ROLE OF INTESTINAL MICROBES IN BODY COMPOSITION IN GERM-FREE, GNOTOBIOTIC AND CONVENTIONAL MICE

Masanori YAMANAKA, Tatsuji NOMURA, and Masao KAMETAKA

2Central Institute for Experimental Animals, 1430 Nogawa, Takatsu, Kawasaki 211, Japan
3Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received September 6, 1976)

Summary To study the role of intestinal microbes in body composition and the effect of Staphylococcus epidermidis (Staph.) on body nitrogen (N) accumulation in host mice, ICR strain male, germ-free (GF) mice, gnotobiotic (GB) mice, obtained from GF mice monocontaminated with Staph. at three weeks of age, and conventional (CV) mice were used. All mice were separated into two groups, one group was killed at five weeks of age (B5) and the other group was killed at eight weeks of age (B8) after being fed an irradiated purified whole-egg protein diet for three weeks. The body weight gains in the three-week period were higher in CV mice than in GF and GB mice. The moisture contents of a carcass per 100 g of body weight were lower in CV mice than in GF and GB mice in both the B5 and B8 groups. The lipid and energy of the carcasses of CV mice in the B5 group were higher than in the other mice. Crude protein of the carcasses of CV mice in the B5 group was higher than in the other mice. Accumulation of dry matter, lipid and energy per mouse for three weeks was higher in CV mice than in GF and GB mice. Crude protein accumulation per unit body weight gains in GB mice was higher than that of GF or CV mice. With respect to N and energy retention per unit food intake by the slaughter method, CV mice tended to show high values. The results of N balance by the balance method showed the similar tendencies as the results of the slaughter method. GF mice showed higher values for dry matter, N and energy of gut contents per 100 g of body weight than those of the other mice. Gross energy of crude protein and lipid in carcass showed no differences among the three groups of mice.

1 This work was used as part of a doctor's thesis submitted by M. Yamanaka to the University of Tokyo.
2 山中聖彦, 野村達次, 亀高正夫
When germ-free (GF) mice were mono- or polycontaminated by five kinds of bacteria, i.e., Bacteroides sp., Eschericia coli (E. coli), Lactobacillus sp., Staphylococcus epidermidis (Staph.) and Streptococcus faecalis, the intestinal microbes had different effects on body nitrogen (N) accumulation as shown in the previous series of experiments. Staph. increased N accumulation of the host mice (I–6).

The purpose of this investigation is to explain the role of intestinal microbes especially Staph. in body composition in GF, Staph. monocontaminated gnotobiotic (GB) and conventional (CV) mice by comparative slaughter methods.

EXPERIMENTAL

Diets. Mice were given an autoclaved CL–2 diet (7, 8) (as in the previous series of experiments) until five weeks of age and thereafter they ate a purified whole-egg protein diet for three weeks (Table 1). This diet contained 10% crude protein (N × 6.25) and it was sterilized by 5 Mrad irradiation with 60Co (Japan Isotope Irradiation Cooperative Society, Tochigi). Diets and water were given ad libitum.

Table 1. Composition of whole-egg protein diet.

|                |          |
|----------------|----------|
| Whole-egg protein | 10.6 g   |
| Corn starch     | 71.4 g   |
| Corn oil        | 8.0 g    |
| Cellulose powder| 4.0 g    |
| Salt mixture     | 4.0 g    |
| Vitamin mixture  | 2.0 g    |

a Provided by Taiyo Foods, Co., Ltd., Yokkaichi, Japan.
b Salt mixture: sodium chloride, 139.3 g; potassium biphosphate, 389.0 g; magnesium sulfate, anhydrous, 57.3 g; calcium carbonate, 381.4 g; ferrous sulfate, 27.0 g; manganese sulfate, 4.01 g; potassium iodide, 0.79 g; zinc sulfate, 0.548 g; cupric sulfate, 0.477 g; cobalt chloride, 0.023 g. (U. S. Pharmacopeia XVIII)
c Vitamin mixture: vitamin A, 1,000 IU; vitamin D₃, 100 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; riboflavin, 1.0 mg; thiamine, 0.5 mg; pyridoxine, 0.4 mg; pantothenic acid, 4.0 mg; niacin, 4.0 mg; choline, 200 mg; inositol, 25 mg; para-aminobenzoic acid, 10 mg; vitamin B₁₂, 2 μg; biotin, 0.02 mg; folic acid, 0.2 mg. Add sufficient cellulose to make 1 g. (J.A. Campbell: Method for determination of PER and NPR)

