Student’s t test or Cochran-Cox’s method (14). Results were considered significant when p was less than 0.05.

Preparation and sucrose density gradient centrifugation of polysomes. Rats were killed by decapitation on days 1, 3 and 7 of the test period, and livers and gastrocnemius muscles were quickly excised. The tissues were immediately frozen in liquid nitrogen and kept in a ultra-freezer at −70°C until use.

Livers were homogenized in 3 volumes of ice-cold 0.35 M sucrose containing buffer A (200 mM Tris-acetate, pH 8.5, 50 mM KCl, 10 mM magnesium acetate and 6 mM 2-mercaptoethanol) with a Potter-Elvehjem-type homogenizer. The homogenate was centrifuged at 10,000 × gav for 10 min and the resulting supernatant (postmitochondrial supernatant) was made 1% each in sodium deoxycholate and Triton X-100. The detergent-treated postmitochondrial supernatant was centrifuged at 105,000 × gav for 10 min to precipitate glycogen and 3-ml aliquots of the supernatant were layered over discontinuous sucrose gradients consisting of 2.5 ml
each of 2.0 M sucrose and 0.5 M sucrose, both containing buffer A. The gradients were centrifuged for 24 h at 105,000 × gav in a Hitachi RP-65 rotor (Hitachi Koki Co., Tokyo) and polysomes were obtained as pellets.

Muscles were homogenized for 20 sec in 5 volumes of ice-cold buffer B (10 mM Tris-HCl, pH 7.5, 250 mM KCl, 10 mM MgCl2 and 6 mM 2-mercaptoethanol) with a Polytron® homogenizer (Kinematica GmbH, Kriens/Luzern, Switzerland). The homogenate was centrifuged at 10,000 × gav, for 15 min and the resulting supernatant (3 ml) was layered over discontinuous sucrose gradients consisting of 2.5 ml each of 2.0 M sucrose and 0.5 M sucrose, both containing buffer B. The muscle polysomes were obtained by centrifuging the gradients under the same condition as described above for the liver.

The liver and muscle polysomes were suspended in 2-mercaptoethanol-omitted buffer A and B, respectively, and the suspensions, containing 2.0 A260 units, were analyzed on a linear 0.5–1.5 M sucrose gradient containing the same buffer. Centrifugation was carried out in a Hitachi RPS-56T rotor at 50,000 rev/min for 60 min. The absorbance at 254 nm was continuously monitored with an ISCO absorbance monitor (Model UA-5, Instrumentation Specialties Co., Lincoln, Nebraska). Although polysomal profiles shown in the RESULTS represent those from a single animal, reproducibilities among the animals in the same experimental group were excellent. The areas under the curves for polysomes larger than dimers and for monomers-dimers were measured. The values are expressed as % ribosomes (monomers-dimers) as polysomes.

RESULTS

Nutritional status of animals during infusion of complete or deficient infusates

Figure 1 shows the growth of rats infused with various test solutions. The rats in group I gained body weight while animals in other groups lost weight. The loss of body weight was marked in groups II and IV where glucose was depleted from the TPN solution.

The nitrogen balances of rats in each group (Fig. 2) were generally correlated with body weight changes in that increased daily negative balances were observed when glucose-depleted solutions were infused. Since the rats in groups I and II received equal amounts of nitrogen, the differences in nitrogen balance are ascribed to increases in urinary nitrogen excretion resulting from increased tissue catabolism. It should be noted that in group II nitrogen balance was somehow maintained until the third day of infusion and the balance turned negative thereafter. More or less the same trend was observed in group IV. In group III, where amino acids were depleted, the trend was opposite; daily urinary nitrogen excretion gradually decreased toward the end of the experiment.

The laboratory data of blood constituents after 7 days of different parenteral nutrition are listed in Table 2. The concentration of plasma glucose in group IV was considerably lower than that of other groups. Urea nitrogen concentration was
Fig. 1. Body weight changes of rats infused with various test solutions. ○, TPN solution (I); ●, glucose-depleted TPN solution (II); ▲, amino acid-depleted TPN solution (III); △, glucose and amino acid-depleted TPN solution (IV). * Significant difference from group I at p<0.05.

