EFFECT OF CASEIN AND A CORRESPONDING AMINO ACID MIXTURE ON RAT REPLETION AFTER PROTEIN DEPLETION

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The nutritional effect of a diet containing amino acid mixture simulating casein was compared with that of casein diet using the rat repletion method. The repletion of rats fed amino acid diet ad libitum was lower than that with a casein diet whether rats were depleted by the 7-day fasting or by the 20-day feeding of nonprotein diet. The use of space-pair-feeding in the repletion tests resulted in approximately similar repletion responses to both the diets. These results confirm that the inferior repletion response to amino acid diet under ad libitum feeding conditions may be due to the lower food intake when compared to the response to the casein diet.

Recent progress in amino acid production permits the practical use of new purified amino acid mixtures for a diet of undernourished patients suffering simultaneously from impaired digestion and absorption. The chemically defined amino acid diets have also been shown to be very useful for the nutritional therapy of inflammatory bowel diseases and for nutritional support in pre-and post-abdominal surgery (1). On the other hand, in earlier studies in our laboratory (2) the growth rate and food intake of normal rats ad libitum fed amino acid diets simulating casein were found to be lower than those of rats fed casein diet at 3.2% of dietary nitrogen level. The inferior growth rate of rats fed amino acid diet was supposed to be due to the depressed food intake by the space-pair-feeding experiments. However, it is not clear whether these observations are also seen under the conditions of protein deficit which are often observed in convalescence from illness or injury, or in postoperative period. The demands for essential amino acid adequacy are very great under these conditions and are known to be fairly well simulated by the conditions of the repletion method. Therefore, it is considered that the nutritional effects of protein or amino acid mixture under the
conditions of convalescence from illness or injury can be evaluated by the repletion method.

The rat repletion method of evaluating the nutritive value of protein has been well established by CANNON and his co-workers. The qualitative and quantitative requirements of amino acids of rats in protein-depleted state were studied (3-6). However, very little study has been made on the comparison of the nutritional effects of dietary protein and its corresponding amino acid mixture by the repletion method.

The present study was conducted using the rat repletion method in order to acquire informations on the nutritional comparison of a protein with its corresponding amino acid mixture in protein-depleted state such as convalescence from illness or injury.

EXPERIMENTAL

Experiment 1 (repleted with ad libitum feeding). Male adult rats of the Wistar strain were raised on a stock diet (CLEA Japan Inc., Tokyo, Japan) to weigh 280 to 350 g prior to depletion. Rats were housed in individual wire cages in a room maintained at approximately 24°C and they were divided into two groups. To study protein depletion one group was fed a nonprotein diet for 20 days and the other group was fasted for 7 days. At the termination of the depletion period each group was divided into two subgroups of eight rats each having a similar average body weight and weight loss. One subgroup was then fed an amino acid diet, and the other subgroup a casein diet ad libitum for 7 days.

The compositions of the amino acid and casein diets (N, 3.2%) used in the experiment were the same as those of diets B and C previously used (2). The amino acid mixture was patterned after the amino acid composition of casein(2). The nonprotein diet was prepared by replacing casein in diet C with sucrose. Food intake and body weight of the rats were recorded daily until the end of the repletion period.

Experiment 2 (replete with space-pair-feeding). Male adult rats of the Wistar strain weighing 290 to 340 g were used in the experiments. Rats were fasted for 8 days, so as to have the same weight loss with group 1. After the depletion period they were divided into 2 groups of 8 rats each having a similar average body weight and weight loss. Rats were space-pair-fed twice daily, 9:00 AM to 10:00 AM and 5:00 PM to 6:00 PM, for 8 days according to a method described elsewhere(2). Namely, one group of rats was fed amino acid diet ad libitum during each 1 hr feeding period. Each rat of the other group was pair-fed a casein diet while a corresponding rat received an amino acid diet each time. Diets were kneaded into dumplings with an equal weight of distilled water for space-pair-feeding. Weight gain and food intake of the rats were recorded daily. Food intake was calculated on dry basis.
Statistical analysis. Data were treated statistically using student’s t test (7).

RESULTS

Experiment 1 was designed to compare the repletion responses of protein-depleted rats to an amino acid diet with those to casein diet. Depletion of protein was performed by fasting or feeding of nonprotein diet. The results are summa-

Fig. 1. Repletion responses of protein-depleted rats to ad libitum feeding of amino acid (○) and casein (●) diets. Depletion was performed by fasting for 7 days (-----) or by feeding of nonprotein diet for 20 days (——). Each point represents the average of eight rats (the mean ± SEM on days 3 and 7).

Table 1. Weight gain and food intake of protein-depleted rats fed casein and amino acid diets ad libitum.