Animals. ICR strain GF mice, GB mice produced by gastric administration of 0.2 ml of Staph. incubated culture to three-week old GF mice and CV mice were reared in plastic rearing cages (three mice per cage) until five weeks of age. At this time, six mice were chosen from the 12 mice in each group and were sacrificed (at 10 AM) by etherization (B₅ group). Among the mice chosen, the body weights of the 12 mice were measured and these mice were separated into two groups with the same body weight levels as much as possible. The other six mice were reared individually in metabolism cages with the diet shown in Table 1 for three weeks. Food intake was measured for three weeks and the
feces and urine were collected in a solution acidified with 0.1 N HCl. The six mice in each group were killed (at 10 AM) by etherization at eight weeks of age (B₈ group). GF and GB mice were reared in vinyl isolators, and CV mice were kept in an open animal room with a temperature of 23±2°C and a humidity of 50–70%. The gastrointestinal tracts from stomach to rectum were removed and their contents were washed out immediately. Carcasses including the gastrointestinal tract and gut contents were stored at –20°C.

Bacteriological tests. In checking the GF status, fresh feces and a swab from the walls of the vinyl isolator were cultured for seven days in thioglycolate medium at 37°C and room temperature. The GF groups were tested at three weeks of age and again at eight weeks of age, and the GB groups were tested at the age of three weeks before Staph. monocontamination (1). Two weeks after the Staph. monocontamination at the age of five weeks, the population of established Staph. was cultured in nutrient broth at 37°C for 24 hours on fresh fecal specimens (2, 3).

Analyses. The carcasses including the gastrointestinal tract were dried at 60°C after spraying with 0.1 N HCl solution. The dried carcasses were ground in a mortar. The gut contents were thawed and blended in a mixer. The feces and urine were dried at room temperature with the urine soaking into the feces. The dried feces and urine were ground in a mortar.

The samples of carcasses, gut contents and excreta were analyzed as follows; moisture, total N, ash, lipid and energy in the carcasses; dry matter, total N and energy in the gut contents; and total N in the feces and urine were estimated. Analyses of samples were performed by routine methods (9), e.g., total N was estimated by the semi-micro Kjeldahl method, ash was measured by incandescence in an electric furnace (550°C), lipid was measured by the Folch extraction method (10) and energy of carcass, gut contents and lipid extracted from the carcass were measured with a calorimeter (Shimadzu-Nenken Recording Bomb Calorimeter CA–2).

RESULTS

In these experiments, the GF state was maintained in the GF group until the experiments were completed. GB mice were also in the GF state until three weeks of age when they were contaminated with Staph. Logarithmic viable counts per g of feces two weeks after Staph. monocontamination were in the range of 7.4–9.1. This Staph. was well established and these results agreed with those of the previous experiments (2, 6). The increases in body weight from 5 to 8 weeks of age are shown in Fig. 1. Body weight gains of CV mice in the experimental period were higher (P<0.001) than those of GF or GB mice. The body weights at 5 and 8 weeks of age were as follows: GF: 23.6±0.6 g (mean ± standard error) and 30.1±0.5 g; GB: 23.7±1.5 g and 29.3±0.9 g and CV: 22.7±0.4 g and 32.9±0.8 g, respectively.

Body components of B₅ and B₈ mice per 100 g of body weight and per 100 g
Fig. 1. Growth curves of germ-free, *Staphylococcus epidermidis* monocontaminated gnotobiotic and conventional mice fed whole-egg protein diet for an experimental period of three weeks. GF, germ-free mice; GB, *Staphylococcus epidermidis* monocontaminated gnotobiotic mice; CV, conventional mice.

Moisture contents per 100 g of body weight in both the $B_5$ and $B_8$ groups of CV mice were lower ($B_5$: CV vs GF and GB $P<0.001$, $B_5$: CV vs GF $P<0.02$, CV vs GB $P<0.01$) than those of GF and GB mice. Crude protein of CV mice of the $B_5$ group was higher ($P<0.001$) than that of GF or GB mice. In the $B_8$ group, lipid contents of CV and GF mice were higher than those of GB mice (CV vs GB: $P<0.01$, GF vs GB: $P<0.02$). The $B_8$ group of GB mice showed a higher ($P<0.05$) ash value than that of CV mice, but there were no differences among mice in the $B_5$ groups. Energy contents showed similar tendencies as the results of lipids. Body components per 100 g of lipid-free body weight showed similar tendencies.

