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By

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Ruminants secrete large volume of well-buffered alkaline saliva, which is the important factor to stabilize the ruminal fermentation and to maintain the rumen constancy. It is said that the flow rate of ruminant saliva increases with the ingestion or rumination of the feed and is changed by the sort of diet (1, 2). It was observed that an elimination of parotid saliva was accompanied by the change of the fermentation and the rapid decrease of pH in the rumen (3). Thus, since the saliva is an important factor to regulate the rumen environment, the change of pH and of the volatile fatty acids (VFAs) concentration in the rumen fluid may be induce the change of the rate of the saliva secretion. On this point, some investigators have hitherto observed the effect of the addition of VFA solution to the rumen on the rate of saliva secretion. Bailey and Balch (2) found that no obvious effect on the rate of secretion of mixed saliva in the cattle when sufficient acetic acid was introduced into the rumen to lower the pH from 6.8 to 5.4 and to increase the total VFAs from 93 to 167mEq/*l*. Boda *et al.* (4) reported that the rate of saliva secretion decreased with time when acetic acid was added to the empty rumen. According to Yarn *et al.* (5), a tendency of increase in the salivary secretion rate was shown during the infusion of sodium salt of VFA to the rumen of the steer. The observation in which flow rate of parotid saliva increases when VFA is added to the rumen has been reported only by Ash and Kay (6). According to them, the introduction of fatty acid solutions into the rumen to make the pH between 3.7 and 4.0 caused a trasient increase in the secretion of parotid saliva.

In the present paper, we describe the results of an experiment in which the rate of parotid saliva secretion, which is a major component of the saliva of ruminant, was investigated during the course of recovery of rumen pH which was made as low as physiological limit (pH 5) by adding of VFA and hydrochloric acid at the start of experiment.

Materials and Methods

Two female sheep were used in this experiment. Both animals had permanent parotid fistulas (3) and ruminal fistulas. Body weights of the sheep were maintained at 35 kg by daily feeding of 400 g of hay, 300 g of the commercial concentrate for dairy use and 50 g of sodium bicarbonate which was sufficient to compensate for the loss of sodium from the parotid saliva through fistula (7).

During the experiment, saliva-collecting masks were attached to the sheep (Plate 1). The experiments were always started at 19th hours after feeding in order to make the environment of the rumen as constant as possible through the experiment. Sixty minutes after the experiment started, one of the following acids (warmed to 40°C), 0.5 M/*l* acetic, propionic, butyric and hydrochloric acids was introduced into the rumen through the rumen fistula to reduce the ruminal pH to 5. As sham treatment, one liter of warmed water was introduced.

The control flow rate was checked for one hour before the each treatment was started. The experiment was finished when ruminal pH recovered to the normal range. The rumen-pH was automatically recorded with an electric polyrecorder (Towa EPR-2T type) by inserting a glass electrode directly into the rumen (Plate 2). The amount of saliva was measured every 30 minutes. The pH of the saliva was determined by a glass electrode pH meter (Towa HM–5A). The bicarbonate concentration in the saliva and total VFAs concentration in the rumen were determined by Conway's micro-diffusion technique (8). The sodium and potassium concentration in the saliva were measured by the flame photometric method (Lange Flame Photometer Model 2A).

Results

Figure 1 shows the changes of the rate of saliva secretion without treatment from the 19th to the 23rd hour after feeding in both sheep. As the rumination occured at 2.5 hours after the beginning of the experiment in sheep B, the rate of secretion increased. It is recognized by many investigators that the rate of parotid saliva secretion increases by rumination (1, 9).

The mean amount of saliva secreted during one hour before the start of treatment was calculated from the data obtained from the whole treatment. It was 28.4 $\pm$14.4 *ml*/30min in sheep A and 10.5$\pm$2.7 *ml*/30 min in sheep B. In Fig. 1, the mean values of total VFAs concentration in the rumen through the experiment in sheep A and sheep B were about 550 mg/*dl* and 350 mg/*dl*, respectively. Also the pH of the rumen fluid was about 7 in both animals.

Water administration to the rumen: In sheep A, the rate of the secretion of saliva before water administration seems too high as compared with the values of the other experiment. However, it might be said that the flow rate decreased with the water infusion. In sheep B, the flow rate once decreased and recovered to

