ENZYME ACTIVITIES IN THE MUCOUS MEMBRANE OF DIGESTIVE CANAL OF THE RUMINANTS I. ACTIVITIES OF SUCCINIC DEHYDROGENASE, GLUTAMIC DEHYDROGENASE, ALKALINE PHOSPHATASE, KETONE BODIES FORMATION ENZYMES

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I. ACTIVITIES OF SUCCINIC DEHYDROGENASE, GLUTAMIC DEHYDROGENASE, ALKALINE PHOSPHATASE, KETONE BODIES FORMATION ENZYMES

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(Received 30, June 1964)

Introduction

Recent development of nutritional-physiological studies on the ruminants has demonstrated the absorption of several substances through the rumen epithelium (1) and their metabolism in the rumen wall (2). Pennington (3) and Seto (4) proved the formation of the ketone bodies from acetic and butyric acid by the incubation of rumen epithelium slices with these two substrates.

Annison (5) reported that butyric acid hardly appeared in the portal vein and it, therefore, scarcely reached to the liver. Though several workers found that about two-thirds to three-quarters of energy required by the animal for maintenance can be supplied in the form of volatile fatty acid (6, 7). Ambo *et al.* (8) found that only one third of the necessary energy appeared as volatile fatty acid in the portal vein. According to a series of these experiments, it may be said that the rumen epithelium and other mucous membranes of the digestive tract play important roles on the metabolism of the contents including volatile fatty acids in the digestive canals. Up to now, few reports have been published on the enzyme activities in the epithelium of the digestive canal of the ruminants.

In this paper, several enzyme activities in the rumen epithelium are compared with these of the liver and of the other digestive canals.

Enzyme activities determined were succinic dehydrogenase, glutamic dehydrogenase, alkaline phosphatase and ketone bodies formation enzymes (which means such a group of enzymes concerning on the formation of ketone bodies as acetoacetate decarboxylase and β-hydroxybutyric acid dehydrogenase, etc.).

Materials and Methods

The tissues used for the experiments were obtained from the adult cow and calf (7 days old) killed at the slaughter house, and from sheep killed in our laboratory.

Mucous membrane homogenate: A suitable amount of tissue of each digestive canal was separated into two parts; mucous membrane and muscle. The "mucous membrane" described in this paper contained tunica epitheliaris, germinal layer, tunica propria, lamina and musclaris mucosae. This mucous membrane was cut into about 3 mm square pieces by scissors. Seven g of these pieces were put into the homogenizer in which 14 g of pure water was previously provided, and were homogenized for five minutes. The homogenate obtained was centrifuged for five minutes at 3000 r. p. m. The supernatant was used immediately as the enzyme preparation for the determination of the activities of succinic dehydrogenase, glutamic dehydrogenase and alkaline phosphatase.

Determination of dehydrogenase activity: Succinic and glutamic dehydrogenases activities were determined with the method of Thunberg (8) in which the time required to decolorize from methylene blue to leuco-metylene blue was measured. One ml of 0.005 per cent methylene blue and 1 ml of each substrate (100 μM/ml sodium succinate or sodium glutamate) were added to the side-arm of Thunberg tube, and 1.5 ml of Krebs-Linger phosphate buffer (pH 7.2) and 0.5 ml of enzyme solution were added into the main tube. The side-arm was tightly sealed with Valap cream (vaseline : lanoline : paraffine = 2 : 2 : 1 plus a small quantity of gum). The air inside of the tube was evacuated. This tube was prewarmed at 37°C in a water bath for three min. Then, the reagents of the side-arm were poured into the main tube and the reaction was started.

Determination of alkaline phosphatase activity : Bodansky's method (10) in which the amount of liberated inorganic phosphorus from β-glycerophosphate used as the substrate was determined, was applied to this experiment. Instead of the blood serum described in the original paper, the enzyme solution mentioned above was used.

Determination of ketone formation enzymes activity: One g (wet weight) of sliced mucous membrane was weighed accurately, and then put into the incubation flask (50 ml) in which 9.5 ml of Krebs-Ringer phosphate buffer (pH 7.2) and 0.5 ml of a substrate (100 μM/0.5 ml sodium acetate or 50 μM/0.5 ml sodium butyrate) were contained. The flask, after the saturation of gas phase with oxygen, was shaken in a water bath for three hours at 38°C. At the end of the incubation, the total keton bodies formed were determined by Thin and Robertson's method (11). The data obtained were not deducted from the ones of the control experiments.

Results and Discussion

As the first experiment, the change of enzyme activities of the rumen mucous membrane due to the time lapse after slaughtering was examined.

The mucous membrane of the rumen obtained from the goat immersed in 0.9 per cent NaCl solution was left at room temperature (29°C) for three hours. With regular interval, the changes of enzyme activities were measured but no remarkable changes could be observed (Table 1).

Table 1. Change of enzyme activities of the rumen mucous membrane of goat due to the time lapse after slaughtering.

Enzyme	Time after slaughtering			
	30 min.	1 hr.	2 hr.	3 hr.
Succinic dehydrogenase*	100	140	150	160
Alkaline phosphatase	37	37	28	33
Ketone formation enzymes acetate as substrate	1.03	1.03	—	1.55
butyrate as substrate	6.72	6.72	—	6.21

Units: Succinic dehydrogenase; second/g tissue (wet weight)
Alkaline phosphatase; mg P/g tissue
Ketone formation enzymes; μM acetone formed/g tissue

* Since dehydrogenase activity was expressed as the time required for the decolorization of methylene blue, the high value in the data means low enzyme activity.