Table 2. Data on glucose, urea nitrogen, total protein and albumin in the plasma after 7 days of different parenteral nutrition.

| Group | Glucose (mg/dl) | Urea nitrogen (mg/dl) | Total protein (g/dl) | Albumin (g/dl) |
|-------|-----------------|-----------------------|----------------------|----------------|
| I     | 116.4 ± 5.2     | 8.4 ± 0.5             | 5.28 ± 0.06          | 3.20 ± 0.12    |
| II    | 103.5 ± 9.9     | 30.2 ± 2.7a           | 4.37 ± 0.13a         | 2.69 ± 0.13a   |
| III   | 92.3 ± 12.1     | 2.3 ± 0.3a,b          | 3.90 ± 0.12a         | 2.29 ± 0.06a   |
| IV    | 56.4 ± 8.4a,b   | 34.4 ± 2.1a,c         | 4.50 ± 0.25          | 2.68 ± 0.13a,c |

* Significant difference from I. a Significant difference from II. b Significant difference from III. All differences were at p<0.05 or more.

greatly increased in the glucose-depleted groups II and IV. The concentration of total plasma protein and albumin in amino acid-depleted group III was significantly lower than that in the other groups.
Fig. 2. Nitrogen balance of rats infused with various test solutions. See legend to Fig. 1 for symbols. * Significant difference from group I at $p<0.05$.

Table 3. Liver weight and cell composition after 7 days of different parenteral nutrition.

| Group | Liver weight (g) | Protein (g/liver) | RNA (mg/liver) | DNA (mg/liver) | Protein/DNA | RNA/DNA |
|-------|-----------------|------------------|----------------|----------------|-------------|---------|
| I     | 7.38 ± 0.20     | 1.40 ± 0.02      | 68.2 ± 2.1     | 25.1 ± 0.6     | 55.8 ± 1.5  | 2.72 ± 0.07 |
| II    | 3.79 ± 0.32a    | 0.89 ± 0.08a     | 42.1 ± 4.4a    | 20.2 ± 1.2a    | 44.2 ± 3.3a | 2.08 ± 0.18a |
| III   | 7.89 ± 0.63a-b  | 1.06 ± 0.07a     | 63.3 ± 5.7b    | 23.9 ± 0.8b    | 44.4 ± 2.2b | 2.65 ± 0.22 |
| IV    | 2.77 ± 0.13a-b-c| 0.55 ± 0.02a-b-c | 23.6 ± 0.7a-b-c| 22.7 ± 1.3     | 24.1 ± 0.5a-b-c | 1.05 ± 0.07a-b-c |

* Significant difference from I. b Significant difference from II. c Significant difference from III. All differences were at $p<0.05$ or more.
Protein synthetic parameters in liver

The abundance of ribosomes and other RNA-containing components is generally correlated with the rate of protein synthesis in tissue (4). As shown in Table 3, the RNA contents of liver cells were markedly reduced in the glucose-depleted groups II and IV. The reduced RNA contents should lead to a reduction in protein synthesis. Marked reductions in liver weights and liver protein contents in groups II and IV probably resulted from decreased protein anabolism on the one hand and from increased protein catabolism (cf. nitrogen balance data in Fig. 2) on the other. The RNA contents in the liver of amino acid-depleted group III were not significantly different from that of the TPN-infused group I. Thus amino acid depletion appears to alter not so much the quantity of ribosomes in liver cells as the efficiency of ribosomes in translating the message.
Table 4. Proportion of ribosomes as polysomes from livers of rats infused with complete or deficient infusates.\(^1\)

| Infusion time | Group I | Group II | Group III | Group IV |
|---------------|---------|----------|-----------|----------|
| 1 day         | 10.7    | 26.1     | 19.9      | 28.7     |
| 7 days        | 13.2    | 33.4     | 36.5      | 63.1     |

\(^1\) Values are expressed as % monomers-dimers as polysomes, calculated from the polysomal profiles in Fig. 3 as described in MATERIALS AND METHODS.

