EFFECT OF NITROGEN SOURCE ON NUTRITIONAL MANAGEMENT AFTER SMALL BOWEL RESECTION IN RATS

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Summary

Nutritional effects of dietary nitrogen sources on rats subjected to 65% distal resection of the small bowel were compared at the 2.4% nitrogen level, i.e., by feeding the rats a diet which contains 2.4% nitrogen from various sources. The nitrogen sources used were casein (C), the casein-simulated amino acid mixture (F), the proposed amino acid mixture (T-2), which was devised in our laboratory, and the amino acid-casein mixture (R), in which 25% of the proposed amino acid mixture was replaced by isonitrogenous casein. All the experimental diets were made cellulose-free. Total weight gains of the resected groups during three weeks were less than those of the corresponding unresected groups because of the depressed gains in the first postoperative week. In the resected rats, as well as in the unresected rats, the maximum total weight gain was obtained with diet R and followed weight gains with diet C, diet F, and diet T-2. Fecal weight increased moderately in the resected rats but the degree of the increase was not influenced by the type of dietary nitrogen source. Dry weight of intestinal remnants increased markedly within one week after resection. The extent of hyperplasia was not altered by the type of dietary nitrogen source. These results indicate that an amino acid mixture partially replaced with casein may be a useful dietary nitrogen source for short gut syndrome.

Synthetically formulated, chemically defined diets containing amino acid mixture as the sole source of nitrogen have opened new possibilities in the nutritional management of intestinal disorders which are accompanied by impaired digestion and absorption (1-4). However, the high osmolality of chemically defined diets results in a difficulty of successive intake in sufficient quantities (3, 5). To overcome this problem, carbohydrates of high molecular weight, oligosac-
charides and starch, are used instead of glucose and sucrose. Despite such modifications, however, the problem has yet to be solved.

In our previous studies, the nutritional effect of an amino acid mixture simulating casein was compared with that of intact casein (6, 7). A lower food intake was observed at the 3.2% dietary nitrogen level in the rats fed the amino acid diet. By replacing 25% of amino acids in the amino acid diet with isonitrogenous casein and lowering the dietary osmolality, both food intake and weight gain of rats fed the diet increased significantly and became almost the same as those of rats fed the casein diet. On the other hand, the diet, in which dietary osmolality was lowered by replacing sucrose in the amino acid diet with starch, was not so much more effective for increasing food intake (8). Furthermore, the proposed nitrogen source, in which 25% of amino acid mixture devised in our laboratory was replaced by isonitrogenous casein, was found to be superior to intact casein in supporting growth of young rats (8).

The present study was conducted with the small bowel-resected rats to investigate the usefulness of the above nitrogen source to nutritional management of gastrointestinal disorders.

METHODS

Experimental animals. Male rats of the Wistar strain weighing 170 to 180 g, which had been fed a laboratory chow (CLEA Japan Inc., Tokyo, Japan) ad libitum, were used in the experiment. They were individually housed in wire cages in an air-conditioned room (23±1°C) with a 12-hr light-dark cycle. The animals were divided into 8 groups of 12 rats each. They were fed an equal mixture of 15% casein and 15% casein-simulated amino acid diets for 3 days, and were fasted for 24 hr without water restriction.

Operative procedure. The four groups of the above experimental rats underwent small bowel resection and the others did not. Under sodium pentobarbital anaesthesia, the abdomen was opened by a midline incision, and the small intestine was spread out. Beginning from a point 5 cm proximal to the ileocecal valve approximately 65% of the small bowel was distally resected. The cut ends of the intestine were joined by an end-to-end anastomosis with care to ensure the patency of lumen. The gut was returned to the peritoneal cavity. A 5,000 U dose of benzylpenicillin potassium dissolved in 0.1 ml of 0.9% saline was introduced into the peritoneal cavity and another dose injected into femoral muscle. The abdomen was closed in two layers. The rats were supplied water at all times and fed the experimental diets ad libitum for three weeks from the next day of operation. The unresected rats were also given water and the experimental diets in the same manner as the resected rats.

