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The Effects of the Administration of Volatile Fatty Acid to the Empty Rumen on the Parotid Saliva Secretion of Sheep

Yoshiaki OBARA*, Susumu WATANABE, Yasuyuki SASAKI and Tsuneyuki TSUDA

(*Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai, Japan*)

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Summary

1) The salivary flow from the unilateral parotid gland of sheep was measured while the pH of rumen fluid was maintained at 5 for 2 hours by the administration of acetic, propionic, butyric or hydrochloric acid into the empty rumen.

2) The rate of parotid secretion significantly decreased when butyric acid was administered. With propionic acid administration, the salivary flow showed a tendency to decrease. On the contrary, acetic and hydrochloric acids administrations had no fixed effect on the flow rate.

3) During the experiment, the ketone bodies, CO_2, glucose and total VFA concentration in the carotid arterial blood were determined. Acetic acid administration resulted in a CO_2 level decrease and a total VFA level increase. The increases of glucose and total VFA concentrations were observed by propionic acid administration. The CO_2 level was lowered by the addition of hydrochloric acid. The marked increases in ketone bodies, glucose and total VFA concentrations and the decrease of CO_2 content were observed during the butyric acid administration.

4) The effect of the VFA in the rumen on the parotid saliva secretion were discussed.

The marked characteristics of ruminant salivary gland, especially the parotid gland, are placed on its metabolic and secretory activities and on the inorganic compositions of the secreted saliva.

It was suggested that these characteristics of saliva secretion in ruminant might be closely related to the digestive process of ingested feed in the rumen. To elucidate the relationship between the rumen content and the saliva secretion, the effect of the addition of VFA solution to the reticulo-rumen on the rate of saliva secretion has been studied by several groups of workers (1–5). However, these

* Present address: Fourth Research Division, National Institute of Animal Health, Kodaira, Tokyo, 187, Japan

experimental results did not coincided with each other. Whether or not the chemical stimulation to the rumen induces the changes of the salivary flow seems still to be the unsolved problem.

The present authors have observed the changes of the salivary flow from the unilateral parotid gland of sheep while the ruminal pH was maintained at 5 or 6 for three hours by VFA administration (6). In those experiments, however, it was not clear whether or not the introduced VFA effected the rumen wall or the lower gut and whether or not the obtained results were solely due to the introduced VFA or the combined effect with the rumen contents. The purpose of the present experiment was to clarify the effect of VFA stimulation to the empty rumen on the parotid secretion of sheep.

Materials and Methods

The experimental animals used were two female sheep. Both animals had permanent large ruminal fistulas, carotid arterial loops and unilateral parotid fistulas. Body weights of both sheep were maintained around 40 kg by daily feeding to each animal of 500 g of hay, 400 g of the commercial concentrate for dairy use, mineral mixtures and 50 g of sodium bicarbonate which was sufficient to compensate for the loss of sodium from the parotid fistula.

The trial was carried out in the following manner. The rumen contents were withdrawn as much as possible through the ruminal fistula and reticulo-omasal orifice was plugged by Watanabe's method (7), and 3 liters of artificial ruminal solution which was warmed to 40°C was introduced into the rumen. The composition of artificial ruminal solution was as follows (mg/dl): KH_2PO_4 407.3, Na_2HPO_4 37.6, $NaHCO_3$ 595.3, NaCl 85.0, NH_4Cl 53.4, $MgSO_4 \cdot 7H_2O$ 56.8 and $CaCl_2 \cdot 2H_2O$ 37.0. The shematic drawing of the animal and equipment was shown in Fig. 1. The parotid saliva flowing out of the unilateral parotid fistula was collected every 10 minutes through a stainless funnel into a beaker to measure the secreted volume. The ruminal pH was automatically recorded with an electric polyrecorder (Towa EPR-2T type) by inserting a glass electrode directly into the rumen.

