Effect of a New Amino Acid Solution on Nutritional Status and Nitrogen Metabolism in Rats with Chronic Renal Failure Undergoing Hyperalimentation

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Summary We studied the effects of the new amino acid solution MRX-III on the nutritional status and nitrogen metabolism of rats with chronic renal failure (CRF) in comparison with those of a general amino acid solution (MPR-F). The essential amino acids/non-essential amino acids ratio was 3.21 for MRX-III and 1.09 for MPR-F. Rats with CRF, induced by 7/8 renal ablation, were divided into 6 groups of 8 rats each receiving total parenteral nutrition (TPN) containing MRX-III or MPR-F at a non-protein calorie/nitrogen ratio (Cal/N) of 300, 600 or 900 for 7 d. The rats were infused with test solutions containing the same amounts of non-protein calories. The cumulative nitrogen balance, as a nutritional index, in the MRX-III group was significantly higher than that in the MPR-F group at the Cal/N of 600 or 900, and the plasma albumin level at the Cal/N of 300. The plasma transferrin levels at the Cal/N of 900 in the MRX-III groups were significantly higher than those in the corresponding MPR-F groups. At all Cal/N, the MRX-III groups showed low levels of blood urea nitrogen and urinary excretion of ammonia and urea nitrogen as compared with the MPR-F groups at the same Cal/N. The plasma amino acid concentration profiles in the MRX-III groups after TPN showed greater similarity to that in the Normal group as compared with the profiles in the corresponding MPR-F groups. No aggravation of renal failure was observed in any TPN groups during TPN. These results indicate that, in rats with CRF undergoing hyperalimentation, the effects of MRX-III on the nutritional status and nitrogen metabolism are superior to those of the general amino acid solution, MPR-F. It is suggested that MRX-III could safely provide adequate amounts of nitrogen during hyperalimentation.

Key Words hyperalimentation, chronic renal failure, amino acid solution, rat, nutrition

Patients with renal failure have malnutritional status with abnormalities of protein metabolism, disturbances of water and electrolytes balance, and excess
catabolism (1). In the management of patients with chronic renal failure (CRF), it is necessary to prevent the production of uremic toxins and supply adequate energy and protein sources. Hyperalimentation therapy, which supplies adequate nutrients and energy, has also been utilized for patients with renal failure. The supply of essential amino acids (EAA) in the treatment of patients with acute renal failure has been reported to have beneficial effects such as improvement of survival rates, weight gain and positive nitrogen balance (2, 3). An amino acid solution for use in hyperalimentation therapy for patients with renal failure, based on the requirements for EAA and histidine proposed by Rose (4), has been used clinically. However, since total parenteral nutrition (TPN) is so prevalent, complications such as hyperammonemia and disturbance of consciousness associated with TPN containing EAA have been reported (5-7). These adverse effects have been observed at a non-protein calorie/nitrogen ratio (Cal/N) of approximately 300 during hyperalimentation (5, 6). Kikuchi et al (8) reported that, in uremic rats, TPN containing EAA and histidine produced hyperammonemia and severe distortion of plasma amino acid concentration at the Cal/N of 300 at higher frequency. Additionally, they reported that TPN containing both EAA and non-essential amino acids (NEAA), used a general amino acid solution, did not produce the adverse effects at a low Cal/N (8).

We have produced a new amino acid solution for renal failure, MRX-III, which is safe, easy to use, and does not produce adverse effects. An amino acid composition of MRX-III is designed by decreasing lysine, methionine, phenylalanine and threonine, of which concentration in the blood is elevated during TPN containing EAA and histidine, and adding histidine, tyrosine and serine as required in renal failure as well as arginine as required for activation of the urea cycle. MRX-III has a composition similar to that of the general amino acid solution MPR-F in regards to containing EAA and NEAA, and no difference in EAA/NEAA ratio (E/N). The purpose of this study was to clarify the effects of MRX-III on the nutritional status and nitrogen metabolism of rats with CRF undergoing hyperalimentation as compared to those of the general amino acid solution, MPR-F.

MATERIALS AND METHODS

Animals and materials. Male Sprague-Dawley rats weighing approximately 70 to 90 g (4 wk old) were obtained from Charles River Japan, Inc. (Atsugi, Japan). The rats were acclimatized for 1 wk. They were given a commercial diet (CRF-1, Oriental Yeast Co., Tokyo, Japan), allowed water ad libitum and housed in a temperature (23±2°C) and humidity (55±15%)-controlled room with artificial lighting from 07:00 to 19:00.