Experimental diets. The compositions of diets used in the experiment are shown in Table 1. All the diets contained 2.4% nitrogen, and 0.5% corn oil as
Table 1. Compositions of experimental diets (N, 2.4%).

| Diet                  | C (%) | F (%) | T-2 (%) | R (%) |
|-----------------------|-------|-------|---------|-------|
| Casein                | 17.5  | —     | —       | 4.4   |
| Amino acid mix<sup>a</sup> | —     | 17.0  | 16.4    | 12.4  |
| Sucrose<sup>b</sup>   | 77.0  | 77.5  | 78.1    | 77.7  |
| Corn oil             | 0.5   | 0.5   | 0.5     | 0.5   |
| Salt mix<sup>c</sup>  | 4.0   | 4.0   | 4.0     | 4.0   |
| Soluble vitamin mix<sup>d</sup> | 0.85 | 0.85  | 0.85    | 0.85  |
| Choline chloride     | 0.15  | 0.15  | 0.15    | 0.15  |

| Retinyl acetate       | 2,000 IU/100 g diet |
| Ergocalciferol        | 200 IU/100 g diet   |
| All-rac-α-tocopherol  | 10 mg/100 g diet    |

<sup>a</sup> The compositions of amino acid mixtures are shown in Table 2.
<sup>b</sup> Sucrose was added as required to complete the mixture to 100%.
<sup>c</sup> The compositions were the same as those used in the previous experiment (7).

Table 2. Compositions of amino acid mixtures in the experimental diets.

| Diet                  | F (%) | T-2 (%) | R (%) |
|-----------------------|-------|---------|-------|
| L-Arginine·L-Glutamate<sup>a</sup> | 1.10  | 1.38    | 1.11  |
| L-Histidine           | 0.52  | 0.46    | 0.33  |
| L-Isoleucine          | 0.77  | 0.96    | 0.77  |
| L-Leucine             | 1.40  | 1.11    | 0.76  |
| L-Lysine·L-Aspartate<sup>a</sup> | 0.89  | 0.73    | 0.50  |
| L-Lysine·L-Glutamate<sup>a</sup> | 1.49  | 2.29    | 1.91  |
| L-Methionine          | 0.47  | 0.64    | 0.52  |
| L-Cystine             | 0.06  | 0.52    | 0.30  |
| L-Phenylalanine       | 0.78  | 0.80    | 0.61  |
| L-Tyrosine            | 0.83  | 0.59    | 0.38  |
| L-Threonine           | 0.65  | 0.75    | 0.59  |
| L-Tryptophan          | 0.17  | 0.32    | 0.27  |
| L-Valine              | 0.96  | 0.95    | 0.71  |
| L-Alanine             | 0.61  | 0.34    | 0.19  |
| L-Asparagine          | 0.64  | 0.45    | 0.29  |
| L-Glutamic acid       | 0.43  | 0.11    | —     |
| L-Glutamine           | 1.67  | 1.28    | 0.86  |
| Glycine               | 0.28  | 1.40    | 1.33  |
| L-Proline             | 1.58  | 1.00    | 0.60  |
| L-Serine              | 0.83  | 0.35    | 0.14  |
| Diammonium citrate    | 0.85  | —       | —     |
| Total                 | 16.98 | 16.43   | 12.37 |

<sup>a</sup> Basic amino acid salt with acidic amino acid.
an essential fatty acid source but no cellulose component. The amino acid compositions of diets are shown in Table 2. The pattern of amino acid mixture in diet F was simulated that of casein (9). The amino acid compositions of the proposed diets, diets T-2 and R, which were devised in our laboratory, were described in detail in the previous paper (8). The diet R contained 25% of dietary nitrogen in a form of casein-N. Each amino acid mixture was made hydrochloride-free by using basic amino acid salts with acidic amino acids as previously reported (9).

**Experimental measurements.** Body weight and food intake of 6 rats of each group were recorded daily through the 3 week experimental period. At the termination of the period they were decapitated for measurement of dry weight of intestinal segment by the method as described below. The other rats of each group were decapitated for the intestinal analysis on day 7 after the initiation of feeding.

