Influences of Protein Malnutrition on Amino Acid Composition, Trace Metal Elements and Tensile Strength of Rat Hairs

Chizuko SHIMOSHIMA, Chihiro NISHIOKA, Kazuyoshi TAKIYAMA,1 Osamu YUGE,2 and Yoshiho KATAYAMA3.*

1Food and Nutrition, Mukogawa Women's University, Ikebirakicho, Nishinomiya 663, Japan
2Laboratory of Textile Materials, Department of Clothing and Textiles, Faculty of the Sciences of Living, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan
3Laboratory of Nutritional Physiology and Biochemistry, Department of Food Sciences and Nutrition, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606, Japan

(Received August 14, 1987)

Summary We examined hair properties from determinations of diameter, amino acid composition, trace metal elements, and tensile strength of the hairs from protein-malnourished rats. The coarse or medium hair diameters of the experimental protein-malnourished rats had a tendency to decrease more than those of the control. Total contents of amino acids in the hairs had a tendency to decrease in the protein-deficient rats. Cystine content of rat hairs definitely decreased only in 5% wheat- and 5% rice-pattern amino acid mixture diet groups but not in 5% gluten diet and 5% casein diet groups. And many of the low-protein diet groups were significantly lower in methionine content than the control except for the 5% casein diet group. The Mg, Zn, and Fe contents in the hairs considerably increased in protein-deficient rats against the control. Tensile strength of coarse hairs in the experimental protein-malnourished rats was significantly lower than that of the control. The changes in amino acid composition of rat hair proteins are more likely to be influenced by various qualities of dietary protein or different compositions of amino acid mixtures in the diets at a similar dietary protein level. It should seriously be considered from our data whether the reduced cystine content in the hair can be regarded as an indicator of nutritional status.

Key Words rat hair, amino acids, tensile strength, trace metal elements, wheat, rice, casein, diameter of hair

1下志万博鶴子，西岡千尋，滝山一善，2 弓削 治，3 片山吉穗
* To whom correspondence should be addressed.
As described by Lea and Luttrell (1) changes in color and texture of hair had been suggested in kwashiorkor, a protein-calorie malnutrition syndrome. Three decades ago, Close (2), Platt and Nagchaudhuri (3), and Bigwood and Robazzi (4) reported that the cystine and cysteine content of hair of malnourished children, such as kwashiorkor, definitely decreased. There were, however, some contrary observations (5, 6). Subsequently in 1960 Koyanagi et al. also found that hair of undernourished children and the skin of vitamin A-deficient rats contained less cystine than in those of well-nourished ones. This resulted in hair being used as an indicator of nutritional status in children (7, 8).

When examining the trace metal elements in hair, Gopalan et al. (9) reported that Indian children with kwashiorkor had considerably less copper content in the hair, but Lea and Luttrell (1) proposed that kwashiorkor was not necessarily accompanied by a reduction in the copper content in the hair. On the other hand, Erten et al. (10) found that hair zinc levels in protein-deficient children were significantly higher than in a group of healthy subjects in the same age range, although Briggs et al. (11) and Amador et al. (12) showed that low zinc levels were associated with malnutrition and malnourished children with acrodermatitis enteropathica, while Bradfield et al. (13) found no difference in hair zinc levels of malnourished subjects compared with the control group.

Hartman et al. (14) showed that hair diameter and cystine content in hair of Negro children with protein deficiencies were considerably decreased. As pointed out by Hartman et al. (14), hair samples have been frequently used as a possible indicator, because hair is an easily accessible, continuously growing, high protein tissue. Koyanagi’s studies on the effect of diet on cystine content of hair reported that changes in the cystine content of hair correlated closely with the consumption of animal protein (8, 15).

When we determined the metabolic changes occurring in rats under various nutritional conditions at a low level of dietary protein or various amino acid mixtures (16–19), we observed changes in the rate of hair growth and texture of these rat hairs.

We examined hair properties from determinations of diameter, amino acid composition, trace metal elements, and tensile strength of the hairs from the protein-malnourished rats.