Table 5. Gastrocnemius muscle weight and cell composition after 7 days of different parenteral nutrition.

| Group | Muscle weight (g) | Protein (mg/muscle) | RNA (mg/muscle) | DNA (mg/muscle) | Protein/DNA | RNA/DNA |
|-------|-------------------|---------------------|-----------------|----------------|-------------|---------|
| I     | 2.48 ± 0.08       | 401 ± 14.5          | 3.63 ± 0.12     | 1.50 ± 0.06    | 270 ± 12.8  | 2.35 ± 0.15 |
| II    | 1.62 ± 0.04\(^a\) | 267 ± 7.7\(^a\)    | 1.91 ± 0.12\(^a\)| 1.05 ± 0.04\(^a\)| 255 ± 6.8   | 1.82 ± 0.08\(^a\) |
| III   | 2.04 ± 0.06\(^ab\)| 327 ± 10.3\(^ab\)  | 2.32 ± 0.15\(^a\)| 1.17 ± 0.07\(^a\)| 283 ± 18.6  | 1.98 ± 0.05 |
| IV    | 1.65 ± 0.09\(^ac\)| 245 ± 18.4\(^ac\)  | 1.49 ± 0.25\(^a\)| 0.94 ± 0.08\(^a\)| 262 ± 3.3   | 1.73 ± 0.19\(^a\) |

\(^a\) Significant difference from I. \(^b\) Significant difference from II. \(^c\) Significant difference from III. All differences were at \(p < 0.05\) or more.

As seen in Fig. 3, the size spectrum of liver polysomes remained essentially the same during 7 days of infusion with the TPN solution (group I) with the proportion of ribosomes representing about 10% of total polysomes. Compared with control group I, infusion of deficient solutions induced progressive disaggregation of liver polysomes. A shift of heavy polysomes to oligomers was more marked in glucose-depleted group II than in amino acid-depleted group III. The depletion of both glucose and amino acids (group IV) induced the most extensive disaggregation. The proportion of ribosomes as polysomes from the livers of rats infused with various test solutions was calculated from the polysomal profiles and the results are shown in Table 4. It should be noted that the polysome breakdown and the resulting increase in the ribosome proportion are evident in all the deficient groups as early as 1 day after the initiation of infusion with test solutions.
Fig. 4. Sedimentation patterns of gastrocnemius muscle polysomes during infusion of various test solutions.

Table 6. Proportion of ribosomes as polysomes from gastrocnemius muscles of rats infused with complete or deficient infusates.\(^1\)

| Infusion time | Group I | Group II | Group III | Group IV |
|---------------|---------|----------|-----------|----------|
| 1 day         | 24.7    | 41.6     | 41.6      | 53.4     |
| 7 days        | 24.4    | 59.1     | 27.9      | 60.8     |

\(^1\) Values are expressed as % monomers-dimers as polysomes, calculated from the polysomal profiles in Fig. 4 as described in MATERIALS AND METHODS.

Protein synthetic parameters in muscle

Table 5 shows that the weight and protein content of gastrocnemius muscles were markedly reduced in all the deficient groups. The glucose-depleted groups II
and IV suffered more severely than the amino acid-depleted group III. The wasting of muscles was reflected in urinary creatinine excretion; the amounts of creatinine excreted on the last day of infusion were 5.06 ± 0.12, 4.29 ± 0.09, 4.62 ± 0.37 and 4.14 ± 0.15 mg/day in groups I, II, III and IV, respectively.

The RNA contents decreased more or less in correspondence with the changes in muscle weights. Unlike liver, the DNA content in the muscle decreased during infusion of deficient solutions, suggesting that some muscle cells were irreversibly damaged during the infusion.