The parotid flow rate was checked as control for one hour before the each treatment. Thereafter, one of the following acids (warmed to 40°C), 0.5 M acetic, propionic, butyric or hydrochloric acid was introduced into the reticulo-rumen till the ruminal pH reached to 5. The rumen pH was maintained for 2 hours by dripping the acid into the rumen according to the reading of the pH meter.

At thirty minutes after the experiment started, the first arterial blood sample was taken through the carotid loop. Immediately after the administration of each acid, a second sample was taken. A blood sample was taken every 60 minutes for three hours thereafter.

The experiment was finished at 1 hour after the 2 hours pH maintenance period. The withdrawn ruminal contents warmed to 40°C were returned to the

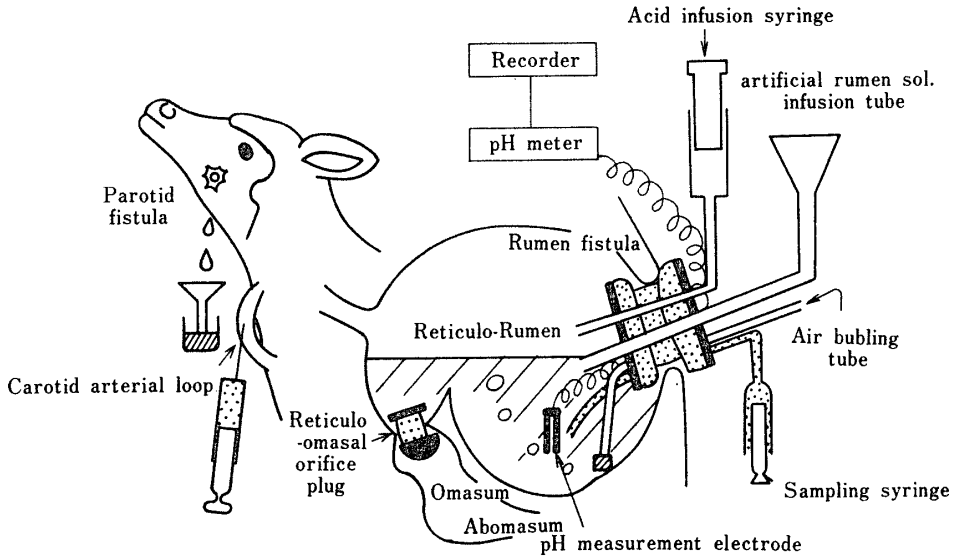

FIG. 1. Shematic drawing of the experimental arrangement of the animal and the equipment.

rumen at the end of each trial. During the experiment, the animal was taken into the experimental cage.

The ruminal and blood VFA concentration were measured by the Friedman Method, modified by Sasaki (8). Blood CO_2 contents, blood ketone bodies and blood glucose level were determined by Conway's micro-diffusion technique (9), Thin and Robertson method (10) and glucose oxidase method of Hugget and Nixon (11), respectively.

Results

In control experiment, in which only the artificial ruminal solution was infused into the empty rumen for three hours, the mean values of the salivary flow in sheep A and B were 11.6+1.3 (±S.E.) and 7.9±1.0 ml/10 min., respectively. Total VFA concentration and pH in the rumen fluid in the control experiment were about 0.5 mM and 7.6, respectively, in both animals.

In Fig. 2, the typical salivary flow changes were shown when the ruminal pH was maintained at 5 by the addition of butyric acid continuously flowing into the rumen during the period of 0 to 2 hours. The total amount of butyric acid required to maintain the ruminal pH at 5 for 2 hours was 0.60 and 0.63 moles in sheep A and sheep B, respectively. The saliva secretion rate from the unilateral parotid gland remarkably decreased during the butyric acid infusion period. In the pH recovering period of 2 to 3 hour, the flow rate changes in sheep A and B were quite different. Both experimental animals presented such symptoms as somnolence, caughing and grinding the teeth. They gradually returned to their normal condition as the ruminal pH returned to the normal level.