MATERIALS AND METHODS

Diets and animals. Amino acid mixtures: Two kinds of amino acid mixtures were prepared from 17 kinds of crystalline L-amino acids and glycine (Ajinomoto Co., Inc., Tokyo), simulating the composition of wheat gluten and rice protein. Additionally, the Miyazaki-pattern amino acid mixture produced by Miyazaki et al. (20–22) which is considered suitable for normal growth of rats, was also prepared. The compositions of the amino acid mixture of each pattern were presented in our previous papers (16–19). These amino acid mixtures were used as a
Table 1. Composition of diets.

| Protein Source | Low-protein diets | Control diet |
|----------------|-------------------|--------------|
|                | 5% as amino acid mixtures | 5% as gluten | 5% as casein | 20% as casein |
| Corn oil* (%)  | 5                 | 5            | 5            | 5            |
| Salt mixtureb (%) | 5       | 5            | 5            | 5            |
| Vitamin mixturec (%) | 1       | 1            | 1            | 1            |
| Choline chloride d (%) | 0.4    | 0.4          | 0.4          | 0.4          |
| Corn starch* (%) | 83.6        | 83.6         | 83.6         | 68.6         |
| Total (%)       | 100.0          | 100.0        | 100.0        | 100.0        |

These diets contained 15,000 IU of retinol and 37.5 μg of ergocalciferol per kg of each diet, respectively. a Ajinomoto Co., Inc. (Tokyo). b, c, d Tanabe Amino Acid Research Foundation (Osaka). e Amylalpha produced by Chuo Shokuryo Co. (Inazawa-shi).

b The composition (%) of salt mixture: CaCO₃, 29.29; CaHPO₄·2H₂O, 0.43; KH₂PO₄, 34.31; NaCl, 25.06; MgSO₄·7H₂O, 9.98; Fe(C₆H₅O₇)·6H₂O, 0.623; CuSO₄·5H₂O, 0.156; MnSO₄·H₂O, 0.121; ZnCl₂, 0.020; KI, 0.0005; (NH₄)₆Mo₇O₂₄·4H₂O, 0.0025.

c The composition (%) of vitamin mixture: thiamine·HCl, 0.059; riboflavin, 0.059; nicotinic acid, 0.294; calcium pantothenate, 0.235; pyridoxine·HCl, 0.029; menadione, 0.006; biotin, 0.001; tetrahydrofolic acid, 0.002; cyanocobalamin, 0.0002; myoinositol, 1.176; ascorbic acid, 0.588; lactose, 97.551.

Diets: As shown in Table 1, the experimental diets were 20% casein diet for the control group and three kinds of low-level diets (5% level) consisting of the wheat-, rice-, and Miyazaki-pattern amino acid mixtures, and also wheat gluten and casein diets at low level (5% level). These diets contained adequate amounts of vitamins and choline chloride.

Animals: Male rats of the Charles-River CRJ strain (Nihon Charles-River Inc., Atsugi) 4 weeks of age, weighing 65 to 70 g, were used in the experiment. They were individually housed in wire cages in a room which was automatically light-controlled to provide a 12-h light: dark cycle with lights on at 08:00, at 22°C and the relative humidity at 60%. The rats were given standard rat pellets (CE-2, purchased from Nihon Clea Inc., Osaka) for about 1 week before the experiment and were divided into six groups: control group, wheat-pattern diet group (W-group), rice-pattern diet group (R-group), Miyazaki-pattern diet group (M-group), gluten diet group (G-group), and low casein diet group (LC-group). The rats were then maintained on a 20% casein diet as a control and three kinds of low-level diets consisting of the wheat, rice-, and Miyazaki-pattern amino acid mixtures and two kinds of low-protein diets of gluten and casein for 4 weeks. They were fed each diet ad libitum with free access to tap water. Daily food intake and body weight for each animal were measured between 09:00 to 10:00.

Hair samples of the rats were clipped with stainless steel scissors from the part closest to the skin in the interscapular region at day-27. These samples were stored...
in a desiccator into which 32% sulfuric acid was put to keep relative humidity at 60% (23).

The diameters of the hair were measured with a Digit Outside micrometer (Mitutoyo Mfg. Co., Ltd., Tokyo) or by using a microscope with an ocular micrometer.

*Determination of amino acid compositions of the hair.* The hair samples were boiled under reflux together with hydrochloric acid (6 N) as an azeotrope substance for 24 h at 125°C. The contents of amino acids were measured from hydrolyzed samples using a Hitachi Amino Acid Autoanalyser (model KLA-5) using a citrate buffer.

*Determination of tensile strength of the hair.* The tensile strength of the hairs was estimated with the Tensile Tester (model TCM-200, Shinkoh Tsushin Co., Ltd., Fujisawa).