Feces were collected weekly from each animal and dried in an oven for 24 hr at 100°C. Dry weight of feces per 100 g of food intake was calculated.

For the intestinal analysis the abdomen was opened and the small bowel, from the pylorus to the ileocecal valve, was removed in one piece. After fat and mesentery were dissected away, the intestine was suspended vertically with a 10 g weight attached to its lower ileal end to ensure uniform stretching. The ten centimeter segment of the intestine distal to the point 10 cm from the pylorus was removed. The segment was dried in an oven for 24 hr at 100°C. Dry weight per centimeter length per 100 g body weight was calculated.

**Statistical analysis.** Data were treated statistically using Student’s t-test (10).

**RESULTS**

**Body weight change**

Body weight changes during the experimental period are shown in Table 3.

In the unresected rats, the body weight gain on the proposed amino acid diet (diet T-2) was slightly less than that on the casein-simulated amino acid diet (diet F), although the difference was not significant (p > 0.05). When 25% of amino acids in the proposed amino acid diet was replaced by isonitrogenous casein, with unchanged amino acid pattern, the resultant weight gain of rats significantly (p < 0.05) increased as compared with those of both the groups fed diets F and T-2, and reached about the same level as in the casein diet group. Weight gains were directly related to food intakes. Food intake of the diet T-2 group was less than that of the diet F group, although the difference was not significant (p > 0.05). On the other hand, food intake of the diet R group significantly (p < 0.05) increased as compared with those of the diet F and T-2 groups and reached about the same level as in the diet C group.

In the resected groups, total weight gains were less than those of the corre-
Table 3. Body weight gain and food intake of rats fed experimental diets at 2.4% nitrogen level.\(^1\)

| Group\(^2\) | Initial body weight (g) | 1st week | 2nd week | 3rd week | Total |
|-------------|-------------------------|----------|----------|----------|-------|
|             | Weight gain (g)          | Food intake (g) | Weight gain (g) | Food intake (g) | Weight gain (g) | Food intake (g) | Weight gain (g) | Food intake (g) |
| Unresected  | Diet C | 160.2±2.1 | 59.0±3.3 | 127.2±5.4 | 51.0±4.9 | 150.7±8.9 | 38.5±2.9 | 145.4±9.1 | 148.5±9.9\(^a\) | 423.3±22.7\(^a\) |
|             | Diet F | 159.7±1.8 | 47.8±2.6 | 116.1±3.5 | 30.8±2.1 | 121.5±1.6 | 30.8±0.9 | 123.0±2.8 | 109.5±4.4\(^b\) | 360.6±7.3\(^b\) |
|             | Diet T-2 | 159.5±1.7 | 42.3±5.8 | 98.8±9.5 | 23.2±3.9 | 107.6±8.5 | 25.0±3.5 | 111.3±8.1 | 90.5±11.7\(^b\) | 317.8±25.2\(^b\) |
|             | Diet R | 159.8±1.5 | 65.0±2.6 | 133.6±5.4 | 51.3±3.3 | 148.3±6.7 | 43.5±1.3 | 143.1±2.3 | 159.8±5.3\(^a\) | 425.0±12.0\(^a\) |
| Resected    | Diet C | 158.5±3.8 | 18.3±4.9 | 64.6±5.9 | 35.2±4.7 | 116.6±8.1 | 27.2±2.4 | 117.4±6.1 | 80.7±9.3\(^b\) | 298.5±17.4\(^b\) |
|             | Diet F | 158.0±2.4 | 21.3±5.0 | 72.6±3.8 | 28.5±2.1 | 107.5±6.6 | 28.3±1.7 | 113.4±4.9 | 78.2±7.7\(^d\) | 293.6±14.2\(^d\) |
|             | Diet T-2 | 156.8±2.0 | 19.5±4.8 | 61.2±7.1 | 26.0±3.5 | 100.0±7.3 | 26.2±2.6 | 109.7±6.1 | 71.7±8.8\(^c\) | 271.0±17.9\(^c\) |
|             | Diet R | 157.3±2.2 | 22.3±3.6 | 67.2±5.3 | 42.0±3.3 | 118.0±8.9 | 35.0±2.1 | 125.1±5.4 | 99.3±7.4\(^a\) | 310.4±18.0\(^a\) |

\(^1\) Each value represents the mean±SEM of six rats. Means with different superscript letters in a column are significantly different (\(p<0.05\)).

