ENZYME ACTIVITIES IN THE MUCOUS MEMBRANE OF DIGESTIVE CANAL OF THE RUMINANTS II. INFLUENCE OF STARVATION UPON ENZYME ACTIVITIES

ENZYME ACTIVITIES IN THE MUCOUS MEMBRANE OF DIGESTIVE CANAL OF THE RUMINANTS

II. INFLUENCE OF STARVATION UPON ENZYME ACTIVITIES

By

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Introduction

It is recognized that the membrane of the digestive canal is not only the site of absorption of nutrients but also the site of the metabolism of them. Particulary in the ruminant, the total area of the mucous membrane is remarkably expanded due to the development of the rumen. Therefore, the amount of metabolite which is formed during the course of absorption through the digestive canal is expected to be considerably higher than that of monogastric animal. This fact should be mentioned as one of the nutritional features of the ruminant.

Several investigators reported (1–5) on the process of metabolism of volatile fatty acids and of the amount of the metabolite produced in the course of absorption. In the previous paper (6), 4 kinds of enzyme activities in 8 portions of digestive canals were reported using a calf, cow and sheep which were all under conventional feeding regimen. The relationship between the age of animal and the enzyme activity was briefly discussed in the same paper.

In the present paper, the change of enzyme activities in the digestive canal of sheep were measured during the course of starvation in order to learn the enzyme activity in response to the gradual decrease of substrate supplied from the digestive canal.

Materials and Methods

Animals: Five female sheep weighing about 40 kg were used in the experiment. Two of them were alloted as control animals and the other three were assigned as 10, 20 and 30 days starvation animals. They were fed hay (1.2 kg/day) and commercial concentrate (300 g/day) before the onset of the starvation. They were stalled in an environmental controlled chamber from five days before the onset of

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the starvation to the end of the experiment in order to equalize the environmental effect on them.

The temperature and relative humidity in the chamber called "zootron" were maintained 25°C and 60% respectively throughout the experimental period. Water was supplied *ad libitum*. The body weight, fecal and urinal weights and the amount of water intake were determined every morning.

Homogenate: The sheep were killed by bleeding without anesthesia on 0, 10, 20 and 30 days after the starvation. A suitable amounts of tissues were sampled from rumen, reticulum, omasum, abomasum, duodenum, jejunum, caecum and rectum. The mucous membranes which were separated from the muscle layer of the aforementioned digestive canal were cut into fine pieces and homogenized in the Waring Blendor for 5 minutes. After centrifugation, the supernatants were used as the enzyme preparation.

Determination of enzyme activity: The enzymes measured were succinic and glutamic dehydrogenases, alkaline phosphatase and ketone body formation enzymes. The determination method of each enzyme activity was quite the same as mentioned in the previous report (6). The activity was all expressed as the one per 100 mg N of the used tissue.

Determination of blood constituents: Blood ketone bodies, VFAs and sugars were determined by Thin and Robertson's (7), Conway's (8) and Hagedorn-Hensen's (9) methods respectively.