In Fig. 3, the summarized data of the changes of saliva secretion rate were

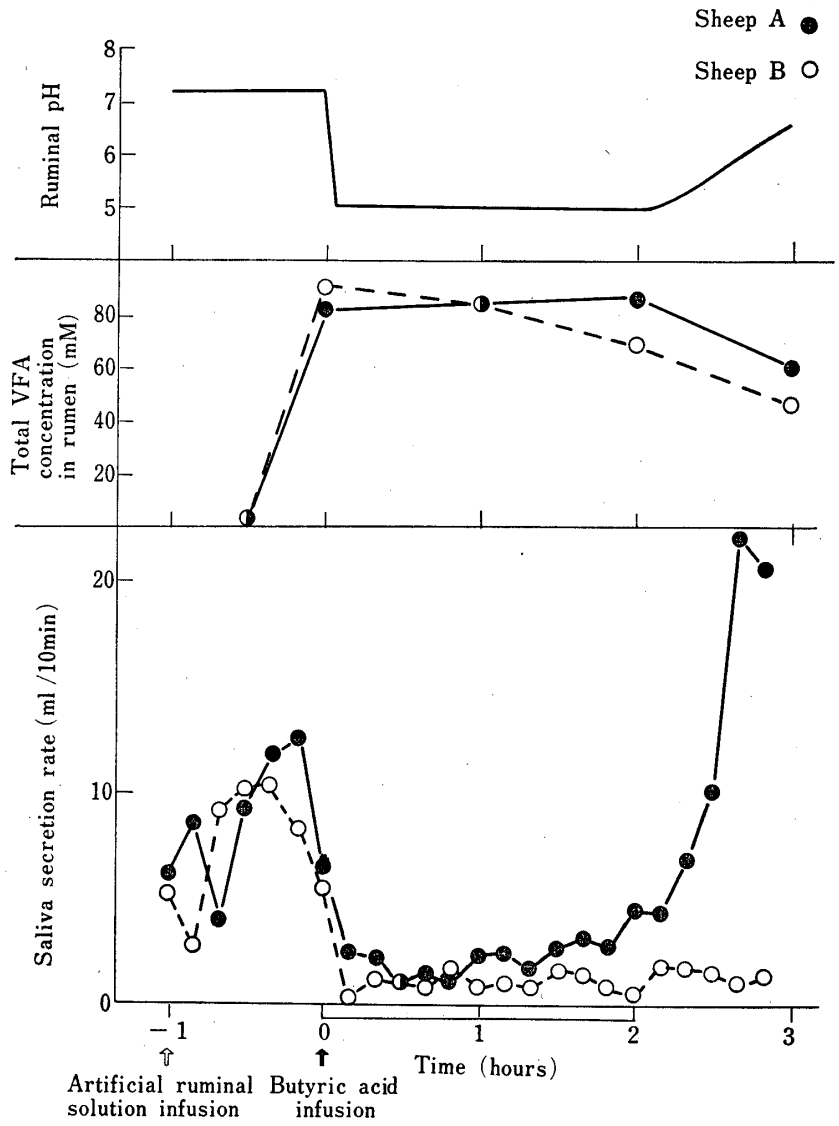

FIG. 2. Changes of the salivary flow and the ruminal condition accompanied with butyric acid infusion into the empty rumen.
Between 0 and 2 hour ruminal pH was maintained by the dripping of butyric acid.

shown when acetic, propionic, butyric or hydrochloric acid were administered into the empty rumen. In each VFA administration, the total VFA level of rumen fluid increased to about 80 mM. The addition of acetic acid had no obvious effect on the rate of saliva secretion. The salivary flow indicated a tendency to decrease during the propionic acid infusion period. On the contrary, the saliva secretion rate significantly decreased to 2.3 ± 0.7 ml/10 min. in sheep A and $1.8\pm$ 0.8 ml/10 min. in sheep B during the butyric acid infusion period. Hydrochloric acid administration had no obvious effect on the salivary flow in sheep A. In sheep B, however, the secretion rate was obviously decreased. The amount of hydrochloric acid used to maintain rumen pH at 5 was 0.26 moles which was about a half that of VFA.