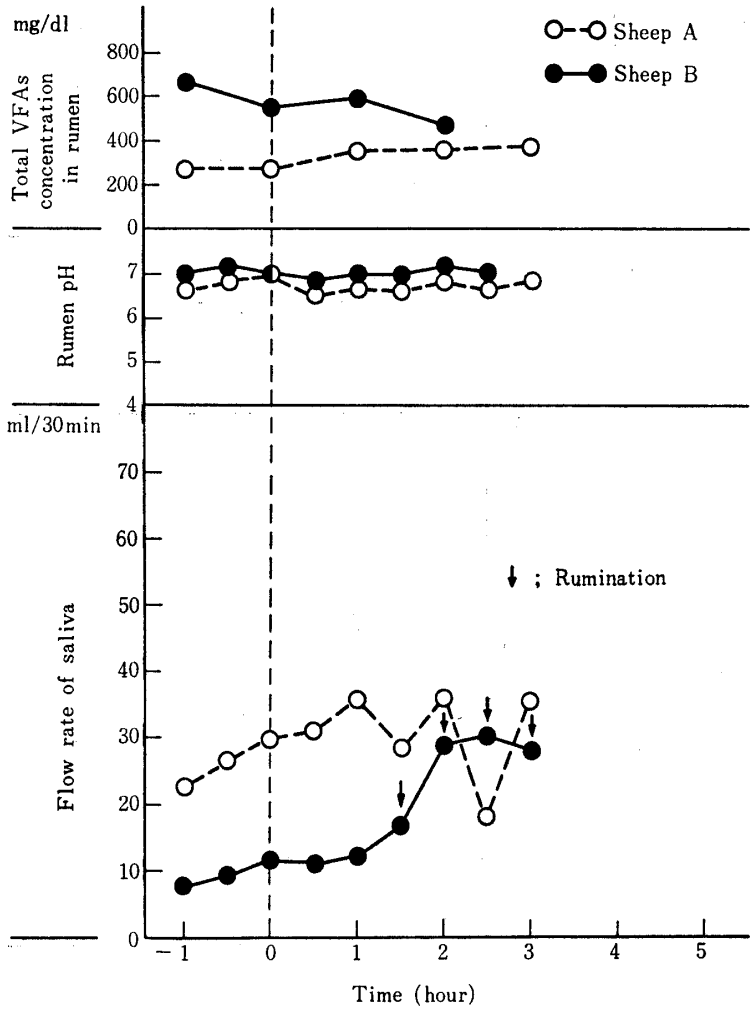

Fig. 1. Changes of the salivary flow and the ruminal condition without treatment.

the normal value at the 2nd hour after infusion. In spite of the addition of one liter of water, the decrease of the total VFAs concentration in the rumen could not be observed in both sheep (Fig. 2).

Acetic acid administration: In spite of the fact that the acetic acid infusion was started at the same time (20th hour after fed), the amount required to lower the pH of rumen fluid to about 5 was 0.25 moles and 0.5 moles in sheep A and B, respectively. As the body weight and the pH of the rumen fluid in both animals before the administration of acid were about same, it was expected that the volume and the fermentative condition of the rumen were generally similar. Therefore, the reason for the difference in required amount of acetic acid may be due to the differences of the buffer action of the rumen fluid and the flow rate of saliva into the rumen. This phenomenon was also found in the case of the administration of butyric acid. The addition of acetic acid to the rumen, where the pH was lowered to 5 and total VFAs was increased from 350 mg/*dl* to 600 mg/*dl*, had no obvious

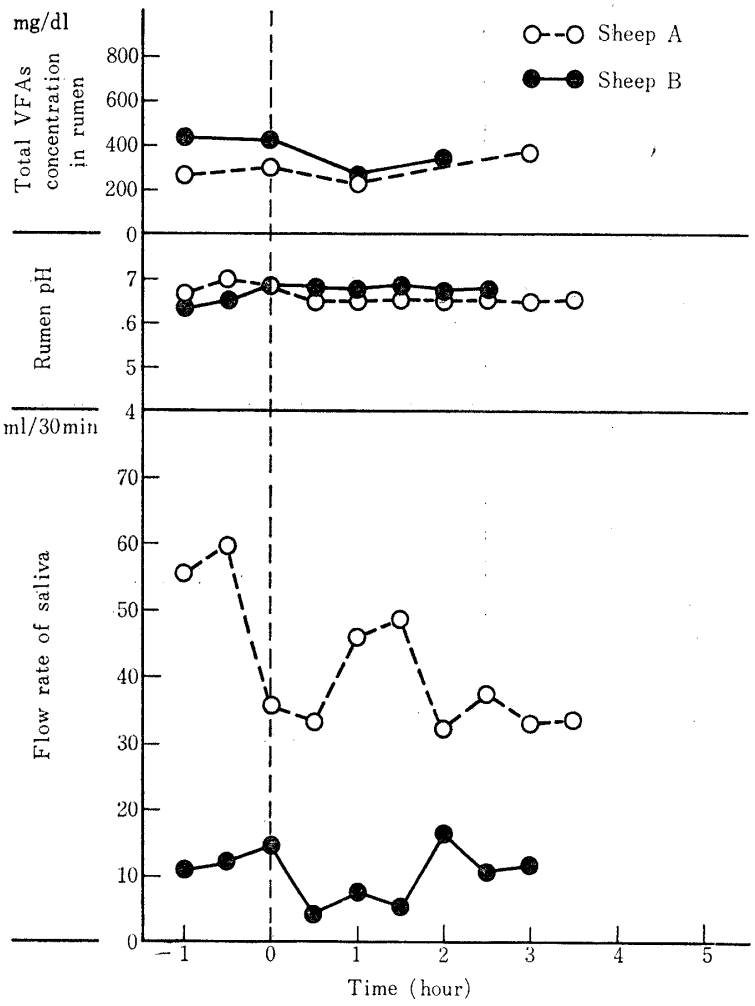

Fig. 2. Changes of the salivary flow and the ruminal condition accompanied with water administration.
The amount of water administered into the rumen is 1 *l* both in sheep A and B. 0 hour indicates the starting time of the treatment.