Results and Discussion

The average rate of body weight decrease (body weight at the time of

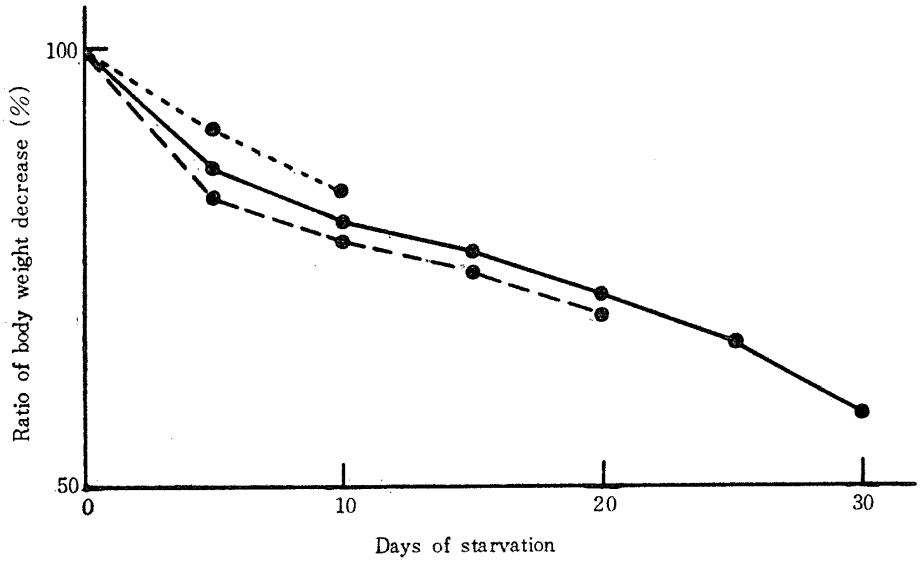

Fig. 1. Ratio of body weight decrease of sheep in the course of 30 days starvation period.
•·····•10 days starved, •– – –• 20 days starved, •——• 30 days starved sheep

measurement/initial body weight × 100) at 0, 10, 20 and 30 days of starvation was 100, 80.6, 71.0 and 58.8 per cent respectively. (Fig. 1).

The fecal weight excreted per day decreased rapidly the first two or three days after the starvation and maintained thereafter an amount less than 100g per day.

The intake of water per day which began to decrease the first one or two days after the starvation was followed by a diverse pattern among the animals during the experimental period. This pattern affected the amount of urine, namely, the sheep which had high water intake showed high urine excretion and vice versa.

The ketone bodies concentration in the blood gradually increased up to 10 days of starvation, but symptoms of ketosis were not observed (Fig. 2).

Though the blood VFAs once disappeared on the third day of the starvation, it again increased to the point where they were measureable with the level of 2 mg/*dl* on 25th day (Fig. 2).