*Determination of trace metal elements in the hair.* The wet-ashing of the hair samples was carried out by the modified method described by Barrett et al. (24) and Abu-Samra et al. (25). The samples weighing between 0.8 and 5.3 mg using a sensitive balance were placed into tubes and 2 ml of concentrated nitric acid was added to them. They were ashed for 2 h in a microwave oven which was equipped with a special aspirator for exhaust gases and corrosive fumes. After wet-ashing, the solvent was evaporated from these decomposed materials. The residues were dissolved into doubly-distilled water and brought up to 20 ml volume with the same fluid. The procedure for blanks was identical. Subsequently, the contents of various metal elements as microgram or mg per gram of hair were assayed with the Atomic Absorption Spectrophotometer (Model Japan Jarrell Ash AA-845 with Flameless Atomizer FLA-10) (26).

*Statistical analysis.* In the tables each value represents the mean ± half range of the confidence interval (confidence limit) at p < 0.05. According to the procedure of Pollard (27) and Masuyama (28) statistical analysis was performed.

**RESULTS**

*Body weight gain and food intake*

The initial body weight was between 130 and 140 g. The growth curves are shown in Fig. 1. The body weights of rats in the control group gained linearly at about 7 g/day throughout the experimental period. A weight loss was observed during the first 2 or 3 days for the R-group and the M-group, and during the first 6 days for the W-group. Thereafter, the growth curves of many of these experimental diet groups showed a linear increase. On the other hand, the body weights of rats in the low-protein diet groups (G- and LC-groups) decreased during the first 5 or 7 days and remained at this lower level until the final experimental day. The weight gain of the rats fed each of the experimental low-level diets (the three amino acid mixture diets and two low-protein diets) was significantly lower than that of the control group (p < 0.05). During the 27-day experimental period, the control rats
gained 210 g, while the M-group, R-group, W-group, LC-group, and G-group gained 58, 23, 0, −5, and −15 g, respectively. Average food intakes of the rats fed the various experimental diets were almost similar to that of the control rats. The other biochemical properties of the serum and liver tissues were previously reported elsewhere (19).

**Hair diameters and distributions of rat hair in unit weight (1 mg)**

According to hair diameters, we have classified the rat hairs into four groups: coarse (>55 μm in diameter), medium (25–54 μm), fine (12–24 μm), and very fine (<11 μm). In these hair samples, the diameters were distributed in a range of 4.3 to 112 μm as shown in Table 2. The diameters of coarse hair were 77 to 112 μm, those of medium hair 25 to 48 μm, those of fine hair 15 to 22 μm, and those of very fine hair 4.3 to 9.1 μm. When the hairs of the control group and the low-nitrogen diet groups were compared, the coarse or medium hair diameters of the latter rats were significantly smaller than that of the control rats except for the coarse hair in R-group, M-group, and LC-group, although there was no difference in diameter among the hair of rats fed the low-nitrogen diets. Furthermore, the fine and very fine hair diameters of the experimental protein-malnourished rats were considerably smaller than those of the control except for the fine hair in the W-group and R-group, or very fine hair in the G-group. On the other hand, the number of very fine hairs in the unit weight (1 mg) had a tendency to increase in the order of W-group,
Table 2. Diameter and distribution of rat hairs in unit weight (1 mg).

|                | Control group | W-group | R-group | M-group | G-group | LC-group |
|----------------|---------------|---------|---------|---------|---------|----------|
| Diameter (µm)  |               |         |         |         |         |          |
| Coarse hair    | 112 ± 20      | 77 ± 17*| 91 ± 10 | 82 ± 26 | 79 ± 15*| 80 ± 29  |
| Medium hair    | 48 ± 5        | 35 ± 4* | 39 ± 4* | 36 ± 3* | 37 ± 2* | 25 ± 1*  |
| Fine hair      | 22 ± 2        | 20 ± 2  | 19 ± 2  | 15 ± 1* | 19 ± 2* | 18 ± 1*  |
| Very fine hair | 9.1 ± 0.9     | 5.5 ± 0.8*| 6.8 ± 0.9*| 5.6 ± 1.1*| 7.2 ± 1.0| 4.3 ± 1.3*|

|                | Coarse and medium hair | Distribution (%) of rat hairs in unit weight (1 mg) |
|----------------|------------------------|---------------------------------|
|                |                         | 9  | 8  | 7  | 6  | 10 | 5  |
| Fine hair      |                         | 6  | 3  | 3  | 2  | 6  | 2  |
| Very fine hair |                         | 85 | 89 | 90 | 92 | 84 | 93 |
| Total          |                         | 100| 100|100|100|100|100|

Values are mean ± half range of confidence interval (confidence limit) at 95% level.
* Indicates significant difference from the control at 95% level.