The polysomal profiles of gastrocnemius muscles under different nutritional conditions are essentially similar to those observed in the liver except that the proportion of ribosomes in control group I represented about 25% of the total polysomes (Fig. 4 and Table 6). The apparent partial recovery of ribosomal proportion in the muscles of group III animals on day 7 of infusion was accompanied by an obvious shift of the average polysome size toward lighter aggregates.

DISCUSSION

The TPN technique offers a unique opportunity to modulate the quality and quantity of nutrient influx into animals and human patients. In the present study, we examined the effects of glucose and/or amino acid depletions from a TPN solution on the nutritional status of rats.

The infusion of nutritionally deficient solutions caused a loss of body weight, negative nitrogen balance, a reduction in plasma protein concentration and decreases in RNA contents in the liver and gastrocnemius muscles. The depletion of glucose (group II) generally caused more severely adverse effects on various parameters than the depletion of amino acids (group III). However, the concentration of plasma proteins, particularly albumin, in group III was lower than that in group II. The hypoalbuminemia in group III is the result of amino acid depletion from the infusate. We recently found that extracellular amino acids are directly utilized for the synthesis of secretory proteins in liver cells without mixing completely with the intracellular free amino acid pool (15). The synthesis of albumin may therefore be more sensitive to reduced amino acid supply from external sources than the synthesis of intracellular proteins in the liver. However, the situation may not be so simple as the above argument in view of a recent report by Lunn and Austin (16), who found that for hypoalbuminemia to occur, not only must dietary protein be inadequate, but it is also necessary for energy consumption to be in excess of requirements. The hypoalbuminemia in group III may thus be the composite result of amino acid deficiency on the one hand and relative excess glucose intake on the other.

With respect to daily urinary nitrogen excretion, the glucose-depleted group II maintained better nitrogen balance than the amino acid-depleted group III until the third day of infusion, after which the balance turned gradually negative. When
energy intake is restricted, the body first draws upon glycogen and fat reserves to correct any deficiency in the metabolic energy pool and, after these reserves are exhausted, utilizes protein reserves as an energy source as evidenced by increased urinary nitrogen excretion with concomitant weight loss. It should also be noted that daily nitrogen excretion in amino acid-depleted group III gradually decreased during parenteral nutrition. A similar trend was observed in an earlier study by Tao et al. (17).

Since protein biosynthesis is carried out on ribosomes, the rate of protein synthesis is primarily determined by the amount and activity of ribosomes in a given tissue. The amount of ribosomes can be estimated by simply determining the total RNA content because ribosomal RNA constitutes the majority of cellular RNA. We found that the weights of liver and gastrocnemius muscles after 7 days of infusion with different nutrient compositions were fairly well correlated with the RNA/DNA ratios in those tissues. These observations suggest that the loss of organ weight and, therefore, the loss of whole body weight during infusion of deficient nutrients are the result of reduced protein synthesis in various organs.

However, as stated earlier, the rate of protein synthesis is determined not only by the amount but also by the activity of ribosomes. The functional activity of ribosomes in vivo can be conveniently assessed by observing the size spectrum of polysomes. Many authors have shown that reduction of protein synthesis under unfavorable nutritional conditions is often accompanied by disaggregation of polysomes in various tissues (for review see Refs. 4 and 5). A previous report from our laboratory (18), for instance, demonstrated that feeding a protein-free diet to rats caused an extensive disaggregation of liver polysomes, presumably because the initiation step of protein synthesis was more severely depressed than the translocation step in the liver of protein-depleted rats. Yokogoshi and his associates (19, 20) reported that the analysis of liver polysomal profiles might be helpful as a measure of dietary protein quality and quantity.

We found in the current investigation that the infusion of nutritionally deficient solutions to rats caused progressive disaggregation of polysomes in both the liver and gastrocnemius muscles, indicating variable degrees of impairment of protein synthesis in these tissues. The changes in polysomal profiles were rapid and sensitive; the polysomal disaggregation was evident in both the liver and muscles as early as on the next day infusate switch when most other parameters, including body weight, and blood and tissue compositions, show no significant changes. The polysomal profiles differentially indicate the state of protein synthesis in individual tissues, which otherwise is difficult to estimate. Thus the determination of polysomal profiles in various tissues may prove to be a useful method as a means to evaluate the optimal composition of TPN solutions and the amounts of different nutrients required for various pathological conditions in experimental animals.