Both MRX-III and MPR-F were obtained from Roussel Morishita Co., Ltd. (Tokyo, Japan). The compositions of the two amino acid solutions are shown in Table 1.

Preparation of rats with CRF. CRF was induced by the method of Platt et
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Table 1. Composition of amino acid solutions.

| Amino acid          | MRX-III (w/v%) | MPR-F (w/v%) |
|---------------------|----------------|--------------|
| l-Isoleucine        | 0.75           | 0.56         |
| l-Leucine           | 1.00           | 1.25         |
| l-Lysine·acetate    | 0.70           | 1.24         |
| l-Methionine        | 0.50           | 0.35         |
| l-Phenylalanine     | 0.50           | 0.935        |
| l-Threonine         | 0.25           | 0.65         |
| l-Tryptophan        | 0.25           | 0.13         |
| l-Valine            | 0.75           | 0.45         |
| l-Alanine           | 0.30           | 0.62         |
| l-Arginine          | 0.30           | 0.79         |
| l-Aspartic acid     | 0.025          | 0.38         |
| l-Cysteine          | —              | 0.10         |
| l-Glutamic acid     | 0.025          | 0.65         |
| Glycine             | 0.15           | 1.07         |
| l-Histidine         | 0.25           | 0.60         |
| l-Proline           | 0.20           | 0.33         |
| l-Serine            | 0.10           | 0.22         |
| l-Tyrosine          | 0.05           | 0.035        |
| Total free amino acids | 5.90         | 10.00        |
| Total free EAA      | 4.50           | 5.20         |
| Total free NEAA*    | 1.40           | 4.80         |
| EAA/NEAA            | 3.21           | 1.09         |
| Total nitrogen      | 0.81           | 1.52         |

*l-Histidine is regarded as NEAA.

al (9). Briefly, 2 wk following 3/4 nephrectomy of the left kidney, total nephrectomy of the right kidney was carried out. The rats were then kept under the same conditions as described above. At 2 wk after the final surgery, blood samples were collected from the tail vein to determine the plasma levels of creatinine (Cr). Rats with a plasma Cr level in the range from 1.5 to 2.6 mg/100 mL were used as the CRF model.

Experimental groups and the composition of test solutions. The rats with CRF were allocated to 6 groups of 8 rats, each receiving TPN containing MRX-III or MPR-F at a Cal/N of 300, 600 or 900. Rats of the same age in the CRF control group (n = 5, rats with CRF) and Normal group (n = 5, normal rats) were given CRF-1 and water ad libitum throughout the experimental period.

The composition of the test solution in each TPN group is shown in Table 2. Although the solutions contained different amounts of amino acids and Cal/N of 300, 600 or 900, they had isocalorie in the same Cal/N solution.
Table 2. TPN composition in each experimental group.

| Composition | MRX-III 300 | MRX-III 600 | MRX-III 900 | MPR-F 300 | MPR-F 600 | MPR-F 900 |
|-------------|-------------|-------------|-------------|-----------|-----------|-----------|
| N-P Cal/N   | 801         | 801         | 801         | 801       | 801       | 801       |
| Glucose (g/L) | 200.32     | 200.32      | 200.32      | 200.32    | 200.32    | 200.32    |
| Amino acid (g/L) | 19.45    | 9.76        | 8.82        | 17.63     | 6.48      | 5.86      |
| Nitrogen (g/L) | 2.67       | 1.34        | 0.89        | 2.67      | 1.34      | 0.89      |
| Na, K, Ca, Mg, Cl, P, A1, B1, B2, B6, B12, C, D, E, K, folic acid, nicotinic acid, pantothenic acid, biotin, Fe, Zn, Cu, Mn, I |

N-P Cal: Non-protein calorie.

**Experimental protocol.** Under anesthesia with sodium pentobarbital, the right jugular vein of rats with CRF was cannulated using a silicone rubber catheter (Daw Corning Co., Michigan, U.S.A.) by the method of Steiger et al (10). After catheterization, test solutions were infused intravenously in incremental fashion at the rate of 1.5 mL/h/rat on day 0, 2.3 mL/h/rat on day 1, and 3.0 mL/h/rat on day 2 and later.

**Measurements.** The body weight of the rats was measured on days 3 and 7 of TPN. Urine samples were collected daily for the determination of the levels of total nitrogen, urea nitrogen, ammonia and orotic acid. After TPN for 7 d, all rats were sacrificed for sampling of tissues and blood.