R-group, G-group, M-group, and LC-group. The fact that there was no difference in the distribution of the rat hairs in the unit weight as compared with the control, except for the fine and very fine hair groups, was surprising. The hair distribution of rats fed the experimental low-nitrogen diets was lower for the fine hairs and increased in percentages for the very fine hair group compared to that of the control.

Amino acid compositions of the rat hairs

The amino acid composition of the rat hairs is indicated in Table 3 together with reference values of sheep wool. When the amino acid composition of the hair of rats (control group) and sheep was compared, the rats had smaller amounts of Ile content in their hair than sheep did. On the other hand, His content of the rat hairs was double the content in the sheep wool. Among the other amino acids, contents of the rat hair showed similar or lower values for Thr, Cys, Val, Arg and all of the non-essential amino acids, and somewhat higher for Lys, Tyr, and Phe. Here, where amino acid contents of the rat hairs in W-group and R-group were compared with the control, the contents of Lys, Thr, Cys, Met, Val, and Ile in essential amino acids were significantly lower than the control \((p < 0.05)\), and furthermore, some non-essential amino acids, Asp, Ser, and Glu also decreased considerably \((p < 0.05)\). Among the amino acids, only the Gly content of the rat hair increased markedly in W-group but not in R-group. On the other hand, M-group showed only a reduction of Met and an increase of Gly \((p < 0.05)\). For G-group, significantly lower contents of Met and Ile, and a markedly higher value of Gly against the control were observed. The LC-group had no reductions of any amino acids but on the contrary, increases of Tyr, Phe, and His in essential amino acids and Gly in non-essential.
Table 3. Compositions of amino acids in the rat hair (g/100 g).

|       | Control group | W-group | R-group | M-group | G-group | LC-group | Keratin of wool |
|-------|---------------|---------|---------|---------|---------|----------|----------------|
| Lys   | 3.37 ± 0.17   | 2.84 ± 0.17* | 2.79 ± 0.33* | 3.01 ± 0.61 | 3.04 ± 0.28 | 3.52 ± 0.32 | 2.65          |
| Thr   | 4.88 ± 0.21   | 4.20 ± 0.30* | 4.17 ± 0.25* | 4.45 ± 1.19 | 4.59 ± 0.46 | 4.80 ± 0.47 | 6.40          |
| Cys   | 10.65 ± 0.87  | 9.12 ± 0.61* | 9.02 ± 0.76* | 9.72 ± 2.04 | 9.87 ± 1.13 | 10.18 ± 1.32 | 11.90         |
| Met   | 0.83 ± 0.19   | 0.50 ± 0.03* | 0.49 ± 0.07* | 0.46 ± 0.16* | 0.45 ± 0.08* | 0.88 ± 0.14 | 0.70          |
| Val   | 4.15 ± 0.24   | 3.48 ± 0.52* | 3.60 ± 0.40* | 3.65 ± 1.04 | 3.83 ± 0.38 | 4.28 ± 0.45 | 4.80          |
| Ile   | 2.83 ± 0.08   | 2.43 ± 0.26* | 2.43 ± 0.28* | 2.32 ± 0.77 | 2.46 ± 0.26* | 2.88 ± 0.28 | 11.30         |
| Leu   | 6.53 ± 0.29   | 6.06 ± 0.56  | 5.86 ± 0.72  | 5.97 ± 1.03 | 6.12 ± 0.69 | 8.02 ± 0.53 | —             |
| Tyr   | 5.59 ± 0.16   | 5.41 ± 0.72  | 4.86 ± 3.01  | 5.52 ± 0.71 | 6.00 ± 0.62 | 6.36 ± 0.29* | 4.65          |
| Phe   | 4.01 ± 0.40   | 3.53 ± 0.68  | 3.54 ± 0.61  | 3.53 ± 0.66 | 3.69 ± 0.43 | 4.40 ± 0.28* | 3.78          |
| His   | 1.65 ± 0.09   | 1.78 ± 0.37  | 1.84 ± 0.42  | 1.55 ± 0.84 | 1.71 ± 0.23 | 1.82 ± 0.11* | 0.70          |
| Arg   | 8.19 ± 0.35   | 8.21 ± 1.08  | 7.95 ± 1.25  | 8.31 ± 1.77 | 8.49 ± 0.68 | 8.28 ± 0.75 | 10.40         |
| EAA Total | 52.68 | 47.56 | 46.56 | 48.49 | 50.25 | 54.22 |
| Asp   | 6.07 ± 0.23   | 5.36 ± 0.38* | 5.28 ± 0.46* | 5.71 ± 0.91 | 5.81 ± 0.61 | 6.27 ± 0.52 | 6.57          |
| Ser   | 7.97 ± 0.39   | 7.43 ± 0.23* | 7.34 ± 0.40* | 7.52 ± 1.87 | 7.52 ± 0.63 | 8.10 ± 0.65 | 10.30         |
| Glu   | 14.24 ± 0.53  | 12.48 ± 0.76* | 12.39 ± 0.64* | 12.55 ± 3.74 | 13.82 ± 1.64 | 14.57 ± 1.31 | 14.10         |
| Pro   | 5.10 ± 0.49   | 5.56 ± 0.76  | 5.62 ± 1.07  | 5.59 ± 1.06 | 5.39 ± 0.44 | 5.05 ± 0.48 | 6.80          |
| Gly   | 5.89 ± 0.20   | 6.69 ± 0.39* | 6.55 ± 1.05  | 6.92 ± 0.07* | 7.37 ± 0.79* | 6.85 ± 0.36* | 6.50          |
| Ala   | 3.61 ± 0.16   | 3.79 ± 0.25  | 3.52 ± 0.83  | 3.75 ± 0.59 | 4.03 ± 3.43 | 3.68 ± 0.31 | 4.13          |
| NEAA Total | 42.88 | 41.31 | 40.70 | 42.04 | 43.94 | 44.52 |