\(^2\) As nitrogen source, diets C, F, T-2 and R contained intact casein, an amino acid mixture of casein pattern, of test pattern and a mixture (the same amino acid pattern with diet T-2) of intact casein and an amino acid mixture, respectively.
sponding unresected groups because of the depressed gains during the first week following distal resection. The order of degree of weight gains in the resected groups fed four experimental diets was the same as that in the unresected groups; weight gain of the diet R group was largest (99.3±7.4 g/3 weeks) and that of the diet T-2 group least (71.7±8.8 g/3 weeks). However, the difference in weight gain between the two groups (R and T-2) was less than that between the corresponding unresected groups.

Food intakes of each resected group were lower than those of the corresponding unresected group, particularly earlier days after resection. This trend was most clearly observed in the intake of rats fed the diets C and R during the first week after resection. The relationship between weight gain and food intake was also observed in the resected rats.

**Fecal excretion**

Dry fecal weight per 100 g of food intake is shown in Table 4. Since all the resected rats had diarrhea for several days postoperatively, their feces could not be collected in this period. The diarrhea subsided within one week after resection in all animals. Fecal excretion in the resected groups tended to increase moderately as compared with that in the corresponding unresected groups both during the second week and during the third week after resection. The increase of fecal weight in the resected group was independent of the type of diets.

**Dry weight of intestinal segment**

Dry weight of intestinal segment per centimeter per 100 g of body weight is shown in Fig. 1. Weight increase was apparent in the segment from the resected animals 7 and 21 days after the initiation of feeding. These results indicate that

| Group\(^b\) | Fecal weight (dry weight, g/100 g food intake/week) |
|-------------|-------------------------------------------------|
|             | 1st week | 2nd week | 3rd week |
| Unresected  |          |          |          |
| Diet C      | 1.67±0.04\(^a\) | 1.87±0.15\(^a,b\) | 2.07±0.24\(^a,b\) |
| Diet F      | 1.49±0.10\(^b\) | 1.45±0.13\(^a,e\) | 1.93±0.08\(^b\) |
| Diet T-2    | 1.50±0.06\(^b\) | 1.64±0.21\(^a,d\) | 1.81±0.13\(^a,e\) |
| Diet R      | 1.30±0.08\(^b\) | 1.47±0.16\(^a,e\) | 1.64±0.13\(^a,d\) |
| Resected    |          |          |          |
| Diet C      | —        | 2.33±0.45\(^b,c,d,e\) | 2.71±0.36\(^b\) |
| Diet F      | —        | 2.31±0.20\(^b\) | 2.16±0.15\(^b,e\) |
| Diet T-2    | —        | 2.57±0.45\(^b,d\) | 2.54±0.37\(^b,e\) |
| Diet R      | —        | 2.11±0.36\(^b,d,e\) | 2.67±0.50\(^b,d,e\) |

\(^{1}\) Each value represents the mean±SEM of six rats. Means with different superscript letters in a column are significantly different (\(p<0.05\)).

\(^{2}\) Nitrogen sources of diets are described in Table 3.
DISCUSSION

A considerable amount of information has accumulated concerning the nutrition after small bowel resection. Postoperative weight gain of small bowel-resected rats is influenced by the site and extent of intestine resected (11, 12). Distal resection has greater effect on weight gain than proximal resection. The effect was more pronounced as the resected part was larger. However, the report of comparison of dietary nitrogen sources is very few. TOULOUKIAN et al. (13) compared the nutritional effect of a chemically defined diet containing an amino acid mixture as nitrogen source with that of a laboratory rat chow. Nutritional effect of an elemental diet containing casein hydrolysate supplemented with amino acids was also compared with that of a laboratory chow (14). The results obtained from these rat experiments demonstrated that a chemically defined diet and an elemental diet could support nutrition in the adaptive phase of the short gut syndrome, but not so well as the laboratory chow. However, the diets compared in these investigations were quite different in nitrogen levels and in the type of components other than nitrogen source. In the present study all the experi-