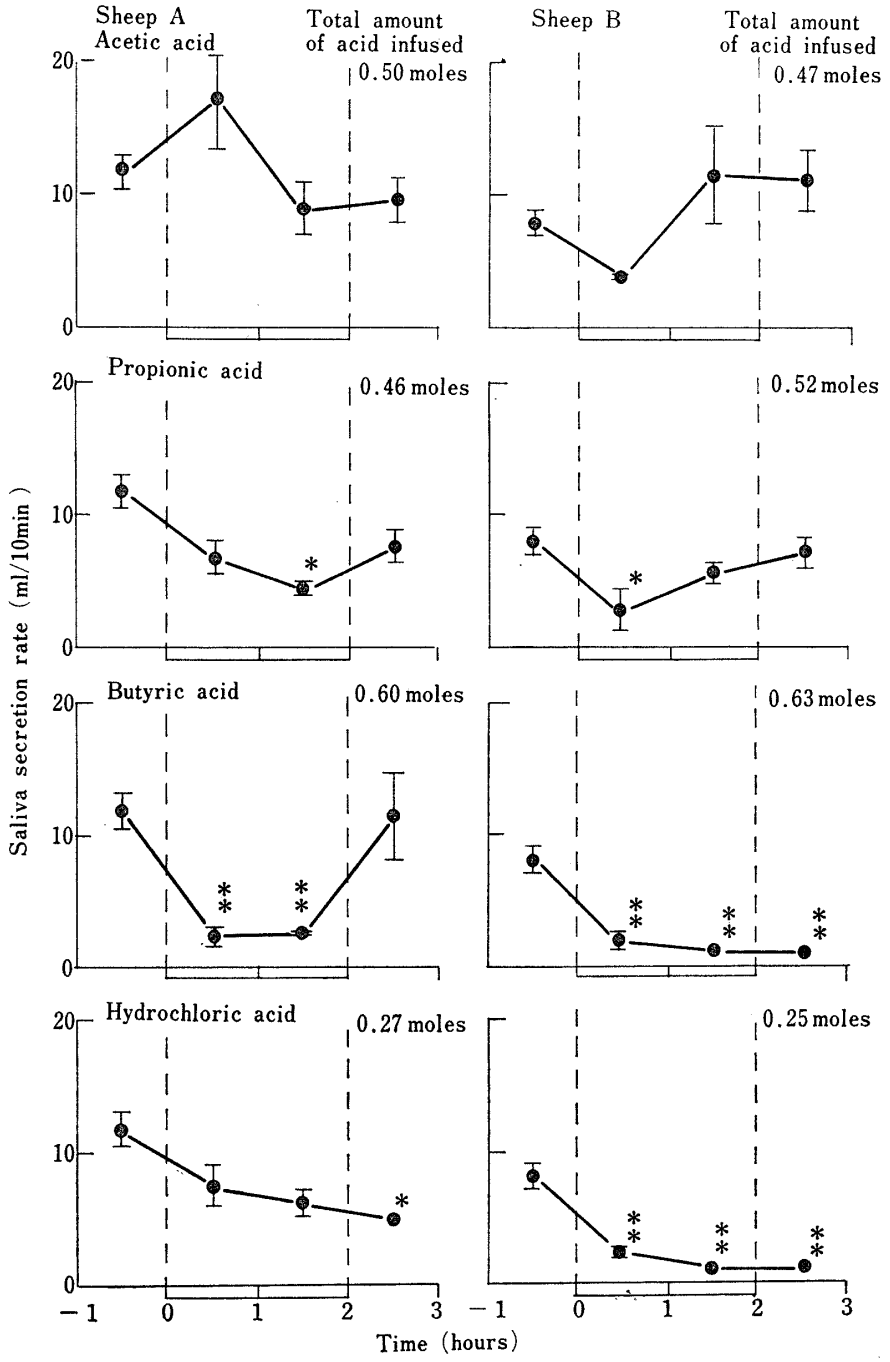

FIG. 3. Changes of salivary flow while ruminal pH was maintained at 5 by addition of VFA or HCl.
The artificial ruminal solution was infused at −1 hour. At 0 hour, each acid was administered. Between 0 and 2 hour ruminal pH was maintained.
Results show the mean±standard error. The control value before acid administration was obtained as the mean of all experiments.
* $P<0.05$ and ** $P<0.01$ Significantly different from the control value.

As shown in Table 1, total VFA, CO_2, ketone bodies and glucose level in carotid arterial blood during the experimental period indicated the different responces by the administration of each VFA and hydrochloric acid. In control

TABLE 1. *Changes of the Arterial Blood Constituents of Sheep A and B Accompanied with Each VFA and HCl Administration into the Rumen*

Acid administered	Arterial blood constituent	hour	VFA (mM) A	VFA (mM) B	CO_2 (Vol%) A	CO_2 (Vol%) B	Ketone body (mg/dl) A	Ketone body (mg/dl) B	Glucose (mg/dl) A	Glucose (mg/dl) B
Acetic Acid	Before inf.	−0.5	0.4	0.6	43.4	55.0	1.8	2.4	73.3	56.1