Each value represents the mean ± half range of the confidence interval at $p<0.05$. * Indicates statistically significant difference against the control group at $p<0.05$. EAA, essential amino acid; NEAA, non-essential amino acid. For all experimental details see the text.
Table 4. Contents of metal elements in the rat hair.

|         | Na (mg) | K (mg) | Ca (µg) | Mg (µg) | Zn (µg) | Fe (µg) |
|---------|---------|--------|---------|---------|---------|---------|
| W-group | 39±12   | 51±32  | 838±1,160| 274±76* | 310±483| 494±269*|
| R-group | 29±22   | 29±19  | 308±59  | 417±222*| 390±38* | 146±69  |
| M-group | 8±6     | 18±16  | 266±75  | 166±137 | 264±69  | 200±194 |
| G-group | 7±4     | 12±7   | 236±105 | 231±134*| 279±30  | 222±88* |
| LC-group| 46±12   | 30±51  | 719±25* | 389±124*| 541±208*| 245±215 |
| Control | 28±17   | 32±32  | 370±218 | 65±48   | 236±53  | 143±52  |

|         | Cu (µg) | Mn (µg) | Cr (µg) | Pb (µg) | Ni (µg) |
|---------|---------|---------|---------|---------|---------|
| W-group | 145±96  | 11±8    | 54±54   | 14±13   | 36±24   |
| R-group | 81±59   | 8±3     | 18±6    | 25±18   | 19±7    |
| M-group | 60±36   | 6±3     | 13±3    | 38±39   | 32±31   |
| G-group | 67±47   | 4±2     | 15±3    | 58±43   | 9±5     |
| LC-group| 142±27* | 15±4*   | 36±8*   | 77±42*  | 33±5*   |
| Control | 46±35   | 7±4     | 19±8    | 20±12   | 15±10   |

Each value represents the mean±half range of the confidence interval at p<0.05. *Indicates statistically significant difference against the control group at p<0.05.

amino acids (p<0.05). Total contents of amino acids in the hairs had a tendency to decrease in the groups on low-nitrogen diets consisting of wheat-, rice-, and Miyazaki-pattern amino acid mixtures. Cys content of the rat hairs decreased only in W-group and R-group but not in M-group, G-group, and LC-group, and many of the experimental diet groups were significantly lower in Met content than the control, except for the LC-group.

Contents of metal elements in the rat hair

In the Na and K contents of the rat hair, there was no significant difference between the experimental protein-malnourished rats and the control. In many experimental groups, however, Mg, Zn, and Fe contents in the rat hair had a tendency to increase; in particular, the W-group, R-group, G-group, and LC-group were significantly higher than the control. Furthermore, Cu, Mn, Cr, Pb, and Ni contents in the rat hair of LC-group increased considerably (Table 4).