Blood from the abdominal aorta was collected into heparinized tubes, and then the wet weights of the liver, gastrocnemius and epididymal fat pads were determined. The blood obtained was used for biochemical determinations after centrifugation (3,000 r.p.m., 10 min, 4°C). The urinary total nitrogen level was determined using a YANACO MT-1600 CN CORDER (Yanagimoto Co., Ltd., Tokyo, Japan). The nitrogen balance on days 0–3 and days 4–7 during TPN was calculated for the TPN groups.

The blood biochemical examinations included measurements of blood urea nitrogen (BUN), Cr, ammonia (NH₃), glutamate oxaloacetate-transaminase (GOT), glutamate pyruvate-transaminase (GPT), albumin (Alb), transferrin (Tf), glucose (Glu), triglyceride (TG), total cholesterol (T-Chol), and phospholipid (PL) using a Hitachi 7150E auto-analyzer (Hitachi Co., Ltd., Tokyo, Japan), respectively. The levels of ammonia and urea nitrogen in the urine were also measured using the Hitachi 7150E auto-analyzer. The level of orotic acid in the urine was measured by the method of Stajner et al (11). After deproteinization by sulfosalicylic acid,

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plasma amino acids were analyzed by an amino acid analyzer (JLC-300, JEOL, Tokyo, Japan). Then, the degree of similarity of the plasma amino acid concentration profile in each TPN group to that in the Normal group was evaluated using the following equation (12): 

\[ S(A, B) = \cos \theta = \frac{\sum_{i=1}^{n} a_i \cdot b_i}{\sqrt{\sum_{i=1}^{n} a_i^2 \cdot \sum_{i=1}^{n} b_i^2}} \]

Pattern similarity \( S(A, B) \) between pattern \( A(a_1, a_2, a_3, \cdots, a_n) \) in the Normal group and pattern \( B(b_1, b_2, b_3, \cdots, b_n) \) in one of the TPN groups was regarded as the cosine of the angle (\( \theta \)) between vector \( \overrightarrow{OA} \) and vector \( \overrightarrow{OB} \) in \( n \) dimensional space. When pattern \( A \) is equal to pattern \( B \), the pattern similarity \( S(A, B) \) is 1, but when patterns \( A \) and \( B \) do not contain any mutual components at all, \( S(A, B) \) is 0.

**Statistics.** All values are expressed as mean \( \pm \) standard error (SE). A statistical comparison between the pairs of MRX-III and MPR-F groups at the same Cal/N was conducted using analysis of variance followed by unpaired Student's t-test by employing a statistical program system (Toukei Library Ver. 5, Yukms, Tokyo, Japan). A statistical comparison between the CRF control group and the Normal group was also performed using unpaired Student's t-test. Differences between the experimental groups were considered significant at the level of \( p<0.05 \).

**RESULTS**

**Nutritional effects of amino acid solution**

During TPN, one rat in each of the MPR-F groups at the Cal/N of 300 and 600 was excluded because of trouble during the care of TPN. One rat in the CRF control group died due to the aggravation of CRF on day 6.

The changes of body weight and cumulative nitrogen balance during TPN are shown in Table 3 and Fig. 1, respectively. The two TPN groups at the Cal/N of 300 showed the highest body weight gain. There were no significant differences in weight gain between the pairs of MRX-III and MPR-F groups at the same Cal/N. The CRF control group showed lower weight gain than the Normal group.

The cumulative nitrogen balances in the TPN groups improved in association with the decrease in Cal/N on both days 0–3 and days 4–7. The MRX-III groups at the Cal/N of 600 and 900 showed significantly more positive nitrogen balance on days 4–7 as compared to the corresponding MPR-F groups. On the other hand, the balance on days 0–3 was negative in all groups and there were no significant differences between the pairs of MRX-III and MPR-F groups at the same Cal/N. The CRF control group showed lower weight gain than the Normal group.