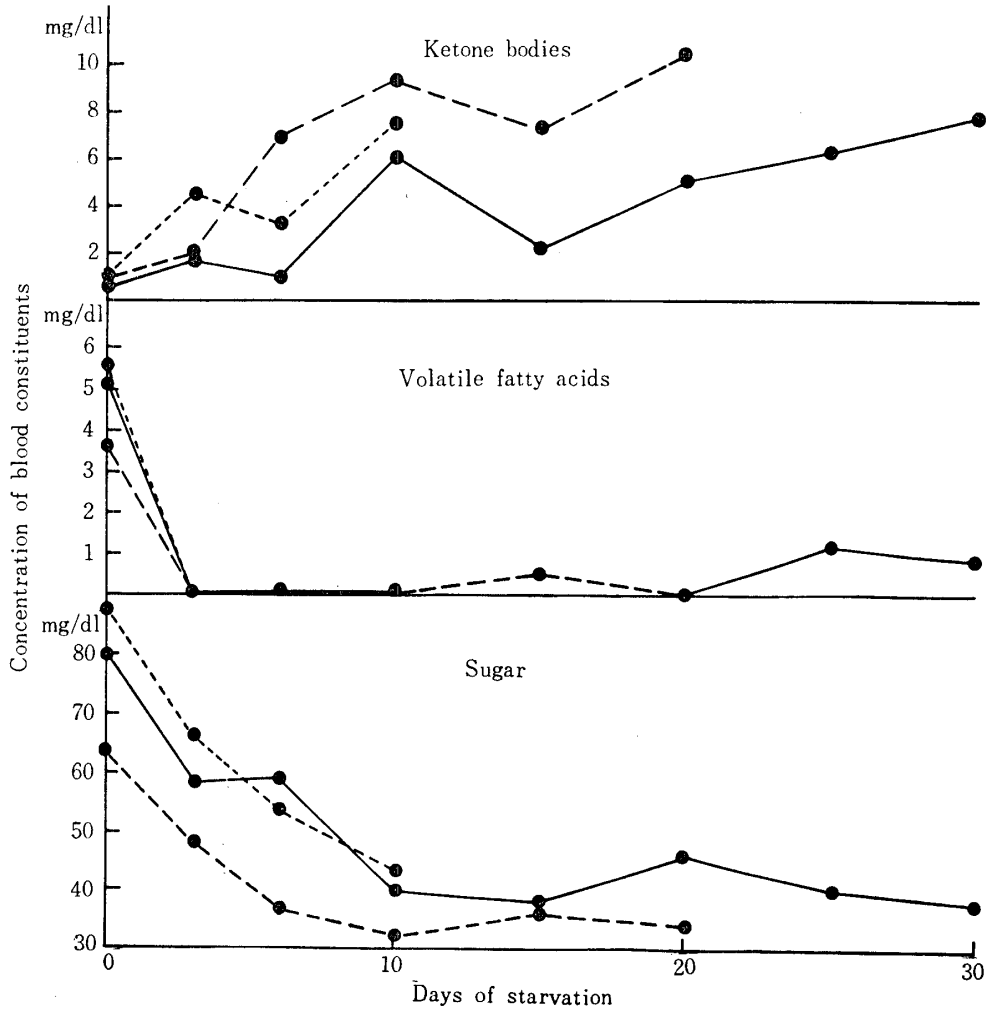

Fig. 2. Concentration changes of ketone bodies, volatile fatty acids and sugar in the blood of sheep during the 30 days starvation period.
•····• 10 days starved, •– –• 20 days starved, •——• 30 days starved sheep.

The blood sugar level which decreased until 10 days after the starvation maintained a level as low as 35–40 mg/*dl* to the end of the experiment.

The contents of each digestive canal gradually decreased as the starvation period lapsed, though remains were found in all digestive canals even on the 30th day of the starvation. The dry matter of the contents in the rumen and in the small intestine (duodenum and jejunum) at the beginning of experiment were 750 g and 40–50 g, respectively. On 30th day of the starvation, they decreased to 10 g and 1.4 g respectively.

Succinic dehydrogenase activities: In the control animals, the succinic dehydrogenase activities were about the same level throughout the digestive canals examined (Fig. 3). In the mucous membranes of rumen and reticulum, the activities so decreased as the starvation proceeded that they could hardly be measured on the 30th day of starvation. These results were also found in the rectum. In the other parts of digestive canal with the exception of the above mentioned three, the activities exhibited a tendency to lower to a limited extent as the starvation went on.

Glutamic dehydrogenase activities: In general, glutamic dehydrogenase activities tended to decrease as the starvation proceeded (Fig. 4). This was particularly true in the rumen and the reticulum. It was often observed, however, that the activities increased once within the period from 10 to 20 days after the starvation in the lower part of digestive canal from omasum. The change of activities of both succinic and glutamic dehydrogenase showed the same