Tensile strength of the rat hairs

To measure tensile strength (kg/mm²) using a tensile tester, we divided the hairs into two groups—medium-fine and coarse—because changes in tensile strength resulted from the variances of hair diameters. As shown in Table 5, in the groups on low-nitrogen diets consisting of amino acid mixtures, tensile strength of coarse hair was 43 to 48% of the control level, whereas the other low-protein diet groups showed about 55% of the control. The tensile strength of coarse hair in all of the
Table 5. Comparison of tensile strength (kg/mm²) of rat hair between the experimental low-nitrogen diet groups and the control group.

|                         | Tensile strength (kg/mm²) |
|-------------------------|---------------------------|
|                         | Medium and fine hair (diameter range: 15–48 μm) | Coarse hair (diameter range: 77–112 μm) |
| Control group           | 12.8 ± 2.1                | 7.4 ± 2.9                |
| W-group                 | 15.1 ± 5.9                | 3.2 ± 1.4*               |
| R-group                 | 16.4 ± 6.2                | 3.6 ± 1.2*               |
| M-group                 | 18.6 ± 7.7                | 3.6 ± 1.8*               |
| G-group                 | 16.1 ± 4.2                | 4.1 ± 1.0*               |
| LC-group                | 19.5 ± 4.8*               | 4.2 ± 1.1*               |

Each value represents the mean ± half range of the confidence interval (confidence limit) at p < 0.05. * Indicates statistically significant difference against the control at 95% level.

Diameters of rat hairs, especially coarse hair, decreased more amongst the protein- or amino acid-deficient rats than the control (p < 0.05). On the other hand, many of the tensile strengths of the medium-fine hair group had a tendency to increase slightly above the control. However, these differences from the control had no significance except for the considerable difference in the LC-group (p < 0.05). In the medium-fine and coarse hair groups there were no considerable differences among the experimental low-nitrogen diet groups.

DISCUSSION

Diameters of rat hairs, especially coarse hair, decreased more amongst the protein- or amino acid-deficient rats than the control. In agreement with the findings of Hartman et al. (14) on hair diameters of Negro children with protein-calorie malnutrition, it could be considered that protein deficiencies might cause changes in the diameter of the hair, especially of coarse hair. From the results of our present study, W-group and R-group showed significant reductions of Lys, Thr, Cys, Met, Val, and Ile in essential amino acids and those of Asp, Ser, and Glu in non-essential amino acids. Furthermore, M-group revealed Met reduction only. On the other hand, G-group showed significantly lower contents of Met and Ile. The LC-group, however, did not show any reductions of amino acid contents of rat hair as compared with the control group. In all of our experimental diet groups, only Met reduction was commonly shown in rat hairs except for the case of the LC-group. Platt and Nagchaudhuri (3), Bigwood and Robazzi (4), and Close (2) suggested that cystine content of hair of malnourished children, such as kwashiorkor, decreased substantially. Subsequently, Koyanagi et al. (8, 15) also found a lower cystine content in the hair of undernourished children than in that of the well-nourished ones and regarded hair as an indicator of the nutritional status of...
children. We used semi-artificial diets which have well-known compositions and origins of nutrients, which allowed us to control the nutrients in the diet more easily. This is far more difficult in the case of human beings such as malnourished children. Many investigators have proposed that reduced cystine content of hair in malnourished children may be regarded as a possible indicator of nutritional status in children. From the results described above, we have found that reduced cystine content of hair was not necessarily related to diet, although the reduction in rat hair occurred by low-nitrogen diets consisting of wheat- or rice-pattern amino acid mixtures. Changes in amino acid composition of rat hair proteins are more likely to be influenced by various qualities of dietary protein or different compositions of amino acid mixtures in the diets at a similar dietary protein level. From our data, it should seriously be considered whether the reduced cystine content in the hair can be regarded as an indicator of nutritional status.

In the W-group and R-group, the changes of amino acid compositions in rat hair certainly reflected, in some instances, the nutritive values of diets which regarded Lys and Thr as the limiting amino acids. In the LC-group, however, there was no reduction among any of the amino acids contained in the casein in the diet although sulfur amino acids were considered as the limiting factors of casein. At present we have no explanation for this phenomenon. The evidence of Koyanagi et al. (8, 15) indicated that cystine content of hair of children fed milk was remarkably higher than that of children not given milk; milk protein (casein) may thus have a special effect on mediators of metabolic changes in the animals.