effect on the rate of secretion at infusion time. At 30 minutes after the infusion, a tendency of the temporary decrease in the flow rate and the inhibition of rumen motility were observed. This flow rate decrease might be due to the inhibition of rumen motility. Shinozaki (10) reported that rumen motility was inhibited by VFA infusion into the rumen. Boda *et al.* (4) observed that the inhibition of rumen motility was accompanied with the decrease of the salivary flow rate. This phenomenon was observed in common during the administration of every VFA. The total VFAs concentration in the rumen recovered to the normal level at one hour after the administration of acetic acid but the recovery of the pH was slower than that of VFAs. The rate of secretion showed a tendency of increase at 1 hour after infusion, when rumen pH was about 5.3. There was a difference in the absolute volume of saliva secreted between animals, however, no difference was observed

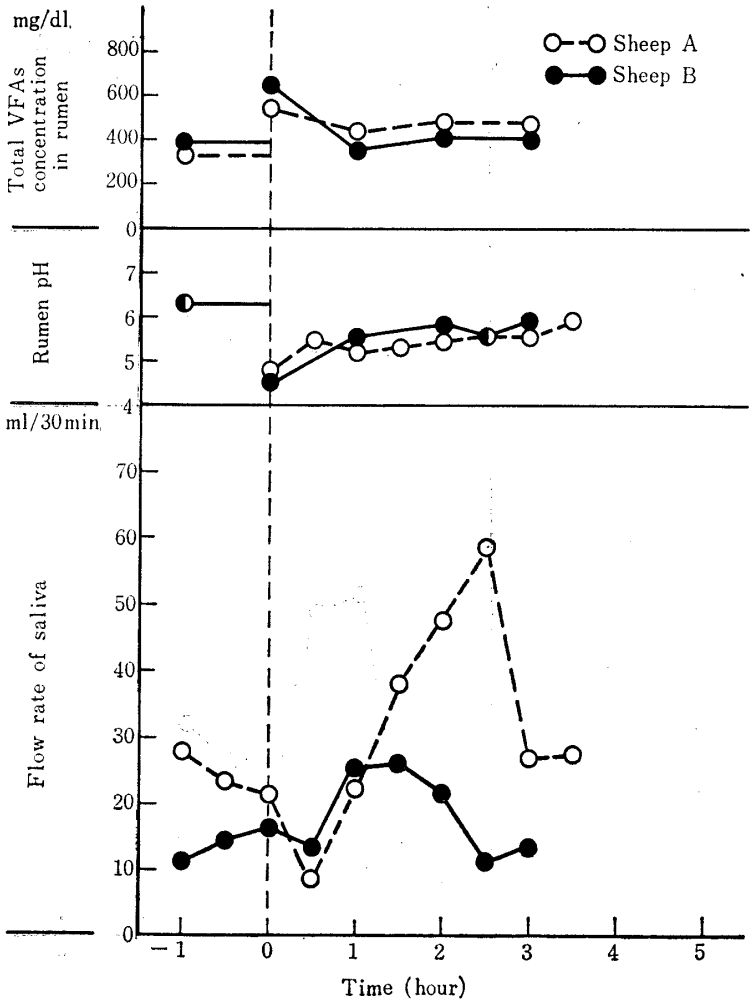

Fig. 3. Changes of the salivary flow and the ruminal condition accompanied with acetic acid administration.
The amount of acetic acid administered into the rumen is 0.25 moles in sheep A and 0.5 moles in sheep B. 0 hour indicates the starting time of the treatment.

in the pattern of the changes with time. The rate of secretion was recovered to the control level at 4th hour after the administration (Fig. 3).

Propionic acid administration: The required amount of propionic acid to lower the pH of rumen fluids to about 5 was 0.38 moles and no difference was seen in the volume of acid required between animals. In sheep A, the rate of salivary secretion doubled at 1.5 hours after infusion. In sheep B, however, only a small increase was shown (Fig. 4).

Butyric acid administration: The amount of butyric acid solution needed to lower the rumen pH about 5 (0.1–0.25 moles) was the least among three VFAs. In sheep A, the rate of secretion began to increase above the value obtained before the treatment at 2 hours after infusion and continued for 2.5 hours, then gradually recovered to the control level. In sheep B, however, the rate of secretion began