The blood biochemical data of each group after TPN are shown in Table 4. The nutritional indices of plasma Alb level and Tf level tended to increase with the decrease in Cal/N in both the MRX-III and MPR-F groups. The plasma Alb level in the MRX-III group at the Cal/N of 300 was significantly higher than that in the corresponding MPR-F group. The plasma Tf level in the MRX-III group at the
Table 3. Changes of body weight in rats with CRF during TPN.

| Group   | Day 0       | Day 3       | Day 7       | Day 0–7 (Gain) |
|---------|-------------|-------------|-------------|----------------|
| Cal/N 300 |            |             |             |                |
| MRX-III | 225 ± 8 (8) | 247 ± 9 (8) | 261 ± 8 (8) | +35.7 ± 1.6 (8) |
| MPR-F   | 229 ± 4 (8) | 253 ± 5 (8) | 264 ± 5 (7) | +33.5 ± 2.3 (7) |
| Cal/N 600 |            |             |             |                |
| MRX-III | 244 ± 6 (8) | 249 ± 5 (8) | 256 ± 5 (8) | +11.7 ± 1.3 (8) |
| MPR-F   | 240 ± 9 (8) | 248 ± 9 (7) | 254 ± 10 (7) | +11.4 ± 1.8 (7) |
| Cal/N 900 |            |             |             |                |
| MRX-III | 233 ± 4 (8) | 240 ± 2 (8) | 241 ± 4 (8) | +8.0 ± 1.9 (8) |
| MPR-F   | 229 ± 7 (8) | 241 ± 6 (8) | 242 ± 6 (8) | +12.9 ± 3.1 (8) |
| CRF control | 228 ± 10 (5) | 238 ± 18 (5) | 255 ± 12 (4) | +27.7 ± 6.2 (4) |
| Normal  | 329 ± 10 (5) | 363 ± 13 (5) | 374 ± 18 (5) | +45.5 ± 8.9 (5) |

Each value represents the mean ± SE (g).
Numbers of rats are shown in parentheses.
**, significantly different at p < 0.01 (unpaired Student’s t-test).

Cal/N of 900 was significantly higher than that in the corresponding MPR-F group. However, under other conditions, there were no significant differences between the pairs of TPN groups.

The BUN level, as an index of nitrogen metabolism, in each MRX-III group was significantly lower than that in the corresponding MPR-F group. The plasma Cr level, as an index of renal function, was lower in each TPN group than that in the CRF control group, but there were no significant differences between the pairs of MRX-III and MPR-F groups at the same Cal/N. At the Cal/N of 300, the plasma Glu level in the MRX-III group was significantly lower than that in the MPR-F group; at the Cal/N of 600 and 900, the plasma T-Cho levels in both MRX-III groups were higher than those in the MPR-F groups; and at the Cal/N of 900, the plasma PL level in the MRX-III group was significantly higher than that in the MPR-F group. However, the plasma BUN, Cr, T-Cho and PL levels in the 6 TPN groups and the Normal group were lower than those in the CRF control group. No significant differences were observed in other indices of blood biochemistry. The plasma GPT, Alb and Tf levels in the control group were significantly lower than those in the Normal group.

The wet organ weights in each group after TPN are shown in Table 5. At the Cal/N of 600 and 900, the liver weights in the two MRX-III groups were significantly higher than those in the corresponding MPR-F groups. For the weights of other organs, there were no significant differences between the MRX-III and MPR-F groups. Each organ weight in the CRF control group was significantly lower than
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Fig. 1. Cumulative nitrogen balance in rats with CRF during TPN. Each column represents the mean ± SE. 

☐: MRX-III (n = 7–8), ■: MPR-F (n = 7–8).

* and **, significantly different at p < 0.05 and p < 0.01, respectively (unpaired Student's t-test).

that in the Normal group.

**Urinary excretion of nitrogen metabolites during TPN**

The urinary excretion of ammonia, urea nitrogen and orotic acid on day 7 is shown in Fig. 2. Each MRX-III group showed significantly lower urinary excretion of ammonia and urea nitrogen compared to the corresponding MPR-F group. At the Cal/N of 300, the urinary excretion of orotic acid in the MRX-III group was significantly higher than that in the MPR-F group, but these values were both lower than that in the Normal group. The urinary excretion of ammonia and urea nitrogen in the CRF control group was significantly lower than that in the Normal group.

**Pattern similarity of plasma amino acid concentration after TPN**

The pattern similarity of the plasma amino acid concentration profile after TPN to that in the Normal group is shown in Table 6. The pattern similarity in each MRX-III group was significantly greater than that in the corresponding MPR-F group.