Table 2 gives the comparison of the enzyme activities of the mucous membrane of the rumen with those of the liver. Though succinic dehydrogenase activity of the adult cow rumen was slightly lower than that of the liver, the difference seemd to be not so great. On the other hand, succinic dehydrogenase activity of the calf rumen mucous membrane was one sixth of that of the calf

Table 2. Enzyme activities of mucous membrane of the rumen and liver of both adult cow and calf.

Enzyme	No. of Exp.	Adult cow		Calf (7 days old)	
		Rumen	Liver	Rumen	Liver
Succinic dehydrogenase	4	29	18	55	9
Alkaline phosphatase	4	92	14	65	24
Ketone formation enzymes acetate as substrate	4	3.10	5.17	0.17	0.34
butyrate as substrate	4	12.41	8.28	2.07	1.21

Units: Succinic dehydrogenase; second/g tissue (wet weight)
Alkaline phosphatase; mgP/g tissue
Ketone formation enzymes; μM acetone/g tissue

liver, and was one half of the adult cow rumen. These data suggest that seven days old calf rumen mucosa could not exhibit all of its possible functions.

Alkaline phosphatase activity of the adult cow rumen epithelium was higher than that of the bovine liver, their values of both adult cow and calf were not so high as the rumen.

There was a great difference of the activities of ketone bodies formation enzymes between the adult cow and calf. In the adult animal, the amounts of ketone bodies produced were higher when sodium butyrate was used as the substrate than that of sodium acetate in both the rumen and liver. These results were the same in the case of the calf.

In the comparison between the adult cow and calf, the ketone bodies formed were found to be 15 times higher in the adult cow than in the calf. From these data, it is suggested that the activity of the ketone formation enzyme system may be increased as the animal grows.

The dehydrogenase activities in the mucous membranes of each digestive canals on both adult cow and sheep are shown in Table 3.

Table 3. Dehydrogenase activities in the mucous membranes of the digestive canals of the adult cow and sheep.

Enzyme	No. of Exp.	Rumen	Reticulum	Omasum	Abomasum	Duodenum	Jejunum	Caecum	Rectum
Adult cow									
Succinic dehydrogenase	4	29	54	58	30	50	—	—	—
Glutamic dehydrogenase	4	69	124	86	77	65	—	—	—
Sheep									
Succinic dehydrogenase	2	44	83	75	47	95	77	100	169
Glutamic dehydrogenase	2	163	285	580	635	420	483	738	755

Units; sec/g tissue (wet weight)

Succinic dehydrogenase activities were found in all parts of the digestive canals examined. In the case of the adult cow, the activities found in the rumen and abomasum were higher than that of reticulum and omasum. In the case of the sheep, the activities were from 15 to 45 sec/g lower than those of the adult cow. However, the order from high to low of the activities found in the examined organs was the same in both species. The activity of glutamic dehydrogenase found in the rumen was about the same as in the duodenum in the adult cow and was highest in the sheep. Generally, the glutamic dehydrogenase activities were remarkably higher in the adult cow than the sheep. The activities of succinic dehydrogenase were higher than glutamic dehydrogenase in the digestive tract examined. In both dehydrogenase, the rumen membrane had highest activities among the examined digestive canals.

The alkaline phosphatase activities in the digestive canals are shown in Table 4.

Table 4. Alkaline phosphatase activities of mucous membranes of digestive canals of both adult cow and sheep.

	No. of Exp.	Rumen	Reti-culum	Omasum	Abo-masum	Duo-denum	Jejunum	Caecum	Rectum
Adult cow	4	92	54	34	23	118	—	—	—
Sheep	2	27	21	23	60	204	125	24	23

Units; mg P/g tissue (wet weight)

In the adult cow, duodenum showed the highest activities followed by the rumen, reticulum, omasum and abomasum in descending order. In the sheep, the duodenum and jejunum had higher values than the other parts of the digestive tract.

It was recognized that the alkaline phosphatase activities were high in the intestinal parts in the ruminant as well as in the simple stomach animal. It was also noticed that the alkaline phosphatase activity in the rumen of the adult cow was about 3.5 times higher than that of sheep.

The amount of ketone formed from acetate were highest in the rumen. In all other parts, about the same relatively lower values were obtained(Table 5).

Table 5. Ketone formation enzymes activities of mucous membranes of the digestive canal of the sheep.

Substrate	No. of Exp.	Rumen	Reticulum	Omasum	Abomasum	Duodenum	Caecum
Sodium acetate	2	6.7	3.1	3.1	4.1	—	4.6
Sodium butyrate	2	17.1	8.3	15.5	4.1	4.1	3.1

Unit; μM acetone/g tissue (wet weight)

When butyrate was used as the substrate, the activities were obviously high in the rumen and omasum in comparison with other parts of the digestive canal. The omasum membrane showed the same activities as the rumen. These results reconfirmed the results of Pennington (3) and Seto (4).

Summary

The activities of succinic dehydrogenase, glutamic dehydrogenase, alkaline phosphatase and ketone bodies formation enzymes in the digestive canals of both cow and sheep were examined.

1) The activities of succinic dehydrogenase were higher in the liver than in the rumen epithelium in both adult cow and calf. In the comparison of succinic and

glutamic dehydrogenase activities, the former was always higher than the latter in the digestive canal examined.

2) Glutamic dehydrogenase activities were considerably higher in the adult cow than in the sheep.

3) Succinic and glutamic dehydrogenase activities in the rumen epithelium were higher in general than in the other parts of the digestive canals.

4) Alkaline phosphatase activities were higher in the rumen epithelium than in the liver in both the adult cow and sheep. Between the digestive canal examined, duodenum and jejunum showed higher values of alkaline phosphatase activities than that of the other parts.

5) The activities of ketone formation enzymes in the new born calf were considerably lower than the adult cow. In the adult cow, rumen and omasum epithelium showed higher ketone formation activities than the other parts, particulary butyrate was used as the substrate.

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