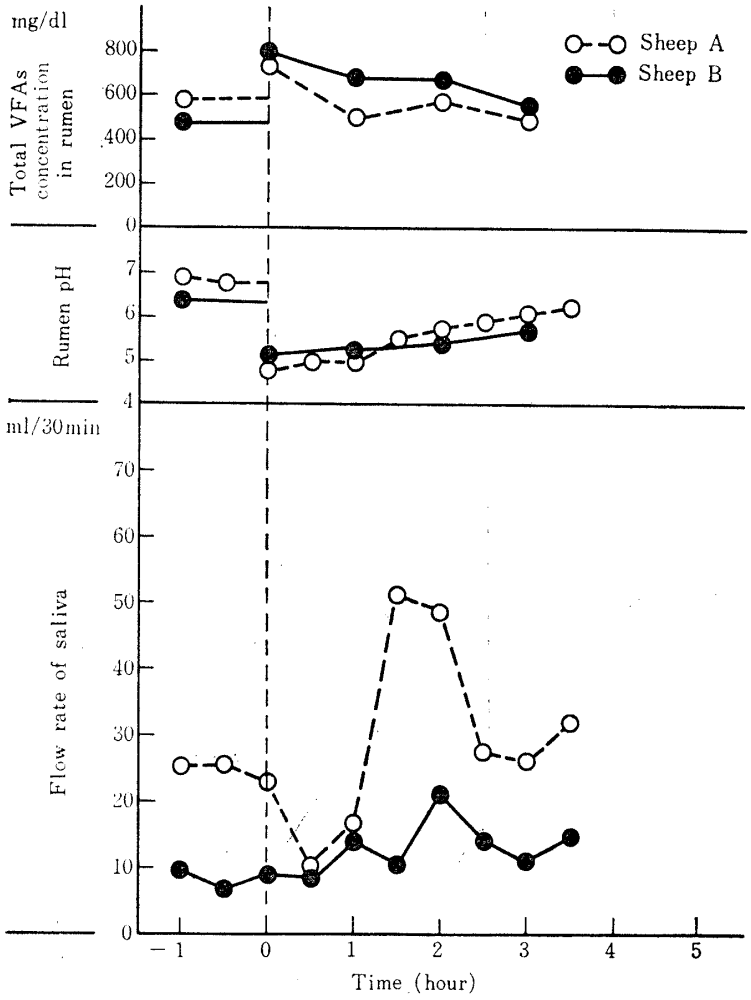

Fig. 4. Changes of the salivary flow and the ruminal condition accompanied with propionic acid administration.
The amount of propionic acid administered into the rumen is 0.38 moles both in sheep A and B. 0 hour indicates the starting time of the treatment.

to increase above the value obtained before the treatment at one hour after infusion though the pH of rumen fluid was too low as expected. The rate of secretion as well as the pH of rumen fluid recovered at 6 hours after infusion (Fig. 5).

Hydrochloric acid administration: Although the volume of hydrochloric acid used to lower the pH of the rumen fluid to about 5 was larger than any VFA used, rather rapid rumen pH recovery was observed at 3 hours after the experiment. The fluctuation of pH was more intense as compared with that of other acids, especially for the first 30 minutes after infusion. The total VFAs concentration in the rumen decreased and then maintained with the level of 200 mg/*dl* thereafter. The rate of secretion hardly changed (Fig. 6).

The relationship between the rate of salivation and salivary composition: It is recognized by many investigators that one of main components in the saliva of