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Table 4. Blood biochemical results in rats with CRF after TPN.

| Group          | n  | BUN (mg/100 mL) | Cr (mg/100 mL) | GPT (IU/L) | GOT (IU/L) | NH₃ (µg/100 mL) |
|----------------|----|-----------------|---------------|------------|------------|-----------------|
| Ca/N 300       | 8  | 10.82±1.92      | 1.39±0.08     | 23.5       | 61.1±2.6   | 21.6±2.2        |
| MRX-III        |    |                 |               |            |            |                 |
| MPR-F          | 7  | 19.14±2.43      | 1.45±0.12     | 35.6       | 53.3±4.5   | 16.2±2.1        |
| Ca/N 600       | 8  | 7.01±0.72       | 1.45±0.10     | 42±9       | 83.6±6.4   | 24.2±1.4        |
| MRX-III        |    |                 |               |            |            |                 |
| MPR-F          | 7  | 12.96±1.63      | 1.54±0.07     | 40±12      | 74.4±7.2   | 23.8±2.6        |
| Ca/N 900       | 8  | 5.84±0.76       | 1.41±0.10     | 41±8       | 78.6±4.5   | 20.9±1.8        |
| MRX-III        |    |                 |               |            |            |                 |
| MPR-F          | 8  | 9.90±0.54       | 1.55±0.14     | 50±9       | 68.2±7.4   | 22.7±2.7        |
| CRF control    | 4  | 86.68±8.37      | 1.87±0.23     | 30±13      | 57.4±5.6   | 28.6±1.7        |
| Normal         | 5  | 19.65±1.17      | 0.59±0.04     | 41±7       | 69.2±4.5   | 45.9±2.2        |

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| Group        | n  | Alb (g/100 mL) | Tf (mg/100 mL) | Glu (mg/100 mL) | TG (mg/100 mL) | T-Cho (mg/100 mL) | PL (mg/100 mL) |
|--------------|----|---------------|---------------|----------------|---------------|------------------|--------------|
| Cal/N 300    | 8  | 2.77 ± 0.06   | 254 ± 10      | 86.6 ± 3.8     | 89.3 ± 11.4   | 100.0 ± 3.2      | 152.8 ± 7.1  |
| MRX-III      |    |               |               |                |               |                  |              |
| MPR-F        | 7  | 2.53 ± 0.07   | 237 ± 18      | 102.7 ± 5.8    | 93.5 ± 11.8   | 96.7 ± 6.5       | 146.4 ± 8.2  |
| Cal/N 600    | 8  | 2.67 ± 0.13   | 209 ± 11      | 75.5 ± 3.0     | 79.4 ± 8.0    | 88.3 ± 6.4       | 134.6 ± 9.9  |
| MRX-III      |    |               |               |                |               |                  |              |
| MPR-F        | 7  | 2.74 ± 0.09   | 192 ± 10      | 76.8 ± 5.1     | 74.4 ± 9.1    | 71.3 ± 5.8       | 115.9 ± 9.0  |
| Cal/N 900    | 8  | 2.42 ± 0.08   | 193 ± 7       | 77.9 ± 4.7     | 73.9 ± 10.7   | 81.3 ± 5.0       | 124.0 ± 7.4  |
| MRX-III      |    |               |               |                |               |                  |              |
| MPR-F        | 7  | 2.36 ± 0.10   | 164 ± 6       | 83.0 ± 6.9     | 67.5 ± 9.0    | 64.2 ± 4.6       | 99.8 ± 6.5   |
| CRF control  | 4  | 2.80 ± 0.18   | 219 ± 28      | 156.0 ± 13.8   | 84.7 ± 24.2   | 123.9 ± 7.8      | 185.9 ± 11.8 |
| Normal       | 5  | 3.42 ± 0.05   | 360 ± 11      | 180.6 ± 7.7    | 108.0 ± 18.2  | 52.3 ± 2.9       | 111.4 ± 5.4  |

Each value represents the mean ± SE.
* and **, significantly different at p < 0.05 and p < 0.01, respectively (unpaired Student's t-test).
DISCUSSION

Motil et al (5) have reported that TPN containing only EAA as an amino acid source produced adverse effects in children with acute renal failure such as hyperammonemia. They indicated that the adverse effects by these amino acids resulted from abnormalities in the nitrogen metabolism due to the injection of an excessive amount of EAA. However, sufficient supplementation of amino acid as well as high energy has been considered to improve the nutritional status as patients with renal failure are in a severely catabolic state. Subsequently, it was also reported that these patients need not only EAA but also NEAA as amino acid sources for maintenance of the nutritional status and prevention of these adverse effects (8). For these reasons, we constructed a new amino acid solution, MRX-III, for use in renal failure. Feinstein et al (13) presumed that the E/N ratio in a TPN solution should be about 3.0 to 4.0 to maintain the nutritional status of patients with renal failure. We have shown that the optimal E/N ratio of an amino acid solution is between 1.6 and 3.2 for therapy for CRF (14), and that MRX-III with an E/N ratio of 3.21 showed beneficial nutritional effects in rats and dogs with renal failure undergoing hyperalimentation as compared to a solution based on EAA and histidine (unpublished observation). In the present study, in order to elucidate the suitability of MRX-III for nutritional support in renal failure, we compared the nutritional effects of MRX-III with those of a general amino acid solution in rats.
Fig. 2. Urinary excretion of ammonia, urea nitrogen and orotic acid on day 7 in rats with CRF. Each column represents the mean ± SE.