The magnesium, zinc, and iron contents in rat hairs of some of the experimental dietary groups significantly increased in comparison with the control group. As described above, many investigators showed different results for metal elements in the hairs of protein-malnourished children (1, 9, 10, 12, 13). There is no common agreement among these data. As pointed out by Suzuki (29), the changes of trace metal elements in hair should be cautiously regarded as an indicator of nutritional status.

We do not know of any study which examines the relationship between changes of tensile strength of the hair and nutrition. In our data on tensile strength of rat hair, every group fed on low-level experimental diets showed less tensile strength in coarse hairs when compared to the control group. The tensile strength is influenced by temperature and humidity. We carried out the experiments under standard conditions (at 20°C, relative humidity at 60%). As pointed out by Hayashi et al. (30) tensile strengths decrease linearly according to increases of hair diameters. We compared the tensile strengths of rat hairs at almost similar diameters. If the diameter of the protein-malnourished rat hair had corresponded to that of the control rat hair, the differences between the experimental rat hair and the control rat hair in our data might have been clearer. From the reference (31) of sheep wool composition, rat hair is composed of three parts, that is, cuticle and medulla with cortex, the latter being the most important part for tensile strength. The cortex consists of intercellular substances and cortical cells which contain matrix and

J. Nutr. Sci. Vitaminol.
microfibril composed of bundles of protofibrils. According to the postulation of Hayashi et al. (30), the cuticle itself can be considered to be harder and stronger than the cortex. Whereas the micelles (crystalline regions) of keratin play a major role in tensile strength, the lack of tensile strength in the experimental rat hairs might be caused by disturbances of micelle-formations of keratin or of cortical cell formation.

We would like to thank Dr. Yasumi Yugari and Ajinomoto Co. Inc. for his scientific advice, kind co-operation and for providing all of the crystalline amino acids. We also thank Dr. Takeo Okumura, Kao Soap Co., Ltd., for his technical advice, and Miss Hiroko Tamai for her technical assistance.

REFERENCES

1) Lea, Catherine, M., and Luttrell, V. A. S. (1965): Copper content of hair in kwashiorkor. Nature, 206, 413.
2) Close, J. (1958): Les modifications chimiques et morphologiques des cheveux, accompagnant le kwashiorkor (Chemical and morphological changes in hair accompanying kwashiorkor. Ann. Soc. Belge. Med. Trop., 38, 95–102.
3) Platt, B. S., and Nagchaudhuri, J. (1954): Malnutrition and hair pigmentation. Proc. Nutr. Soc., 13, ix–x.
4) Bigwood, E. G., and Robazzi, F. (1955): Amino acid and sulphur content of hair in normal African natives and in kwashiorkor. Voeding, 16, 251–256.
5) Wysocki, A. P., Mann, G. V., and Stare, F. J. (1954): The cystine and methionine content of the hair of malnourished children. Am. J. Clin. Nutr., 2, 243–245.
6) Friedman, M., and Orraca-Tetteh, R. (1978): Hair as an index of protein malnutrition, in Nutritional Improvement of Food and Feed Proteins, ed. by Friedman, M., Plenum Press, London and New York, pp. 131–154.
7) Koyanagi, T., and Odagiri, S. (1960): Effect of vitamin A on the cystine content of the skin of rats. Nature, 186, 809–810.
8) Koyanagi, T., and Takanoashi, T. (1961): Cystine content in hair of children as influenced by vitamin A and animal protein in the diet. Nature, 192, 457–458.
9) Gopalan, C., Reddy, V., and Mohan, V. S. (1963): Some aspects of copper metabolism in protein-calorie malnutrition. J. Pediatr. (Tropical Pediatrics), 63, 646–649.
10) Erten, J., Arcasoy, A., Ayhan, O. Çavdar, and Cin, S. (1978): Hair zinc levels in healthy and malnourished children. Am. J. Clin. Nutr., 31, 1172–1174.
11) Briggs, M. H., Briggs, M., and Wakatama, A. (1972): Trace elements in human hair. Experientia, 28, 406–407.
12) Amador, M., Peña, M., Garcia-Miranda, A., Gonzalez, A., and Hermalo, M. (1975): Low hair-zinc concentrations in acrodermatits enteropathica. Lancet, ii, 1379.
13) Bradfield, R. B., Yee, T., and Baertl, J. M. (1969): Hair zinc levels of Andean Indian children during protein-calorie malnutrition. Am. J. Clin. Nutr., 22, 1349–1353.
14) Hartman, D., Fougere, W., and King, K. W. (1966): Diameter and amino acid changes in hair of Negro children with protein-calorie malnutrition. Proc. Soc. Exp. Biol. Med., 123, 542–544.
15) Koyanagi, T., Hareyama, S., and Takanoashi, T. (1965): Effect of supplementation of vitamin, phosphorus, methionine or skim milk on the cystine content of hair, dark adaptation, creatine-creatinine excretion and growth of undernourished children. Tohoku J. Exp. Med., 85, 108–114.
16) Katayama, Y., Kodama, Y., and Kubo, Y. (1979): Some lipogenic enzyme activities in rat livers in which there was an excessive fat accumulation due to feeding low level amino acid mixture diets. *J. Nutr. Sci. Vitaminol.*, 25, 229–241.