**Table 2.** Moisture, crude protein, lipid, ash and energy contents of a carcass (except gastrointestinal contents) per 100 g of body weight in germ-free, *Staphylococcus epidermidis* monocontaminated gnotobiotic and conventional mice.

|         | $B_5$ group | | | $B_8$ group | | |
|---------|-------------|---|---|-------------|---|---|
|         | GF | GB | CV | GF | GB | CV |
| Number of mice | 6 | 6 | 6 | 6 | 6 | 6 |
| Moisture (g) | 76.0 | 76.3 | 72.8 | 66.8 | 68.7 | 61.8 |
| ±0.2* | ±0.2 | ±0.6 | ±0.8 | ±0.9 | ±1.3 |
| Crude protein (g) | 14.6 | 14.9 | 16.5 | 15.8 | 16.6 | 16.2 |
| ±0.2 | ±0.2 | ±0.1 | ±0.1 | ±0.1 | ±0.3 |
| Lipid (g) | 5.6 | 5.2 | 6.5 | 13.5 | 9.9 | 16.9 |
| ±0.5 | ±0.2 | ±0.5 | ±0.9 | ±0.9 | ±1.6 |
| Ash (g) | 2.7 | 2.7 | 3.0 | 3.1 | 3.2 | 2.8 |
| ±0.1 | ±0.1 | ±0.2 | ±0.1 | ±0.1 | ±0.1 |
| Energy (kcal) | 143 | 138 | 159 | 216 | 190 | 258 |
| ±2.5 | ±2.0 | ±5.9 | ±8.1 | ±7.2 | ±13.7 |

* Mean ± SE.  
* Determined as $N \times 6.25$. $B_5$ group, mice sacrificed at five weeks of age; $B_8$ group, mice sacrificed at eight weeks of age; GF, germ-free mice; GB, *Staphylococcus epidermidis* monocontaminated gnotobiotic mice; CV, conventional mice.
Table 3. Moisture, crude protein, ash and energy contents of a carcass (except gastrointestinal contents) per 100 g of lipid-free body weight in germ-free, *Staphylococcus epidermidis* monocontaminated gnotobiotic and conventional mice.

|                | B<sub>3</sub> group | B<sub>8</sub> group |
|----------------|---------------------|---------------------|
|                | GF      | GB      | CV      | GF      | GB      | CV      |
| Number of mice | 6       | 6       | 6       | 6       | 6       | 6       |
| Moisture (g)   | 80.6    | 80.5    | 77.9    | 77.2    | 76.2    | 74.4    |
| ±0.3<sup>a</sup> | ±0.2    | ±0.3    | ±0.2    | ±0.2    | ±0.3    | ±0.6    |
| Crude protein (g) | 15.6    | 15.7    | 17.7    | 18.2    | 18.5    | 19.5    |
| ±0.2            | ±0.2    | ±0.2    | ±0.2    | ±0.1    | ±0.1    | ±0.6    |
| Ash (g)         | 2.9     | 2.8     | 3.2     | 3.6     | 3.5     | 3.4     |
| ±0.1            | ±0.2    | ±0.2    | ±0.2    | ±0.2    | ±0.1    | ±0.1    |
| Energy (kcal)   | 150     | 145     | 170     | 250     | 211     | 313     |
| ±3.2            | ±2.3    | ±7.2    | ±12.1   | ±10.1   | ±23.5   |

<sup>a</sup> Mean ± SE. Abbreviations see Table 2.

Accumulation of body components per mouse or per 10 g of body weight in the period of purified whole-egg protein diet are shown in Fig. 2. Amounts of dry matter and energy accumulation per mouse for three weeks in CV...
mice were higher \((P<0.01-0.02)\) than those of GF or GB mice and lipid in GB was lower \((P<0.01-0.05)\) than that of GF or CV mice. Crude protein showed no significant differences but there were higher tendencies in CV mice than in GF or GB mice. Ash accumulation in CV mice tended to be lower than that in GF and GB mice.

Dry matter, lipid and energy accumulation per 10 g of body weight gain showed no significant differences among the three groups of mice. Crude protein accumulation showed the highest value in GB mice \((\text{vs GF: } P<0.02, \text{vs CV: } P<0.001)\). Ash accumulation in CV mice was lower than those in GF \((P<0.02)\) and GB \((P<0.001)\) mice.