	Infusion period	0	1.9	1.7	45.4	55.3	1.2	4.9	76.7	43.3
		1	1.4	2.1	32.8	40.5	1.2	3.0	69.3	48.4
		2	1.1	2.3	31.0	39.0	2.4	5.5	46.5	40.0
	After inf.	3	0.8	1.0	42.4	54.0	3.0	4.3	45.0	51.7
Propionic Acid	Before inf.	−0.5	0.4	0.4	51.0	54.0	2.0	3.0	70.0	53.9
	Infusion period	0	1.1	1.9	47.8	52.0	1.2	1.8	85.0	66.7
		1	0.7	0.9	51.2	56.0	1.2	1.2	95.0	85.3
		2	0.7	0.9	48.0	55.0	3.0	2.4	98.3	79.7
	After inf.	3	0.6	0.4	52.6	55.0	1.8	1.8	90.0	68.3
Butyric Acid	Before inf.	−0.5	0.5	0.4	44.8	54.0	1.2	3.1	55.0	62.7
	Infusion period	0	1.5	3.2	45.4	49.0	2.5	4.3	204.6	239.8
		1	1.5	6.0	37.8	36.6	8.0	12.5	147.4	228.8
		2	1.3	8.5	42.8	33.0	11.7	13.5	48.0	60.5
	After inf.	3	0.5	3.4	35.6	56.4	13.0	16.0	48.4	63.8
HCl	Before inf.	−0.5	0.3	0.4	51.4	50.0	3.1	3.1	72.6	63.8
	Infusion period	0	0.3	0.4	46.2	41.6	2.5	2.5	83.6	70.4
		1	0.4	0.4	42.8	40.4	3.1	1.8	70.4	69.3
		2	0.3	0.3	39.4	38.2	2.5	3.7	60.5	36.0
	After inf.	3	0.4	0.4	32.2	36.0	2.5	3.1	83.6	40.0
Control		−0.5	0.5	0.5	45.0	54.2	3.1	1.8	50.0	-
		0.5	0.4	0.6	46.0	59.0	2.0	1.8	47.3	62.7
		1.5	0.5	0.6	47.4	52.0	1.2	1.8	51.3	-
		2.5	0.5	0.6	47.0	54.2	1.2	2.4	61.7	55.0