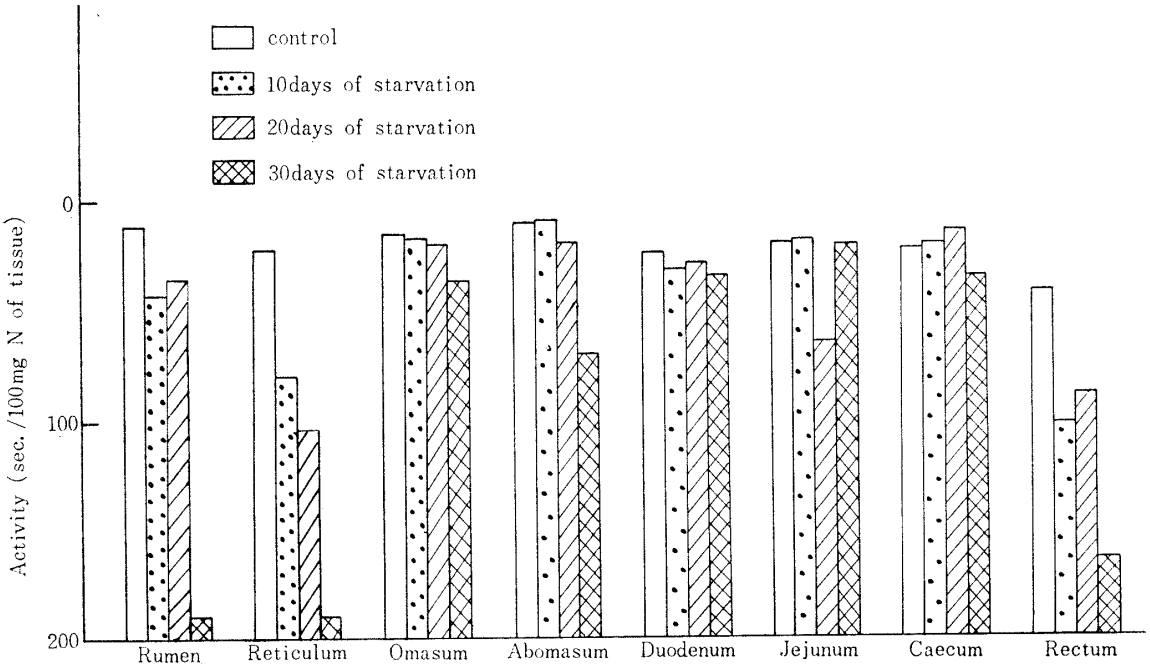

Fig. 3. Succinic dehydrogenase activity in the digestive canal membrane during the course of 30 days starvation of sheep.

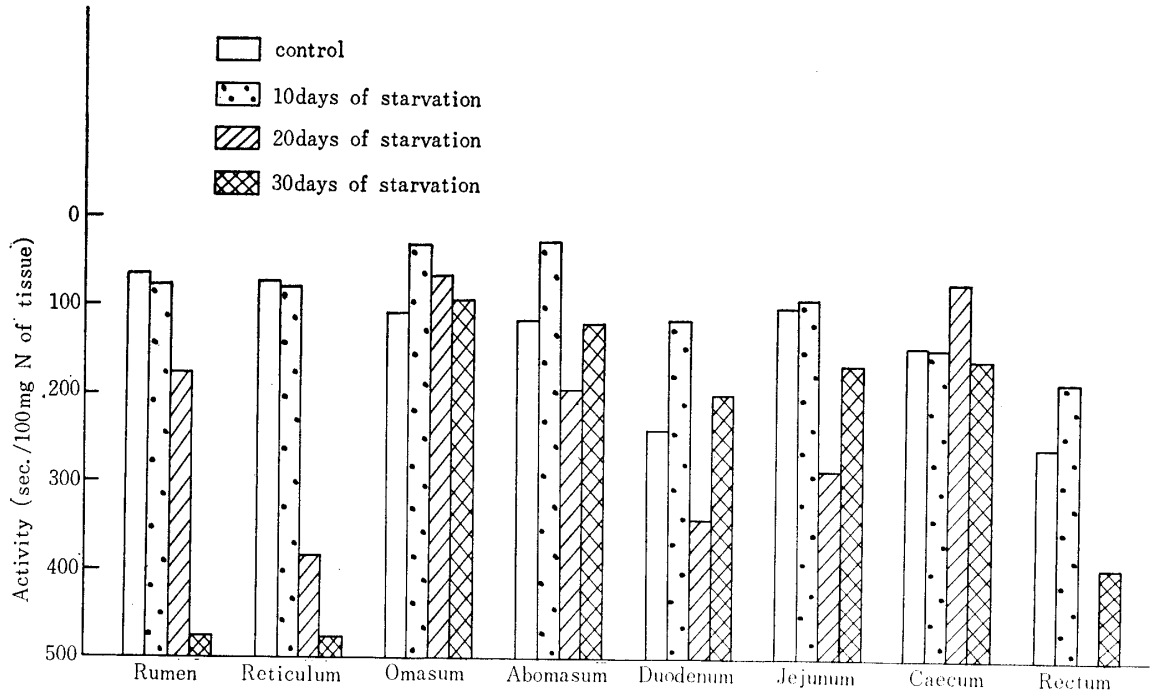

Fig. 4. Glutamic dehydrogenase activity in the digestive canal membrane during the course of 30 days starvation of sheep.

pattern in the examined digestive canal. On the 30th day of starvation, the very low activities in the rumen, reticulum and rectum may be contrasted with the relatively high ones in the omasum, abomasum, duodenum, jejunum and caecum. This phenomena was quite the same as in the case of succinic dehydrogenase. It may be understandable that the activity change in the rumen and reticulum was the same in both dehydrogenase because of the similarities of their tissue organization and the nature of the contents. It was interesting, however, that the activity of the rectum was also the same as the above two, though its reason was hardly explicable.