The ratios of N, ash and energy retention per food intake and N balance per mouse for three weeks are shown in Table 4. N and ash retention observed by the comparative slaughter method showed no differences among the three groups of mice. However, ash retention of CV mice tended to be lower than that of GF and GB mice. On the other hand, energy retention of CV mice was higher than that of GF \((P<0.05)\) and GB \((P<0.01)\) mice. In the results of the balance method, N retention of CV mice was higher \((P<0.02)\) than that of GF mice.

### Table 4. Ratio of nitrogen, ash and energy retention per food intake and nitrogen balance in germ-free, Staphylococcus epidermidis monocontaminated gnotobiotic and conventional mice.

|                   | GF     | GB     | CV     |
|-------------------|--------|--------|--------|
| Number of mice    | 6      | 6      | 6      |
| Nitrogen retention | \(\%\) | 16.6±1.3\(^a\) | 17.5±1.5 | 17.6±1.0 |
| Ash retention     | \(\%\) | 9.9±1.1 | 10.9±0.9 | 8.1±1.3 |
| Energy retention  | \(\%\) | 9.5±0.6 | 7.2±0.9  | 13.3±1.3 |
| Nitrogen retention | \(\%\) | 14.8±2.4 | 23.0±3.0 | 25.3±2.4 |
| Nitrogen balance  | (mg)   | 189±30 | 262±33  | 355±33  |

\(^a\) Mean ± SE. Abbreviations see Table 2.

Retention by slaughter method = \(\frac{B_f-B_i}{I} \times 100\)

\(B_f\): final body nitrogen, ash and energy. \(B_i\): initial body nitrogen, ash and energy.

\(I\): nitrogen, ash or energy intake per mouse for three weeks.

Retention by balance method

\(\text{Nitrogen retention = } \frac{I-E}{I} \times 100\)

\(\text{Nitrogen balance per mouse for three weeks = } I-E\)

\(I\): nitrogen intake. \(E\): nitrogen excretion in feces and urine.

Dry matter, N and energy of gut contents from stomach to rectum per 100 g of body weight are shown in Table 5. The data for CV mice of the \(B_0\) group were significantly lower \((P<0.001)\) than those for the GF and GB mice, and GB mice
**Table 5.** Dry matter, nitrogen and energy of gut contents per 100 g of body weight in germ-free, *Staphylococcus epidermidis* monocontaminated gnotobiotic and conventional mice.

| B₈ group | B₉ group |
|----------|----------|
| GF       | GB       | CV       | GF       | GB       | CV       |
| Number of mice | 6 | 6 | 6 | 6 | 6 | 6 |
| Dry matter (mg) | 5,152 ± 191 | 4,521 ± 104 | 3,164 ± 181 | 3,928 ± 214 | 3,338 ± 100 | 2,094 ± 134 |
| Nitrogen (mg) | 380 ± 11 | 336 ± 4 | 265 ± 10 | 227 ± 7 | 218 ± 4 | 158 ± 9 |
| Energy (kcal) | 23.2 ± 0.8 | 20.3 ± 0.6 | 14.0 ± 0.8 | 17.6 ± 0.9 | 15.6 ± 0.5 | 9.4 ± 0.6 |

Table 6. Gross energy per g of samples of dry matter of defatted carcasses and protein or lipids of carcasses in germ-free, *Staphylococcus epidermidis* monocontaminated gnotobiotic and conventional mice.

| B₈ group | B₉ group |
|----------|----------|
| GF       | GB       | CV       | GF       | GB       | CV       |
| Number of mice | 6 kcal | 6 kcal | 6 kcal | 6 kcal | 6 kcal | 6 kcal |
| Dry matter | 4.64 ± 0.03 | 4.66 ± 0.05 | 4.75 ± 0.04 | 4.62 ± 0.05 | 4.65 ± 0.05 | 4.76 ± 0.04 |
| Protein | 5.84 ± 0.06 | 5.89 ± 0.12 | 5.75 ± 0.10 | 5.92 ± 0.07 | 5.81 ± 0.07 | 5.97 ± 0.05 |
| Lipids | 9.01 ± 0.02 | 9.09 ± 0.02 | 8.95 ± 0.02 | 8.80 ± 0.05 | 8.98 ± 0.03 | 8.86 ± 0.06 |