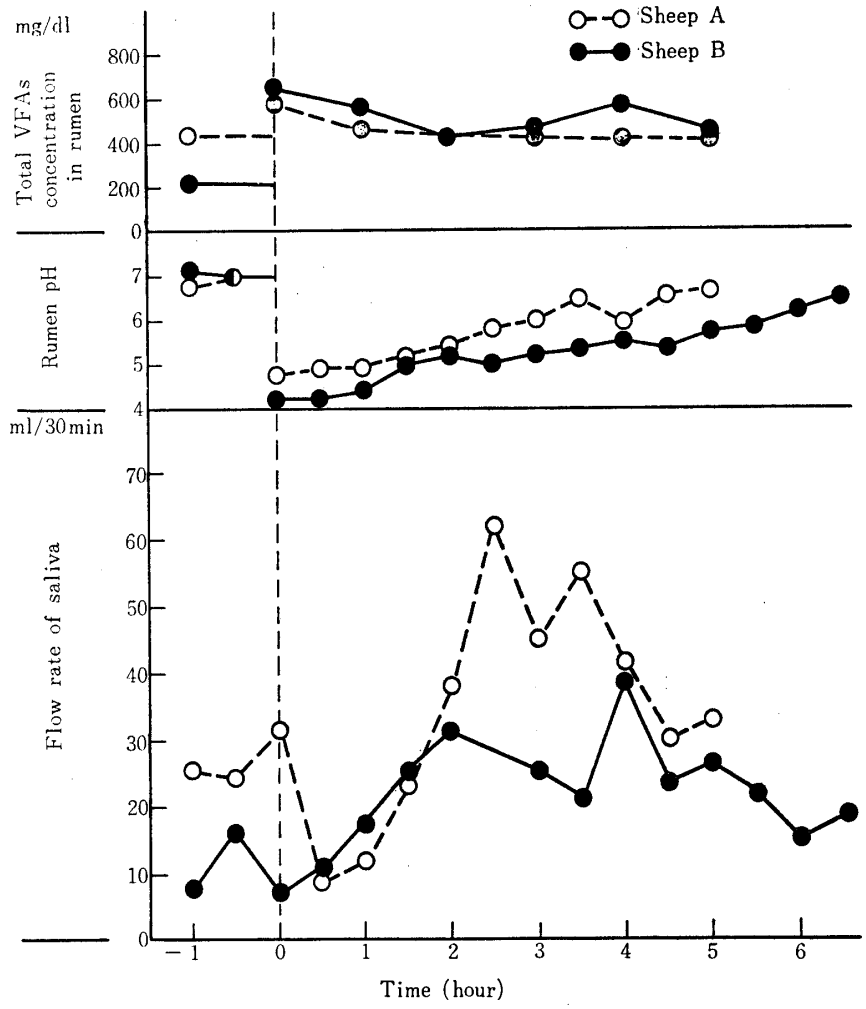

Fig. 5. Changes of the salivary flow and the ruminal condition accompanied with butyric acid administration.
The amount of butyric acid administered into the rumen is 0.1 mole in sheep A and 0.25 moles in sheep B. 0 hour indicates the starting time of the treatment.

ruminants is sodium bicarbonate. The two important factors affecting the composition of inorganic ions in the parotid saliva are the sodium deficiency and the rate of secretion (9). In Fig. 7, a relationship between the sodium and potassium concentrations in the saliva and the rate of secretion was indicated. It was clear that no relation was observed between flow rate and sodium concentration, though a slight difference was observed between the animals. The same results were obtained in potassium. The mean values of sodium concentration in sheep A and B were 169.5±3.9 and 184.2±10.9 mEq/*l*, respectively. For potassium, they were 4.0±0.3 and 6.8±2.5 mEq/*l* in sheep A and B, respectively. As shown in Fig. 8, the bicarbonate concentration in the saliva increased from 100 mEq/*l* to 150 mEq/*l* as the increase of secretion rate, while the salivary pH remained constant at about 9.1, in spite of the increase of the bicarbonate concentration.

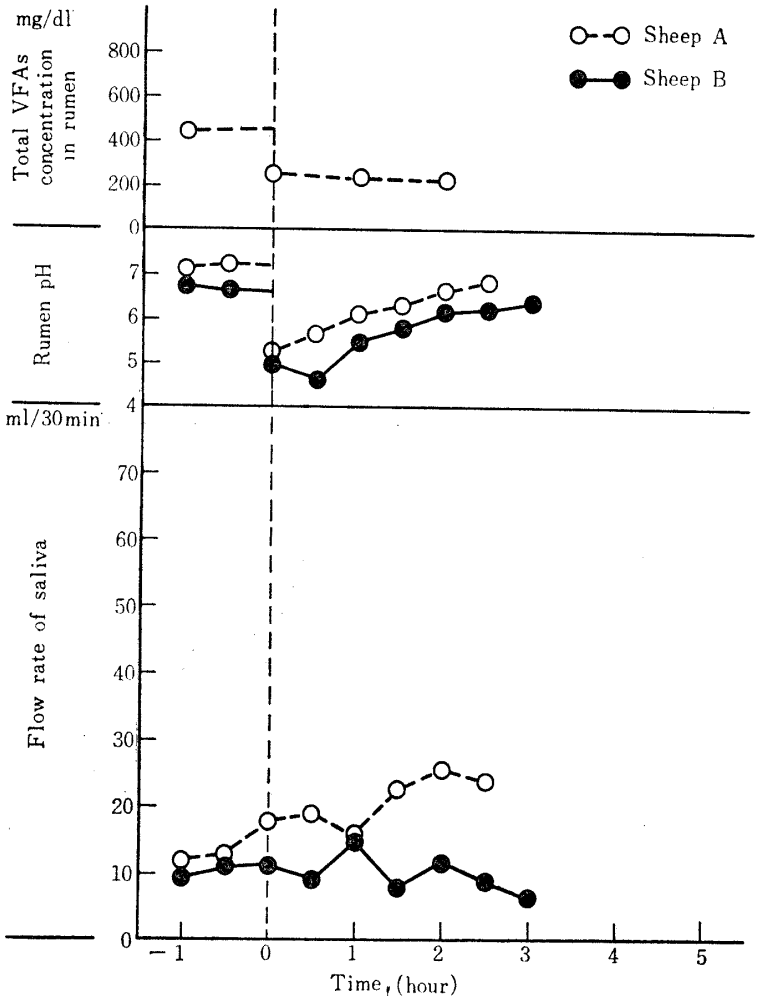

Fig. 6. Changes of the salivary flow and the ruminal condition accompanied with hydrochloric acid administration.
The amount of hydrochloric acid administered into the rumen is 0.4 moles in sheep A and 0.3 moles in sheep B. 0 hour indicates the starting time of the treatment.

Discussion

Some investigators have reported experiments in which the change of flow rate of the saliva was studied during VFA administration to the rumen (2,4–6). However, infused VFA into the rumen in the above experiments were acetic acid or mixed VFAs. Propionic or butyric acid were not used. In addition, there have been few investigations which treated the relation between the pH of rumen fluid and the secretion rate of parotid saliva with time progress. In this paper, the changes of the rate of secretion accompanied by the recovery of the rumen pH which was lowered to 5 by VFA and hydrochloric acid administrations were observed. There was a difference in the rate of salivation between experimental sheep but the pattern of changes accompanied with the administration was similar for both animals.