- MRX-111 (n=7-8), MPR-F (n=7-8), CRF control (n=4), Normal (n=5).

* and **, significantly different at p<0.05 and p<0.01, respectively (unpaired Student's t-test).

with CRF undergoing hyperalimentation. In TPN, a good Cal/N, amount of non-protein calorie required to have amino acids be utilized to smoothly synthesize a protein, is 150-200 in general. However, patients with renal failure should not be supplied a lot of nitrogen to avoid accumulation in the body. Therefore, we need to supply suitable amounts of amino acid and non-protein calories at a higher Cal/N, which is 500-1,000. But adverse effects such as hyperammonemia and disturbance of consciousness associated with TPN containing EAA have been observed at a Cal/N of 300 (5-8). Our group has reported that TPN containing MRX-III at a Cal/N of 150 showed the same adverse effects as mentioned above (15). For these reasons, as the condition of administering nitrogen, we set up the Cal/N of 300, 600 and 900.

In the present study, the CRF model was induced by surgical nephrectomy, producing chronic impairment of renal function. This rat model has been used in many basic studies of renal failure. The rats in the CRF control group showed high plasma Cr and BUN levels, diminished body weight gain and plasma levels of EAA, especially of valine, isoleucine, leucine and tryptophan (data not shown). Therefore,
Table 6. Pattern similarity of plasma amino acid concentration in each group after TPN to that in the Normal group.

| Group       | $n$ | $\cos \theta$          |
|-------------|-----|-------------------------|
| Cal/N 300   |     |                         |
| MRX-III     | 8   | 0.943 ± 0.008           |
| MPR-F       | 7   | 0.680 ± 0.020           |
| Cal/N 600   |     |                         |
| MRX-III     | 8   | 0.869 ± 0.015           |
| MPR-F       | 7   | 0.676 ± 0.016           |
| Cal/N 900   |     |                         |
| MRX-III     | 8   | 0.830 ± 0.021           |
| MPR-F       | 8   | 0.689 ± 0.029           |
| CRF control | 4   | 0.954 ± 0.006           |

Each value represents the mean ± SE.

**, significantly different at $p < 0.01$ (unpaired Student's $t$-test).

it was considered that this model is similar to the CRF seen in human patients (1, 9).

Regarding the nutritional effects, though differences in the changes of body weight were not observed between MRX-III and MPR-F groups, the cumulative nitrogen balance on days 4–7 was more positive in the MRX-III groups at the Cal/N of 600 and 900 as compared with those in the MPR-F groups. The nitrogen balance improved at a lower Cal/N. We speculated from this result that supplementation with less NEAA, which are administered in renal failure, enhanced the utilization of all amino acids. It has been reported that metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes (16), and that muscle protein degradation in metabolic acidosis is partially mediated by glucocorticoid (17, 18). The acid-base status and serum glucocorticoid levels were not determined in this study. Additionally, we did not find any evidence that metabolic acidosis was induced in any rats in these experimental groups. However, additional information may be acquired by determining these indices.

In the MRX-III groups, the plasma Tf level at the Cal/N of 900, Alb level at the Cal/N of 300 and liver weight at the Cal/N of 600 and 900 were significantly higher than those in the corresponding MPR-F group. The indices of nitrogen metabolism, BUN level and urinary excretion of ammonia and urea nitrogen in each MRX-III group were lower than those in the corresponding MPR-F group. These results suggest that MRX-III not only might facilitate nitrogen availability but also may partially improve the nutritional status in CRF, in contrast with MPR-F.
It has been reported that impairment of the urea cycle, especially in arginine deficiency (19–21) and in inborn errors of urea synthesis (22,23), induces hyperammonemia and increases the urinary excretion of orotic acid due to the stimulation of orotic acid synthesis by the excess ammonia (24). An increase in plasma orotic acid level has also been observed in patients with renal failure undergoing hyperalimentation containing EAA without arginine (25). In the present study, the plasma orotic acid was not determined, but the urinary excretion of orotic acid in each TPN group was low and the plasma Cr level as an index of renal function did not increase during the period of TPN. These results suggest that TPN containing NEAA might not aggravate renal failure under these conditions of Cal/N, and that the NEAA are important. In this study, the urinary excretion of orotic acid in the MRX-III group at the Cal/N of 300 was significantly higher than that in the MPR-F group. It has been reported that excess dietary lysine in rats induced an increase in the excretion of orotic acid and ammonia but decreased urea excretion, and that arginine supplementation prevented orotic aciduria (26). The lysine/arginine ratio in the composition of MRX-III is higher than that in MPR-F. This difference might cause the higher excretion of orotic acid in the MRX-III group at the Cal/N of 300, which is the condition of infusing more amino acid. It might be suggested that the lysine/arginine ratio has an effect on orotic acid biosynthesis during the infusion of amino acid solution in renal failure.