The present study has dealt with extreme cases of nutrient shortages caused by complete depletion of glucose and/or amino acids from the infusion mixtures. We are now applying the present method to evaluate the effect of infusion of branched-
chain amino acid-enriched TPN solutions in rats.

REFERENCES

1) Dudrick, S. J., Wilmore, D. W., Vars, H. M., and Rhoads, J. E. (1968): Long-term parenteral nutrition with growth, development, and positive nitrogen balance. Surgery, 64, 134–142.

2) Steiger, E., Vars, H. M., and Dudrick, S. J. (1972): A technique for long-term intravenous feeding in unrestrained rats. Arch. Surg., 104, 330–332.

3) Wretlind, A. (1972): Complete intravenous nutrition. Nutr. Metabol., 14, 1–57.

4) Munro, H. N. (1970): A general survey of mechanisms regulating protein metabolism in mammals, in Mammalian Protein Metabolism, ed. by Munro, H. N., Vol. IV, Academic Press, New York, pp. 3–130.

5) Spadoni, M. A., and Gaetani, S. (1972): Influence of nutritional factors on ribosome dynamics, in Newer Method of Nutritional Biochemistry, ed. by Albanese, A. A., Vol. V, Academic Press, New York, pp. 145–182.

6) Hozumi, K., Tsuji, O., and Kushima, H. (1970): An automatic microdetermination of carbon, hydrogen, and nitrogen in organic compounds using data printing system. Microchem. J., 15, 481–497.

7) Bonsnes, R. W., and Taussky, H. H. (1949): On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 158, 581–591.

8) Marks, V., and Lloyd, K. (1963): Preservation of blood samples for glucose analysis by glucose-oxidase. Clin. Chim. Acta, 8, 326.

9) Searcy, R. L., and Cox, F. M. (1963): A modified technique for ultramicro estimation of urea nitrogen. Clin. Chim. Acta, 8, 810–812.

10) Gornall, A. G., Bardawill, C. J., and David, M. M. (1949): Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177, 751–766.

11) Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971): Albumin standards and the measurement of serum albumin with brom cresol green. Clin. Chim. Acta, 31, 87–96.

12) Karsten, U., and Wollenberger, A. (1972): Determination of DNA and RNA in homogenized cells and tissues by surface fluorometry. Anal. Biochem., 46, 135–148.

13) 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.

14) Cochran, W. G., and Cox, G. M. (1950): Experimental Designs. John Wiley & Sons, New York.

15) Oshita, T., Kawada, T., Endo, Y., and Natori, Y. (1984): Heterogeneity of free valine pools for protein synthesis on free and membrane-bound polysomes in rat liver. J. Biochem., 96, 651–657.

16) Lunn, P. G., and Austin, S. (1983): Dietary manipulation of plasma albumin concentration. J. Nutr., 113, 1791–1802.

17) Tao, R. C., Yoshimura, N. N., Chin, I. B., and Wolfe, A. M. (1979): Determination of intravenous non-protein energy and nitrogen requirements in growing rats. J. Nutr., 109, 904–915.

18) Sato, A., Noda, K., and Natori, Y. (1979): The effect of protein depletion on the rate of protein synthesis in rat liver. Biochim. Biophys. Acta, 561, 475–483.

19) Yokogoshi, H., Sakuma, Y., and Yoshida, A. (1980): Relationships between nutritional quality of dietary proteins and hepatic polyribosome profiles in rats. J. Nutr., 110, 383–387.

20) Yokogoshi, H., Sakuma, Y., and Yoshida, A. (1980): Effect of dietary protein quality and quantity on hepatic polyribosome profiles in rats. J. Nutr., 110, 1347–1353.

J. Nutr. Sci. Vitaminol.