During infusion period, ruminal pH was maintained at 5. inf.: infusion

period, the total VFA, CO_2, ketone bodies and glucose concentration remained unchanged in both sheep A and B. The total VFA concentration increased by acetic, propionic and especially by butyric acid but did not increase by hydrochloric acid administration. In acetic acid infusion, the CO_2 content decreased at first hour after the start of infusion but returned to the normal level by the third hour. The CO_2 level also decreased by butyric acid administration. In hydrochloric acid infusion, however, the lowest CO_2 level was observed at third hour. The marked ketone bodies increase was observed at third hour of butyric acid infusion. The ketone bodies also increased with acetic acid, but decreased or remained unchanged with propionic or hydrochloric acid administrations. The significant rise of glucose level was obtained between 0 and 1 hour after butyric acid administration. A slight rise of glucose level was also observed with the propionic acid addition. The two other acids showed no effect on the glucose concentration.

Discussion

As shown in Fig. 3, the salivary flow showed a different response to the administration of each acid. The results obtained from the present investigation were the same as those of Obara et al. (6). Namely, when the ruminal pH was maintained at 5 with the addition of butyric acid, a signifficant decrease of the salivary flow rate was observed. The decrease of the flow was also observed, though to a lesser degree, by propionic acid infusion. On the contrary, the effects of acetic and hydrochloric acid infusion on the salivary flow were irregular.

Blood CO_2 content was obviously decreased by ruminal administration of acetic, butyric and hydrochloric acid. The present authors (12) have already reported that the decrease of salivary flow was accompanied with the lower blood CO_2 level in sodium deficient sheep. Recently, Juhasz and Szegedi (13, 14) showed that the administration of large quantities of crushed grain or glucose, or the intraruminal infusion of lactic acid to sheep lead to a decrease of ruminal pH and blood CO_2, and caused the changes of the quantity and composition of saliva. In addition, they (14) suggested that the amount of saliva increased with the compensated metabolic acidosis but decreased with uncompensated metabolic acidosis. The inhibition of saliva secretion by ruminal addition of butyric acid might be related to the uncompensated metabolic acidosis. However, the acidosis observed in the case of ruminal infusion of acetic and hydrochloric acid did not necessarily induce a lowered salivary secretion. Therefore, it is improbable that the acidosis alone or such acidosis as observed in this experiment inhibits the saliva secretion.

Pennington (15) found that the presence of butyrate in the rumen led to a several fold increase of ketone bodies level in the blood. In the present experiment, the ketone bodies level in arterial blood increased gradually and reached a maximum at third hour after the ruminal infusion of butyrate. By that time, however, salivary flow returned to the normal level in one sheep. From the present and previous experiments (6), we concluded that ketone bodies in blood may not be the major factors to depress humorally the saliva secretion rate.

The blood glucose concentration increased immediately after the addition of the butyric acid into the rumen. Several investigators (16, 17) reported that the intravenous infusion of butyrate resulted in a marked increase of blood glucose level in sheep. The present authors (18) had already discussed the mechanism of butyric acid hyperglycemia. In our other experiment*, however, the hyperglycemia induced by the intravenous infusion of glucose had not influence on the saliva secretion rate. The decrease of salivary flow developed by the intraruminal infusion of butyric acid, therefore, might not be due to the hyperglycemia.

The VFA produced in the reticulo-rumen are absorbed through the rumen epithelium (19). Under usual feeding regimen, the main VFA which appears in