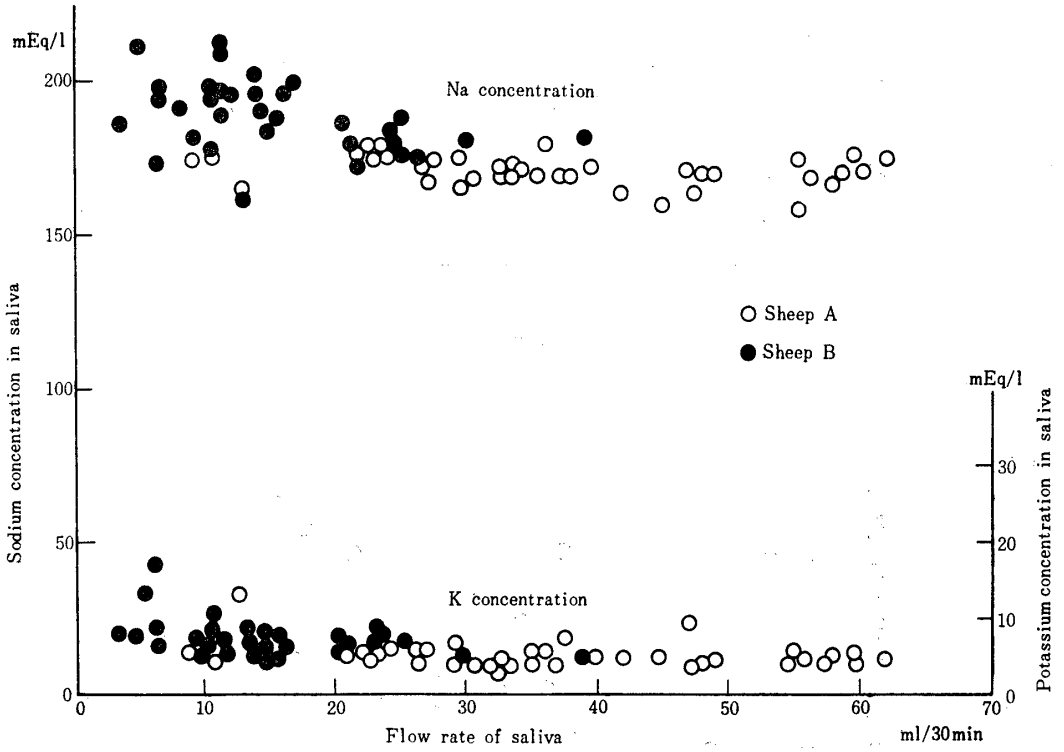

Fig. 7. The relationship between the salivary flow rate and Na and K concentration in saliva.

It was reported that the parotid secretion was depressed in sheep and cattle when the rumen was inflated or distended with water (11, 12). The same result as mentioned above was observed in this paper. This might prehaps be due to the rising of the rumen pressure and the extension of the rumen wall. From the 30th to the 60th minute after each VFA administration, the rate of saliva secretion decreased. This might perhaps be due to the extention of the rumen. Boda *et al.* (4) observed the same result on this point. The increase of the rate of secretion was observed from two to two and a half hours after each VFA administration. In the case of butyric acid, the rate of salivation was the highest among three VFAs, and the least in propionic acid. These results seem to indicate a characteristic of each acid and especially of propionic acid which seems to have a particular metabolism as compared with other VFAs. In every experiment of VFA infusion, the total VFAs concentration in the rumen almost recovered to the normal level in the first hour after administration. It was assumed that acetic acid, propionic acid and butyric acid administered were almost absorbed through the rumen epithelium during the first hour of the experiment (13). Kay and Ash (6) reported that absorptive stimulation of VFA accelerated the secretion of saliva. However, these reports could not explain the results in the present experiment because the increase of saliva secretion was observed around the 2nd hour after infusion. The rate of secretion did not change when the rumen pH was lowered to about 5 by hydrochloric acid. In the other experi-