Discussion

Previous experiments (1–6) indicated that intestinal microbes affected body N accumulation and they had different effects according to the sort of microbes. *Staph.* increased N accumulation of the host mice. In these experiments, however, only an autoclaved CL-2 diet was used. Thus, to examine whether the previous results were caused by the use of the CL-2 diet, the irradiated purified diet...
containing whole-egg protein was used in the present study. The reason for using this protein is that it is considered to be a good quality protein and such a diet can easily be reproduced. Crude protein measurements of the purified whole egg protein diet (10.0%) showed lower values than those of the CL-2 diet (23.9%), while crude fat measurements of the purified whole-egg protein diet (7.9%) showed higher values than those of the CL-2 diet (4.6%). In the results of gross energy measurements, purified whole-egg protein diet showed 4.60 kcal per g of dry matter and autoclaved CL-2 diet 4.37 kcal per g of dry matter. One percent vitamin mixtures are usually used, but the reasons for using a 2% vitamin mixture in the purified whole-egg protein diet were that vitamins might be lost by sterilization and the short experimental period of three weeks.

Body crude protein of CV mice was higher than that of GF mice and GB mice also showed higher value than that of GF mice (Tables 2 and 3). Moisture and ash contents of CV mice were lower than those of GF or GB mice, but lipid and energy contents of CV mice were higher than those of GF or GB mice. The increase in lipid contents in the B₈ group of CV mice was especially remarkable. This might be due to the influence of intestinal microbes, but the details are unknown. Lipid contents in the B₅ group of GB mice were lower than those of the GF mice, and moisture showed the opposite results. The GF and GB mice were not offsprings from the same parents, and therefore these results might be caused by the difference in parents or times of experiments. Moreover, Staph. might have some activities which prevent body lipid accumulation in host mice. For example, gross energy of lipids in GB mice had a tendency to be higher than that of GF mice in both the B₅ and B₈ groups.

STANIER and MOUNT (11) showed a body composition of CV mice as follows: moisture: 65-70 g, lipid: 5–12 g, N: 2.5–2.9 g and ash: 3.1–4.0 g per 100 g of body weight; our experimental results agreed with the above report. ROBINSON and LAMBOURNE (12) reported that the body lipid content of mice was the reverse of the moisture content, but body protein content was stabilized. In the experiments by TANAKA et al. (13) where rats were fed diets with various ratios of protein to energy for three weeks, moisture contents of the carcasses were decreased by aging and body lipids gave opposite results. In the body protein or ash contents showed similar tendencies in the results of our experiments and also in those of CZAJKA-NARINS and HIRSCH (14).

In body component accumulation (Fig. 2), dry matter in CV mice was significantly higher than those in GF and GB mice. In a case not illustrated, lipid accumulation per unit dry matter accumulation in GF or CV mice was higher than that of GB mice and for protein and ash, GF and CV mice gave lower values than GB mice. In the accumulation of body N per unit ash accumulation, CV mice showed higher values than GB and GF mice. The rank was CV, GB and GF mice. In the previous report (6), body N accumulation per 100 g of body weight showed increases in the order of GF, E. coli GB, Staph. GB and CV mice.
Retention ratios of body components per food intake were generally high in CV mice and low values were shown in GB and GF mice in that order. These results supported the previous results obtained by balance method.

Intestinal microbes affect the gut contents more directly than the body components. Dry matter, N and energy of gut contents (Table 5) in GF mice were higher than those of GB and CV mice. The rank was GF, GB and CV mice with significant differences among three groups of mice. These results showed reverse tendencies to body components and agreed with the results of total N of gut contents in the previous report (6). It is well known that the cecum of GF mice is much larger than that of CV mice and the GF cecum has more contents than the CV mice cecum, as seen in Table 5.

No differences in gross energy of protein and lipids of carcasses were observed among three groups of mice. Several researchers have reported the gross energy per g protein in animal bodies as follows: 5.74 kcal for rats (13), 5.348 kcal for pigs, 5.411 kcal for sheep and 5.447 kcal for cows, and also reported on the gross energy per g lipids, i.e. 9.608 kcal for pigs, 9.414 kcal for sheep and 9.499 kcal for cows (15). The value of gross energy of mouse lipid ranged from 8.80 to 9.09 kcal per g of lipid (Table 6). These values were lower than the results of REID et al. (15) for pigs, sheep and cows and the above gross energy of mouse lipid resembles the results of 8.95 kcal per g of rat lipid obtained by TANAKA et al. (13). The value of 5.7 kcal per g of animal protein and 9.5 kcal per g of animal lipid are already known as the values of gross energy. From the results of other researchers and these experiments, it appears that there are species differences in gross energy of protein and lipids.