Alkaline phosphatase activities: It is recognized that the alkaline phosphatase activity is high in the intestinal part in the ruminant as well as in the monogastric animal (6). The activities in the forestomach and rectum, where they were very low even before the experiment, did not change during the course of the starvation. On the contrary, the activities in the duodenum and jejunum increased 3 to 6 times high in comparison with the control period on the 30th day of starvation. This trend was the same in caecum where the activity rose 9 times as high as that of the control (Fig. 5). This seems to suggest that the gradual decrease of dehydrogenase activity is due to the lack of substrate for them as the result of starvation. On the other hand, the reason for the remarkable increase of alkaline phosphatase activity is rather difficult to explain. One of the possible explanations may be that phosphatase is related to the active absorption of nutrient through the intestinal

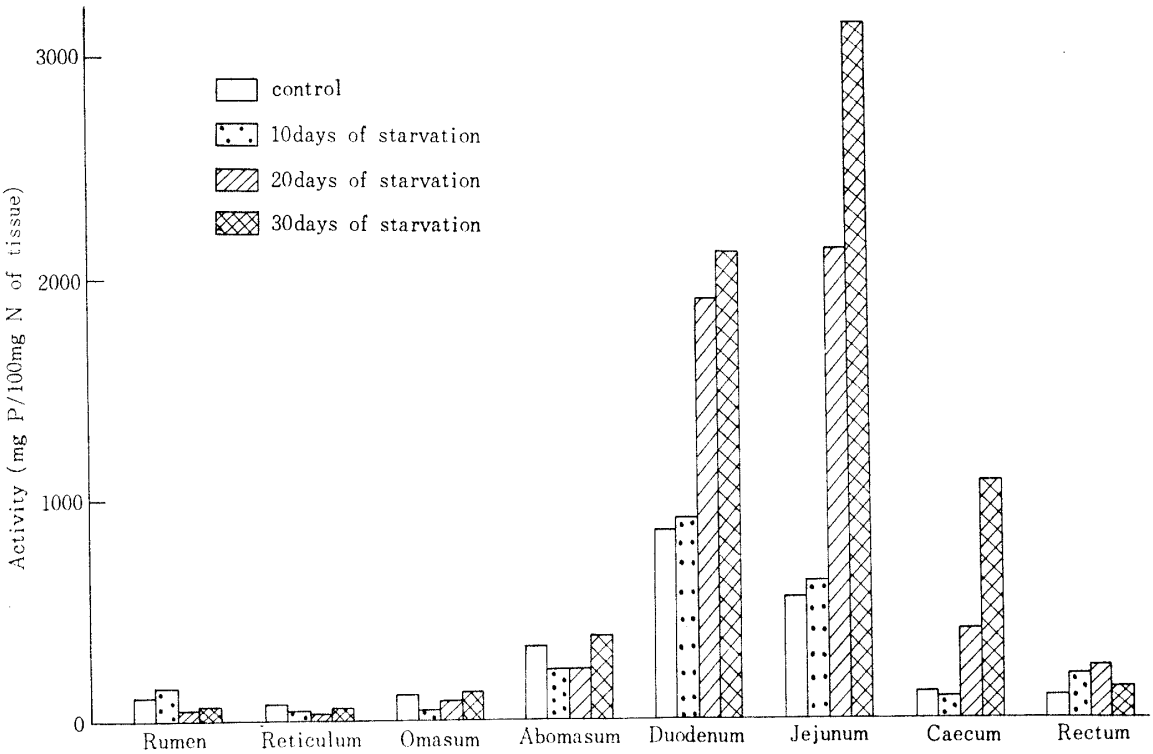

Fig. 5. Alkaline phosphatase activity in the digestive canal membrane during the course of 30 days starvation of sheep.

membrane, therefore, its activity will increase in order to increase the efficiency of nutrient uptake to maintain the homeostatic condition of the body. Itikawa *et al.* (10) reported that inactivation of dehydrogenase was generally observed in a goat starved for 30 days. In their report, however, no investigation was carried out on the digestive canal membrane activity. The intestinal alkaline phosphatase activity, however, increased somewhat within the 30 days starvation length.