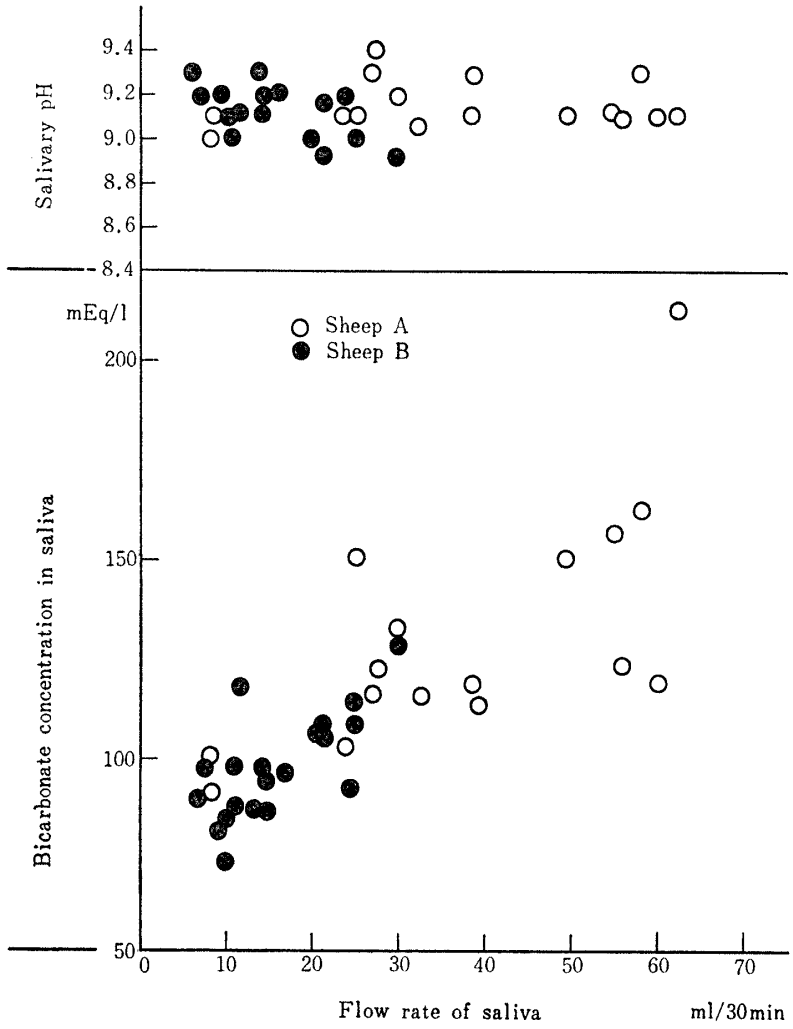

Fig. 8. The relationship between salivary pH and the bicarbonate concentration accompanied with changes of salivary flow.

ment, however, it was observed that when the rumen pH was remarkably lowered by hydrochloric acid infusion (from 7.2 to 0.9), the flow rate rapidly decreased from 59 *ml*/20 min to 9 *ml*/20 min (14).

As to the mechanisms in the increase of the salivary flow rate a few possibilities may be considered.

1) Hill (15–17) postulated that the free nerve endings which were found in several areas of the reticulo-rumen and of the oesophagus by the use of methylene blue staining are probably the receptors for the reticulo-ruminal reflexes. The fact that VFAs and not hydrochloric acid increased the salivary flow rate suggest that the stimulation with VFAs or their metabolite to the chemoreceptors located in the reticulo-ruminal epithelium induced the change in flow rate.

2) On the other hand, the fact that the onset of the flow rate increase was observed 1 or 2 hours after the administration of VFAs suggests that the absrobed VFA or their metabolite in the blood directly regulated the salivary flow. Sasaki

and Umezu (18) reported that the salivary gland slices utilize VFA both in oxygen uptake and in amount of consumption to a considerable extent.

3) It will be kept in mind that the level of pH around which the increase of flow rate was noticed was 5.3. Further investigation will be required to clarify the mechanism on the salivary flow rate increase.

The change of inorganic ion concentration accompanied with the flow rate change was investigated in mixed, parotid and submaxillary saliva on several species of animals. Bailey and Balch in cattle (1), Coat and Wright in sheep (21), Dawes in man (19), Yoshimura *et al.* in dog (20).

It is said that increased saliva flow usually accompanied by the increase of sodium, bicarbonate and sometimes of chloride, and the decrease of potassium and phosphate. However, in the present experiment, sodium and potassium concentrations did not change with the increase of salivary flow, though the values themselves differed between animals. Increased salivary flow was obviously accompanied by the increase in bicarbonate concentration but salivary pH remained constant with the value of 9.1. This result differs from the report on dogs (20) in which parallel increase of total bicarbonate concentration and pH in saliva with the rate of salivary flow was investigated. This might be due to the fact that the activity of carbonic anhydrase which plays an important role in the excretion of bicarbonate (20) differs between sheep and non-ruminants and that the concentration of phosphate, which is contained in the parotid saliva of ruminant, decreases with the increase of salivary flow (2).

Summary

1) The salivary flow from the unilateral parotid gland of sheep was measured during the course of recovery of pH which was lowered at the start of experiment to 5 by the administration of an acid such as acetic, propionic, butyric or hydrochloric.

2) The rate of parotid secretion indicated a tendency to decrease for 30 minutes after administration of each VFA, followed by an increase during the next 1 or 2 hours. Ruminal condition and saliva secretion rate completely recovered to the normal level at 4 hours after administration except in the case of butyric acid. The flow rate did somewhat decrease by water but did not change on the administration of hydrochloric acid.

3) The concentrations of sodium and potassium in the saliva showed hardly any change but that of bicarbonate changed accompanying the increase of the secretion rate. On the other hand, salivary pH stayed at a level of 9.1 regardless of the change in flow rate.