Fig. 1. Dry weight of intestinal segments of rats fed experimental diets for one week (left panel) and for three weeks (right panel). The bars indicate mean values of six rats and vertical lines indicate SEM.

hyperplasia occurred in proximal remnants after distal resection. The degree of hyperplasia was not influenced by the type of dietary nitrogen source.
mental diets used were the same in the above dietary factors influencing on nutritional comparison of them.

McCARTHY and KIM (15) found in the proximally resected rats that significant elevation in fecal excretion occurred in the second postoperative week, falling towards normal by the fourth week. NYGAARD (11) reported with a laboratory chow containing 23% of crude protein and 2.6% of fat that fecal nitrogen excretion did not change by 25% distal resection, but increased progressively as the extent of small bowel resected was larger. Similar effect of the small bowel resection was observed on fecal fat excretion. In our experiment, fecal weight in the first postoperative week could not be determined because of the occurrence of diarrhea in this period. Any fecal nitrogen and fat excreted was not also determined. No detailed information was, therefore, obtained on fecal excretion of ingested nitrogen and fat in the resected rats.

All the rats showed variable diarrhea for several days after distal resection; the diarrhea subsided within one week. Disappearance of diarrhea seemed to be in accord with development of hyperplasia in intestinal remnant. Regarding hyperplasia, it is known that the intestinal remnant after resection undergoes mucosal hyperplasia, particularly in the ileal remnant after proximal resection (12, 15, 16). Past experiments did not make it clear when adaptive hyperplasia occurred in the intestinal remnants since the measurements of intestinal remnants were made after a considerably longer time following resection. The present study revealed that a marked hyperplasia occurred in the proximal remnant within one week after distal resection (Fig. 1). Both the duration of diarrhea and the extent of intestinal hyperplasia were unchanged with the type of dietary nitrogen source; casein, amino acid mixture or a mixture of casein and amino acids (Fig. 1). After intestinal hyperplasia occurred, rats were found to tolerate the experimental diets well, and an optimal diet for normal rats was found to be suitable for the resected rats.

A chemically defined diet and an elemental diet help to overcome some of the limitations associated with the use of customary foodstuffs in various gastrointestinal disorders. One of the interesting characteristics of the diets is that the diets are composed of simple or elemental form of nutrients which minimize the digestive process. The other is that the diets contain no indigestible bulk of fibrous materials which leads to striking diminution in fecal excretion (15, 17). All the diets used in the present study contained no cellulose component. In the preliminary experiment, these diets were found to reduce the fecal weight below one-tenth as compared with a commercial laboratory chow containing 6% of crude fiber. Therefore, even a diet which contains intact casein instead of amino acids as nitrogen source is a low residue diet, if indigestive and nonabsorptive components such as cellulose are not contained. A moderate elevation of fecal excretion in the resected rats, as shown in Table 4, indicates a reduction of the ability of digestion and absorption as compared with the unresected rats. The extent of fecal weight
both in the unresected groups and in the resected groups was unaltered with the type of dietary nitrogen source.

A use of amino acid mixtures requiring no digestion prior to absorption as the sole source of nitrogen seems to be advantageous only when digestive ability in intestinal remnant is evaluated. The classical view that dietary proteins were completely hydrolyzed to amino acids in the lumen prior to absorption had been believed. However, the present predominant view is that there appears to be at least two modes of absorption of protein digestion products: absorption of free amino acids and intestinal mucosal uptake of oligopeptides with intracellular hydrolysis (18, 19). In the present experiment, an amino acid-casein mixture in which 25% of amino acids was replaced by isonitrogenous casein was tolerated by the 65% small bowel-resected rats as well as by normal rats.

From the results, a well-balanced amino acid-casein mixture is expected to be applied to dietary nitrogen source for various gastrointestinal disorders.

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