It has been shown that TPN containing only EAA and histidine produced a decrease of BUN and better nitrogen balance compared to a general amino acid solution under the condition of a Cal/N of a more than 600 in uremic rats (8). TPN containing only EAA and histidine did not have beneficial effects on the status of renal failure at the Cal/N of 300; indeed, it induced adverse effects such as hyperammonemia. However, in the present study, MRX-III did not induce adverse effects under any Cal/N conditions and it was somewhat superior to the general amino acid solution in its nutritional effect in CRF. The EAA comprise 76.3% (w/v%) of the total amino acids in MRX-III and 52.0% of those in MPR-F. We believe that nutritional support with hyperalimentation in renal failure should include a high percentage of EAA. Although the appropriate E/N ratio and amount of NEAA for nutritional support with hyperalimentation in renal failure are unknown, NEAA are indispensable. Moreover, the relationship between the E/N ratio and the Cal/N in the treatment of renal failure remains to be investigated.

Some reports indicate a distortion of the plasma amino acid profile in renal failure suggesting abnormalities in the metabolism of amino acids (5,27,28). Especially, it has been observed that the plasma levels of EAA such as branched-chain amino acids and threonine are decreased and those of NEAA tend to be increased (27). Yugari (29) suggested that the appropriate amino acid composition in hyperalimentation for renal failure might normalize the distortion of the plasma amino acid profile. In the present study, compared to that in the MPR-F groups at the same Cal/N, the plasma amino acid profile in each MRX-III group was similar to that in the Normal group. This result also indicates that the
amino acid composition of MRX-III was more suitable than that of MPR-F for nutritional supplementation in renal failure.

Our observations indicate that, in rats with CRF undergoing hyperalimentation, the effects of MRX-III on the nutritional status and nitrogen metabolism are superior to those of the general amino acid solution, MPR-F. MRX-III may provide an adequate amount of nitrogen with the reduced risk of inducing adverse effects under hyperalimentation. It is speculated that, first of all, about one-third of NEAA to EAA would be better, supported partly the speculation of Feinstein et al (13) and our data (14), and that a decreased amount of NEAA is essential for nutritional treatment in CRF under hyperalimentation. There are differences concerning E/N ratio and patterns of EAA and NEAA between MRX-III and MPR-F. In the present study, we think the reasons for MRX-III being superior to MPR-F are 1) higher E/N ratio and 2) the amino acid patterns of EAA and NEAA. However, the effective amount and ratio of each amino acid in formulation remain unknown. Further studies will be required to elucidate these matters.