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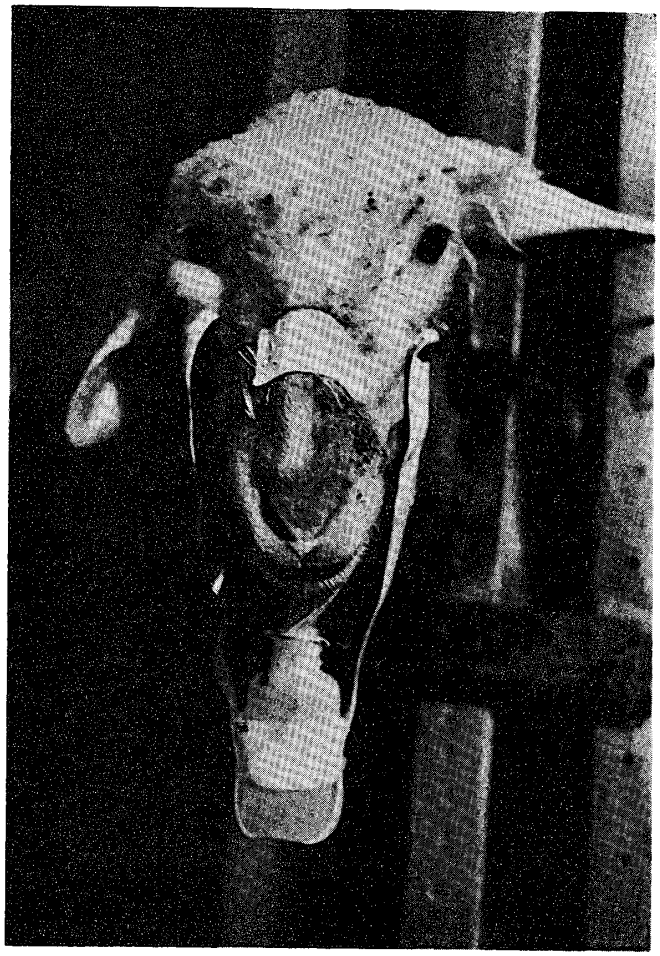

Plate 1. Experimental animal fixed with saliva-collecting mask and bottle.

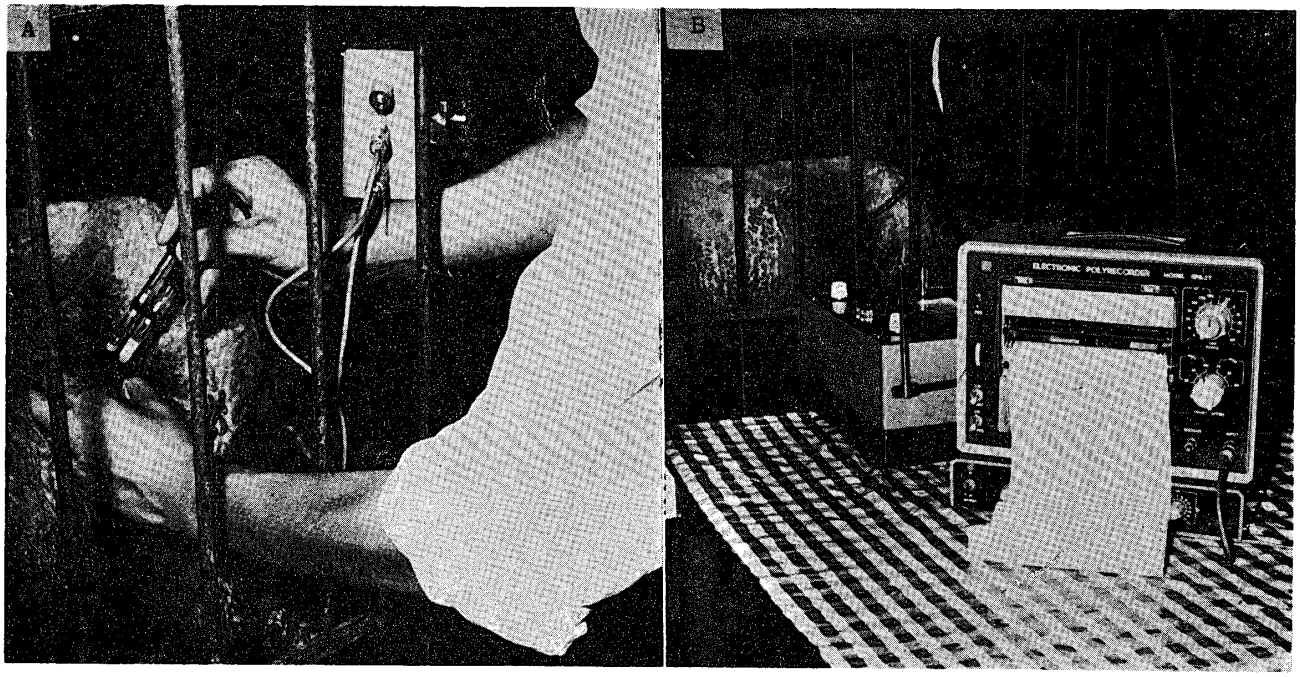

Plate 2. A. Insertion of glass electrode into rumen.
B. Automatical recording of rumen-pH with electric polyrecorder.