The authors are grateful to Mr. T. Ito for his storage of bacteria and the bacteriological tests of established bacteria, and to Professor K. Kikuno, of Wayo Women’s University, for his helpful advice concerning calorimetry.

REFERENCES
1) YAMANAKA, M., IWAI, H., SAIITO, M., YAMAUCHI, C., and NOMURA, T. (1972): Influence of intestinal microbes on digestion and absorption of nutrients in diet and nitrogen retention in germ-free, gnotobiotic and conventional mice. I. Protein and fat digestion and nitrogen retention in germ-free and conventional mice. Jap. J. Zootech. Sci., 43, 272-283.
2) YAMANAKA, M., IWAI, H., SAIITO, M., YAMAUCHI, C., and NOMURA, T. (1973): Influence of intestinal microbes on digestion and absorption of nutrients in diet and nitrogen retention in germ-free, gnotobiotic and conventional mice. II. Protein and fat digestion and nitrogen retention in monocontaminated gnotobiotic mice. Jap. J. Zootech. Sci., 44, 380-387.
3) YAMANAKA, M., IWAI, H., SAIITO, M., YAMAUCHI, C., and NOMURA, T. (1973): Influence of intestinal microbes on digestion and absorption of nutrients in diet and nitrogen retention in germ-free, gnotobiotic and conventional mice. III. Protein and fat digestion and nitrogen retention in polycultivated gnotobiotic mice. Jap. J. Zootech. Sci., 44, 388-396.
4) YAMANAKA, M., IWAI, H., SAIITO, M., YAMAUCHI, C., and NOMURA, T. (1972): Influence of
intestinal bacteria on apparent biological value of protein in diet. *Jap. J. Germfree*, 2, 56–60.

5) YAMANAKA, M., TAKAHASHI, H., ISHIHARA, T., IWA, H., and SAITO, M. (1973): Nitrogen in the cecum and the kidneys in germ-free, gnotobiotic and conventional mice. *Exp. Animals*, 22, 257–262.

6) YAMANAKA, M., NOMURA, T., and KAMETAKA, M. (1974): Role of intestinal microbes on body nitrogen accumulation in germ-free, gnotobiotic and conventional mice. *J. Nutr. Sci. Vitaminol.*, 20, 389–400.

7) NOMURA, T. (1968): International Symposium: The germ-free animal as a tool in research. Belgium.

8) NOMURA, T., SAITO, M., and MATUSAKI, T. (1968): Studies on production of gnotobiotes (in Japanese), in *An Interium Report of Overall Studies on Experimental Animals*, 1 ed., Kagakugijutsucho, Tokyo, pp. 57–76.

9) MITSUDA, H. (1961): General analyses of foods and fodders (in Japanese), in *Jikken Eiyo-kagaku*, revised 2nd ed., Izumishobo, Kyoto, pp. 43–65.

10) FOLCH, J., LEES, M., and SLOANE STANLEY, G. H. (1957): A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.*, 226, 497–509.

11) STANIER, M. W., and MOUNT, L. E. (1972): Growth rate, food intake and body composition before and after weaning in strains of mice selected for mature body weight. *Br. J. Nutr.*, 28, 307–325.

12) ROBINSON, D. W., and LAMBORNE, L. J. (1970): The influence of growth rate and retardation on the nucleic acid and nitrogen concentration in skeletal muscles and whole body composition of the mouse. *Growth*, 34, 235–255.

13) TANAKA, H., YAMAGUCHI, M., and KAMETAKA, M. (1974): Body composition and utilization of protein and energy in growing rats at different dietary protein to energy ratios by use of purified whole-egg protein. *Agric. Biol. Chem.*, 38, 1113–1120.

14) CZAJKA-NARINS, D. M., and HIRSCH, J. (1974): Supplementary feeding during the pre-weaning period. *Biol. Neonate*, 25, 176–185.

15) REID, J. T., BENSADOUN, A., BULL, L. S., BURTON, J. H., GLEESON, P. A., HAN, I. K., JOO, Y. D., JOHNSON, D. E., McMANUS, W. R., PALADINES, O. L., STRoud, J. W., TYRRELL, H. F., VAN NIEKERK, B. D. H., and WELLINGON, G. W. (1968): Some peculiarities in the body composition of animals, in *Body Composition in Animals and Man*, ed. by Agricultural Board Division of Biology and Agriculture, National Research Council, National Academy of Sciences, Washington, D. C., pp. 19–44.