Memory of the Rhythmic Change in Activity of Duodenal Alkaline Phosphatase in Rats

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Summary Studies were made on memory of the rhythmic change in activity of duodenal alkaline phosphatase in rats. During starvation, the peak enzyme activity decreased gradually disappearing in 4 days. The peak activity of duodenal alkaline phosphatase was retained by feeding starch diet for 4 days instead of starvation for 4 days, but not by feeding casein diet for the same period. Starvation for one day after feeding casein diet for 4 days resulted in disappearance of the peak activity. However, the peak activity was still retained after one day of starvation after 4 days on starch diet. Therefore, starch feeding appears to be important in the memory of the rhythmic change in activity of duodenal alkaline phosphatase.

Key Words duodenal alkaline phosphatase, circadian rhythm, anticipation, casein diet, starch diet, memory

The activities of intestinal brush border enzymes show a circadian rhythm and the time of their peak shifts with change in the feeding schedule (1–3). Generally, the amplitude of change in their activities is largest in the segment of the small intestine in which their activities are highest (4, 5). Thus the amplitude of alkaline phosphatase activity is highest in the duodenum and declines markedly toward the ileum. Anticipation of food intake has been suggested to be a trigger for initiation of the rhythms in activities of intestinal brush border enzymes, since the activities begin to increase before the start of feeding (6, 7). When animals are starved for several days (8, 9), the rhythms gradually disappear and on refeeding it takes several days before new rhythms reappear (7).

In the present study, we examined which foodstuff is responsible for memory of the circadian rhythm of intestinal alkaline phosphatase in rats.

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EXPERIMENTAL

Male Sprague-Dawley rats initially weighing about 100–130 g were housed in individual cages and kept in a room lighted automatically from 08:00 to 20:00 hr every day. The animals were given laboratory chow (Oriental Yeast Co., Osaka, Japan) ad libitum or for 6 hr from 13:00 to 19:00 hr for 10 days. Then they were given one of the semisynthetic diets shown in Table 1 for 4 days by the same feeding schedule as before. Water was given ad libitum throughout the experiment. In some experiments, rats with a blind loop including the duodenum (length, about 17 cm) were used (10).

Rats were sacrificed by decapitation and the entire small intestine was removed and washed with cold saline. The first 4 cm length from the pylorus was discarded and the next 14 cm length and the blind loop were used for enzyme assay. The segment was everted and the mucosa was scraped off with a glass slide and homogenized in 10 volumes of cold distilled water with a Teflon homogenizer. The homogenate was diluted appropriately and used for enzyme assay and protein determination. Alkaline phosphatase activity was determined by the method of Forstner et al. (11). Protein was measured by the method of Lowry et al. (12) with bovine serum albumin as a standard. Enzyme activity was expressed as μmoles inorganic phosphate liberated/min/mg protein.

RESULTS

Alkaline phosphatase activity in the duodenum showed a marked circadian rhythm with a peak at 20:00 hr in control rats or rats with a blind loop when the animals were given laboratory chow for 6 hr from 13:00 to 19:00 hr. A similar rhythm with a peak at 00:00 hr was also observed in rats fed ad libitum (Fig. 1).

Figure 2 shows a change in the peak activity of alkaline phosphatase during starvation after supply of laboratory chow ad libitum for 10 days. The peak activity decreased day by day during starvation and disappeared after 4 days.

Next, rats were maintained on laboratory chow for 10 days by a controlled

<table>
<thead>
<tr>
<th>Table 1. Composition of semisynthetic diets (%)</th>
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<tbody>
<tr>
<td>Starch diet</td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Starch</td>
</tr>
<tr>
<td>Salt mixture</td>
</tr>
<tr>
<td>Vitamin mixture</td>
</tr>
<tr>
<td>Cellulose powder</td>
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<tr>
<td>Choline chloride</td>
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*a Purchased from Tanabe Amino Acid Research Foundation (Osaka, Japan).
RHYTHMIC CHANGE OF DUODENAL ALKALINE PHOSPHATASE

Fig. 1. Rhythmic change in activity of duodenal alkaline phosphatase. Meal-fed (from 13:00 to 19:00 hr for 10 days) control rats (○—○). Meal-fed rats with a blind loop (●—●). Control rats fed ad libitum for 10 days (□—□). Values for meal-fed are means ± SE for 5 rats and those for rats fed ad libitum are means ± SE for 3 rats.

Fig. 2. Effect of starvation on the peak activity of duodenal alkaline phosphatase. Enzyme activity was measured at 00:00 hr. Values are means ± SE for 5 rats.

feeding schedule (6 hr from 13:00 to 19:00 hr) and then given a starch diet or casein diet for 4 days by the controlled feeding schedule. On the next day, they were sacrificed at the times shown in Fig. 3. At 12:00 hr, before the start of feeding, the activity of alkaline phosphatase in rats that had been given starch diet for 4 days began to increase, while that in rats given casein diet by the same feeding schedule remained at the basal level.