17) Katayama, Y., and Saimei, M. (1979): Effect of feeding amino acid mixtures on lipid transport from rat liver as measured by liver perfusion. *J. Nutr. Sci. Vitaminol.*, 25, 525–542.

18) Katayama, Y., Fijinaka, M., Nagata, Y., Shimoshima, C., and Saimei, M. (1984): Metabolic changes occurring in the rats with fatty liver produced by amino acid mixture diets at low level. *Ann. Rep. Sci. Living, Osaka City Univ.*, 32, 15–30.

19) Katayama, Y., and Shimoshima, C. (1984): Metabolic changes of the rats fed low protein diets.—Comparison of amino acid mixture diets and natural protein diets. *Ann. Rep. Sci. Living, Osaka City Univ.* (in Japanese), 32, 31–38.

20) Miyazaki, M., Hayakawa, S., and Sakurai, Y. (1964): Shironezumi no seicho ni hitsuyo na aminosan pattern (Required amino acid composition for normal growth of rats). *Rep. Res. Commit. Essent. Amino Acid.* (in Japanese), No. 22, 10–11.

21) Miyazaki, Y., and Hayakawa, S. (1965): Shironezumi no seicho ni hitsuyo na aminosan pattern (Required amino acid composition for normal growth of rats). *Rep. Res. Commit. Essent. Amino Acid.* (in Japanese), No. 25, 89.

22) Miyazaki, M., and Hayakawa, S. (1964): Shironezumi no seicho ni hitsuyo na saiteichissoryo to sono aminosan sosei (Requirement of nitrogen for normal growth of rats and its amino acid composition). *J. Jpn. Soc. Food Nutr.* (in Japanese), 17, 12.

23) Yuge, O., and Tanaka, M. (1960): Technical Note for Textile Materials in Osaka City Univ. (in Japanese, O.C.U., Osaka pp. 10–15.

24) Barrett, P., Davidowski, J., Jr., Penaro, K. W., and Copeland, T. R. (1978): Microwave oven-based wet digestion technique. *Anal. Chem.*, 50, 1021–1023.

25) Abu-Samra, A., Morris, J. S., and Koirtyohann, S. R. (1975): Wet ashing of some biological samples in a microwave oven. *Anal. Chem.*, 47, 1475–1477.

26) Kamakura, M. (1983): A study of the characteristics of trace elements in the hair of Japanese—Reference values and the trace elements patterns for determining normal levels. *Jpn. J. Hyg.*, 38, 823–838.

27) Pollard, J. H. (1977): A Handbook of Numerical and Statistical Techniques, Cambridge Univ. Press, London, pp. 210–231.

28) Masuyama, G. (1978): Shosurei no Matomekata (Statistical Arrangement for Small Samples in Biological Sciences) (in Japanese), Takeuchishoten-Shinsha, Tokyo, I, II, pp. 80–96, 103–119.

29) Suzuki, T. (1985): Mohatsu-Bunseki (Analysis of Hair). *Yueki to Eiyo (Transfusion and Nutrition)*, 7–9.

30) Hayashi, S., Okumura, T., and Ishida, A. (1976): Preliminary study on racial difference in scalp hair, in Biology and Disease of the Hair, ed. by Toda, K. et al., Univ. Tokyo Press, Tokyo, pp. 555–561.

31) Ohoya, S., and Muraoka, Y. (1980): Shin-Seni-Genryogaku (in Japanese), Aikawa-Shobo, Tokyo pp. 185–203.