REFERENCES

1) Kopple JD. 1978. Abnormal amino acid and protein metabolism in uremia. Kidney Int 14: 340–348.
2) Abel RM, Beck CH, Abbott WM, Ryan JA, Barnett GO, Fischer JE. 1973. Improved survival from acute renal failure after treatment with intravenous essential L-amino acids and glucose. N Engl J Med 288: 695–699.
3) Stanley J, Dudrick J, Steiger E, Long JM. 1970. Renal failure in surgical patients. Treatment with intravenous essential amino acid and hypertonic glucose. Surgery 68: 180–186.
4) Rose EC. 1949. Amino acid requirements of man. Fed Proc 8: 546–547.
5) Motil KJ, Harmon WE, Grupe WE. 1980. Complications of essential amino acid hyperalimentation in children with acute renal failure. JPEN 4: 32–35.
6) Grazer RE, Sutton JM, Friedstrom S, McBarron FD. 1984. Hyperammonemic encephalopathy due to essential amino acid hyperalimentation. Arch Intern Med 144: 2278–2279.
7) Rapp RP, Bivins BA, McRoberts JW. 1982. Hyperammonia encephalopathy in a patient receiving essential amino acid/dextrose parenteral nutrition. Clin Pharm 1: 276–280.
8) Kikuchi T, Tanaka H, Kokuba Y, Sato M. 1994. Amino acid supplementation to hyperalimentation in uremic rats: effects of amount and composition of amino acids on nutrition and uremia. Renal Failure 16: 209–220.
9) Platt R, Roscoe MH, Smith FW. 1952. Experimental renal failure. Clin Sci 11: 217–228.
10) Steiger E, Vars HM, Dudrick SJ. 1972. A technique for long-term intravenous feeding in unrestrained rats. Arch Surg 104: 330–332.
11) Stajner A, Suba J, Musil F. 1968. The determination of orotic acid in the blood serum by means of the spectrophotometric method. Experientia 24: 116–117.
12) Tamura S, Osawa F. 1969. Amino acid pattern similarity between foods in Japan. J Jpn Soc Food Nutr 22: 494–495.
13) Feinstein EI, Kopple JD, Silberman H, Massry S. 1983. Total parenteral nutrition with high or low nitrogen intakes in patients with acute renal failure. Kid Int 26: S319–S323.
Fujii Y, Tanaka H, Sasamura T, Yamauchi K, Kameda H, Kawashima M, Kikuchi T. 1996. A study on MRX-III, a new amino acid solution for renal failure (3)—Influence of essential to non-essential amino acids ratio on chronic renal failure rats receiving total parenteral nutrition (Cal/N 300). *Jpn Pharmacol Ther* 24: 735–741.

Fujii Y, Tanaka H, Ikeda A, Kameda H, Kikuchi T, Sato M. 1993. Study on infusional dose of amino acid solution in rats with acute renal failure. *Jpn Pharmacol Ther* 21: 4617–4624.

Mitch WE, Medina R, Grieber S, May RC, England BK, Price SR, Bailey JL, Goldberg AL. 1994. Metabolic acidosis stimulates muscle protein degradation by activating the adenosine triphosphate-dependent pathway involving ubiquitin and proteasomes. *J Clin Invest* 93: 2127–2133.

May RC, Kelly RA, Mitch WE. 1986. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. *J Clin Invest* 77: 614–621.

Garibotto G, Russo R, Sofia A, Sala MR, Robaudo C, Moscatelli P, Deferrari G, Tizianello A. 1994. Skeletal muscle protein synthesis and degradation in patients with chronic renal failure. *Kid Int* 45: 1432–1439.

Milner JA. 1985. Metabolism aberrations associated with arginine deficiency. *J Nutr* 115: 516–523.

Milner JA, Visek WJ. 1973. Orotic aciduria and arginine deficiency. *Nature* 245: 211–213.

Czarnecki GL, Baker DH. 1984. Urea cycle function in the dog with emphasis on the role of arginine. *J Nutr* 114: 581–590.

Brusilow SW. 1984. Arginine, an indispensable amino acid for patients with inborn errors of urea synthesis. *J Clin Invest* 74: 2144–2148.

Matsuda I, Nagata N, Matsuura T, Oyanagi K, Tada K, Narisawa K, Kitagawa T, Sakiyama T, Yamashita F, Yoshino M. 1991. Retrospective survey of urea cycle disorders: Part 1. Clinical and laboratory observations of thirty two Japanese male patients with ornithine transcarbamylase deficiency. *Am J Med Genetics* 38: 85–89.

Visak WJ. 1979. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutr Rev* 37: 273–282.

Nakasaki H, Katayama T, Yokoyama S, Tajima T, Mitomi T, Tsuda M, Suga T, Fujii K. 1993. Complication of parenteral nutrition composed of essential amino acids and histidine in adults with renal failure. *JPEN* 17: 86–90.

Fico ME, Hassan AS, Milner JA. 1982. The influence of excess lysine on urea cycle operation and pyrimidine biosynthesis. *J Nutr* 112: 1854–1861.

Alvestrand A, Furst P, Bergström J. 1982. Plasma and muscle free amino acids in uremia: influence of nutrition with amino acids. *Clin Nephrology* 18: 297–305.

Wilcken DEL, Gupta VJ, Reddy SG. 1980. Accumulation of sulphur-containing amino acids including cystein-homocysteine in patients on maintenance haemodialysis. *Clin Sci* 58: 427–430.

Yugari Y. 1982. Amino acid infusion solution for various diseased states. *J Jpn Soc Food Nutr* 35: 1–13 (in Japanese).