Both unoperated rats and rats with a blind loop were given laboratory chow for 10 days and then given a semisynthetic diet ad libitum for 3 days. The peak
Fig. 3. Effects of starch diet and casein diet on duodenal alkaline phosphatase activity. Activities were measured at the induced times in rats given starch diet or casein diet from 13:00 to 19:00 hr for 4 days after meal-feeding on laboratory chow for 10 days. Values are means ± SE for 5 rats.

Table 2. Peak activities of duodenal alkaline phosphatase in rats on various diets. Unoperated rats and rats with a blind loop were given each diet *ad libitum* for 4 days after 10 days on chow diet.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Starch diet</th>
<th>Casein diet</th>
<th>Cellulose diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated</td>
<td>1.97 ± 0.33</td>
<td>1.47 ± 0.35</td>
<td>0.58 ± 0.12</td>
</tr>
<tr>
<td>Operated</td>
<td>1.78 ± 0.20</td>
<td>0.65 ± 0.09</td>
<td>0.57 ± 0.05</td>
</tr>
</tbody>
</table>

Values for rats fed starch diet or casein diet are means ± SE for 5 rats and those for rats on cellulose diet are means ± SE for 3 rats.

Table 3. Peak activities of duodenal alkaline phosphatase in rats starved for 24 hr after starch diet or casein diet. Rats were given each diet *ad libitum* for 4 days after 10 days on chow diet and then starved on the following day. Enzyme activity was measured at 00:00 hr on the following day.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Starch diet</th>
<th>Casein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-day feeding</td>
<td>1.97 ± 0.33</td>
<td>1.47 ± 0.35</td>
</tr>
<tr>
<td>1-day starvation after 4-day feeding</td>
<td>1.39 ± 0.24</td>
<td>0.50 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 rats.

activity of duodenal alkaline phosphatase (at 00:00 hr) was determined on day 4 (Table 2). Unoperated rats on starch diet or casein diet showed a marked increase in enzyme activity, but in rats on cellulose diet the activity remained at the basal level. In rats with a blind loop the activity in the blind segment was increased markedly by starch diet but not by casein diet or cellulose diet.

When rats were maintained on starch diet or casein diet for 4 days after 10 days on chow diet and then starved on the following day, the peak activity decreased, as shown in Fig. 2. It did not disappear in rats previously given starch diet, but it disappeared in rats previously given casein diet (Table 3).

**DISCUSSION**

Stevenson et al. (13) reported that a circadian rhythm in the activity of intestinal alkaline phosphatase was observed only in rats on a restricted feeding regime, not in rats fed ad libitum. They also reported that the activity was minimal at the start of feeding, rising slowly during feeding and reaching a high level and peak after feeding. On the other hand, Saito et al. (7) reported that the enzyme activity exhibited a circadian fluctuation in rats fed ad libitum and also in rats fed during a 6-hr period from 09:00 to 15:00 hr. They found that the activity of alkaline phosphatase had already increased one hour before the start of feeding when the rats were waiting for food and concluded that anticipation of food intake acts as a trigger to initiate the enzyme rhythm. The present study confirmed the latter observations.

The peak activity of alkaline phosphatase gradually decreased during starvation and had disappeared after 4 days of starvation, as shown in Fig. 2. Similar results have been reported on the activities of intestinal sucrase, maltase and leucine aminopeptidase (8, 9).

When rats were given starch diet for 4 days after meal-fed with laboratory chow for 10 days, a rhythmic change in activity of alkaline phosphatase was observed and the activity began to increase before the start of feeding. However, in rats given casein diet on the same feeding schedule, the activity still remained at the basal level before the start of feeding and gradually increased during feeding showing a peak about 9 hr after feeding (Fig. 3). These results suggested that the starch diet maintained the rhythmic change in activity of alkaline phosphatase but that the rhythmicity disappeared in 4 days on feeding casein diet and that new dietary induction of the enzyme on feeding casein diet occurred as shown in a previous paper (14).

As shown in Table 2, the peak activity of alkaline phosphatase in the blind segment was maintained at a higher level on feeding starch diet for 4 days after laboratory chow for 10 days, and resulted indicated that only the starch diet could maintain the rhythmicity once it had been memorized. The phenomenon cannot be explained by a difference in food intake between rats on casein diet and on starch diet, because rats consumed more casein diet (19±1 g/day) than starch diet (14±2 g/day). Since food intake on feeding cellulose diet was extremely low (6±1 g/day), it is unclear whether cellulose itself could not maintain the rhythmicity or low food intake which caused disappearance of the rhythmicity.

The rhythmicity was maintained in the blind segment on feeding starch diet, showing that excitation of nervous systems and/or secretion of humoral factors...
accompanied by feeding starch diet might play a role for the maintenance.

Disappearance of the circadian rhythmicity in rats on casein diet is clearly shown in Table 3: one day of starvation after 4 days on casein diet following 10 days on chow diet resulted in disappearance of the peak activity. On the other hand, the peak activity was still maintained after one day of starvation following administration of starch diet under the same feeding schedule as casein diet.

These results strongly suggest that circadian rhythmicity of duodenal alkaline phosphatase is memorized through feeding starch diet for several days, but not through feeding casein diet.

REFERENCES