* Unpublished data

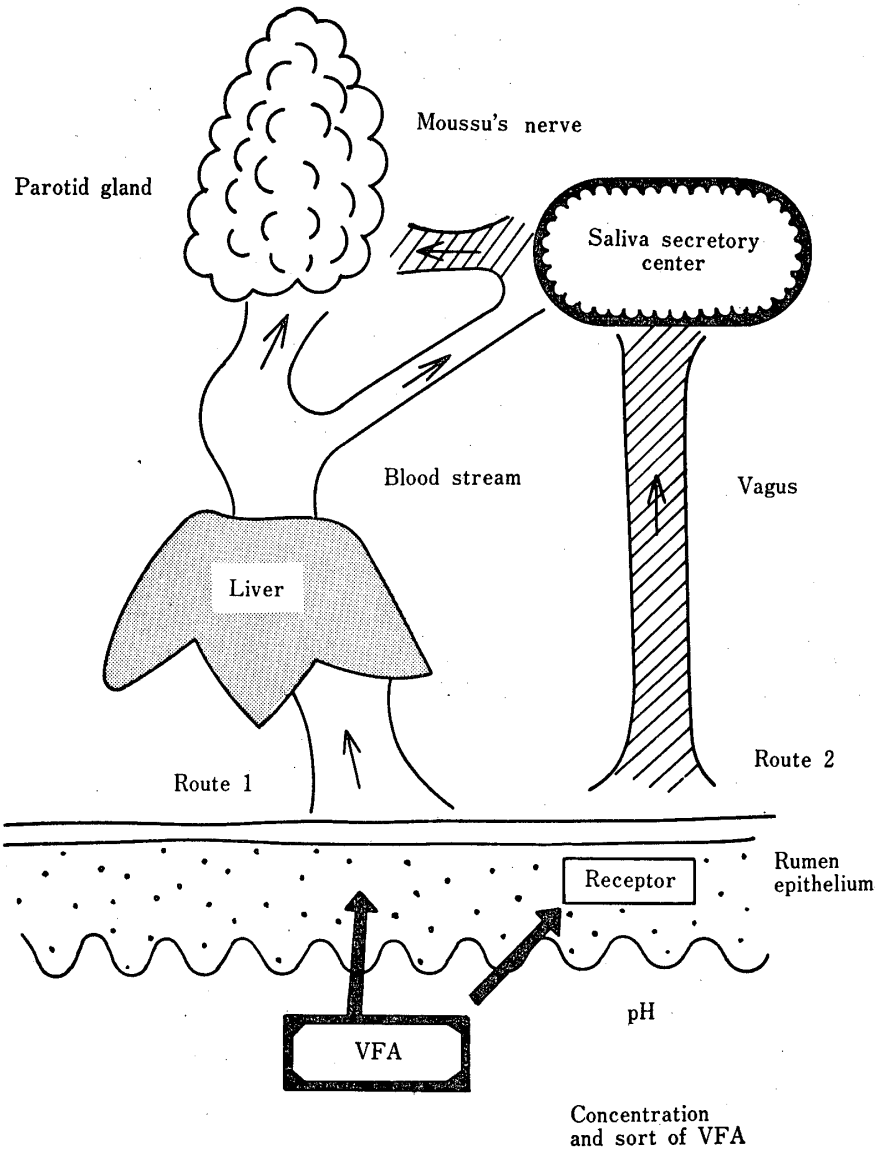

FIG. 4. Schematic drawing of the humoral (Route 1) and nervous (Route 2) regulations of ruminal VFA stimulation on the parotid secretion of sheep.

the carotid arterial blood is not butyrate or propionate but acetate (20). Though the identification of each VFA which appeared in the blood was not determined in the present experiment, the butyrate or propionate in the blood which escaped from the metabolism inside the rumen epithelium or inside the liver might act on the parotid gland directly through the humoral pathway or indirectly through the nervous system to suppress its activity.

Kay (21, 22) has been shown that the vagus nerve forms the afferent pathway originating in the oesophagus and forestomach which controls the reflexes of salivary secretion. It seems likely that VFA solution introduced into the rumen acts on the chemoreceptor located in the reticulo-rumen as to change the activity of the salivary glands through the vagal afferent fibers. The extent of the influence

may depend on the sort of VFA, its concentration and pH.

Through the above discussion, the possible controlling mechanisms of parotid secretion by introduced VFA into the rumen are schematically illustrated in Fig. 4. Route 1 is the humoral pathway in which VFA or its metabolites in the blood act on the parotid gland directly or indirectly. Route 2 is the nervous pathway in which the chemoreceptor located in the reticulo-rumen is influenced by VFA so as to depress the parotid activity. The extent of the importance of the two routes for parotid secretion needs further investigation.

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