<table>
<thead>
<tr>
<th>Depletion</th>
<th>Diet for repletion</th>
<th>Weight gain (g/7 days)</th>
<th>Food intake (g/7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (7 days)</td>
<td>Casein</td>
<td>94.6±4.1a</td>
<td>180.2±3.3</td>
</tr>
<tr>
<td></td>
<td>Amino acid</td>
<td>66.8±4.3b</td>
<td>145.1±5.6b</td>
</tr>
<tr>
<td>Feeding of non-protein diet (20 days)</td>
<td>Casein</td>
<td>93.4±5.3</td>
<td>165.5±5.2</td>
</tr>
<tr>
<td></td>
<td>Amino acid</td>
<td>63.0±5.7b</td>
<td>137.5±6.1b</td>
</tr>
</tbody>
</table>

a Mean ± SEM of eight rats.
b Significantly different from the appropriate casein values at the p < 0.01 level.
rized in Fig. 1 and Table 1. Rats lost 29% of initial body weight during the 7-day fasting period and the daily body weight loss was 12.3 g. On the other hand, 22% of initial body weight, 3.2 g per day, was lost during the 20-day depletion period by feeding with nonprotein diet.

When rats were depleted by fasting, weight gains during the 7-day repletion period were 66.8 g for the amino acid group and 94.6 g for the casein group. This lower repletion response to amino acid diet seems to be due to a lower food intake, 145.1 g per 7 days for the amino acid group vs 180.2 g per 7 days for the casein group. Also, in the repletion tests with rats protein-depleted by feeding of nonprotein diet, rats of the amino acid group gained more slowly and ate a less amount of the diet, 63.0 and 93.4 g gain per 7 days for amino acid and casein group, and 137.5 and 165.5 g intake per 7 days, respectively.

The two depletion methods, fasting and feeding of nonprotein diet, did not influence the subsequent repletion responses. Furthermore, the depletion by fasting markedly shortened the period of depletion suitable for subsequent repletion tests. Fasting prior to the repletion period seemed, therefore, to be more convenient rather than feeding of nonprotein diet as a treatment of protein depletion for the rat repletion method, and the rats in experiment 2 were depleted by fasting.

Fig. 2. Repletion response of protein-depleted rats to space-pair-feeding of amino acid (○) and casein (●) diets. Depletion was performed by fasting for 8 days. Each point represents the average of eight rats.
Experiment 2 with the space-pair-feeding method was designed to clarify whether the inferior repletion response to amino acid diet in experiment 1 was due to a lower food intake. The results of rat repletion tests in experiment 2 are shown in Fig. 2. Rats lost about 30% of initial body weight during the 8-day fasting period. Average daily body weight loss by fasting was 12.5 g. Thereafter, weight gains of protein-depleted rats during the 8-day repletion period (food intake, 97.9 g/8 days) with space-pair-feeding of amino acid and casein diets were closely similar, 36.0 g of the amino acid diet and 40.0 g of the casein diet. This confirms that the inferior repletion response to amino acid diet in experiment 1 was due to a lower food intake. But the repletion rates in experiment 2 were much slower compared with the repletion rate to the amino acid diet in experiment 1.

DISCUSSION

The repletion method, unlike the young rat growth method, allows repeated use of animals and does not require longer experimental period, although rats must be depleted prior to the repletion. In previous investigations using the repletion method, the depletion was mainly performed by feeding a nonprotein diet to subject rats. Frost and Sandy (8) reported that male rats fed a nonprotein diet lost 21 to 28% of initial body weight (140 to 220 g) at the end of the 12-day period. In our experiments with adult rats, a 7-day period of fasting was adequate for 29% loss of initial body weight of 289 to 350 g, whereas a 20-day period was required for depletion by feeding with a nonprotein diet. The much larger body weight loss of fasted rats is due to the increased catabolization of the body protein for helping basal energy expenditures without any exogenous energy supply from food. The results of our repletion experiments (Fig. 1 and Table 1) show that fasting is a more severe and time-sparing treatment for the depletion without causing serious damage which can not be easily repleted any more.

The avidity of depleted rats for essential amino acids was very great, and we expected that the intake of an amino acid diet would approach that of a casein diet under a protein-depleted condition. Contrary to expectations, the results showed a lower intake and an inferior repletion rate in the amino acid group (Fig. 1). However, similar repletion rates were obtained when the rats of two dietary groups were space-pair-fed (Exp. 2), supporting the contention that an inferior repletion response to amino acid diet under ad libitum feeding conditions was due to lower intake of amino acid diet. These findings were the same as those obtained in growth experiments in young rats (2). In our previous report (9) the stomach was found to distend in rats force-fed an amino acid diet, and the stomach distension was suggested to be one of the major factors of low food intake when a certain magnitude was exceeded. Also, in the repletion tests with adult rats it is considered that the stomach distension of rats of amino acid group is one of the
major factors causing depression of food intake.

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