Ketone body formation enzymes: As previously reported (6) the ketone body formation enzyme activity was remarkably higher in the rumen, reticulum and omasum than in the other parts of digestive canal. Though the extent of the changes of the activities were not so great, the general pattern of the changes was a mountain like shape in each part of the digestive canal when acetate was used as substrate. In short, the activities once increased and then decreased from the middle to the end of the experiment. The same pattern was also found in the rumen and reticulum, when butyrate was used as substrate. On the other hand, the activities maintained high in the omasum and low in the caecum throughout the experiment (Fig. 6). The change of the activities of the intestine were omitted in this paper due to their low level under normal feeding conditions (6).

Summary

The activity changes of succinic and glutamic dehydrogenases, alkaline phosphatase and ketone body formation enzymes during the course of a 30 days

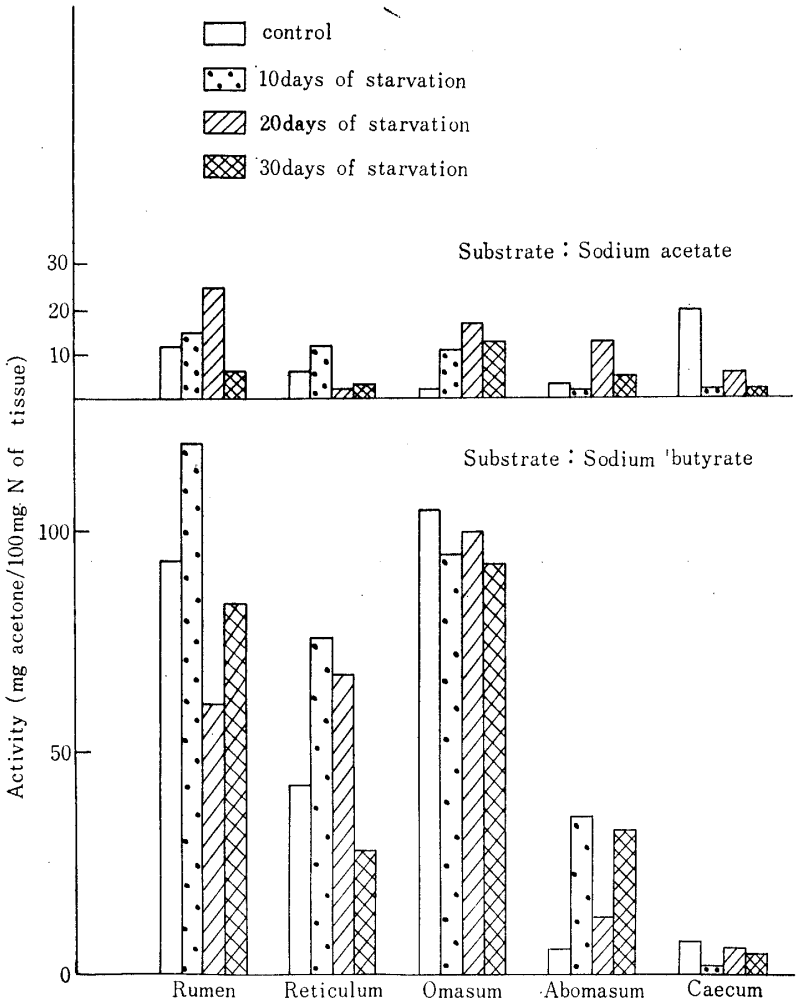

Fig. 6. Ketone formation enzyme activity in the digestive canal membrane during the course of 30 days starvation of sheep.

starvation period were measured on the mucous membrane of rumen, reticulum, omasum, abomasum, duodenum, jejunum, caecum and rectum of sheep.

1) The succinic and glutamic dehydrogenase activities reduced a little toward the end of experiment on all examined digestive cannal membrane except the rumen and reticulum where the activities remarkably decreased.

2) The alkaline phosphatase activities increased 3 to 9 times that of the control value on the duodenum, jejunum and caecum at the end of the starvation. The activities of the other digestive canals except for the above three were maintained as low as control throughout the experiment.

3) The activities of ketone body formation enzymes did not show noticeable changes generally, though each forestomach membrane exhibited its particular pattern to a limited extent.

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