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## The Effects of the Intravenous Infusion of Volatile Fatty Acid on the Parotid Secretion of Sheep

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### Summary

1) The salivary flow from the unilateral parotid gland of sheep was measured while the total volatile fatty acid concentration of the carotid arterial blood was elevated to around 2 mM for 2 hours by intravenous infusion of acetic and butyric acid.

2) The rate of parotid secretion showed a tendency to increase by acetic acid infusion. As the infusion was stopped, the flow rate returned to the normal level. On the contrary, butyric acid infusion did not affect the flow rate during the infusion period.

3) The total VFA, CO<sub>2</sub>, ketone bodies, glucose and lactic acid concentrations in carotid arterial blood were determined. Acetic acid infusion resulted the decrease of CO<sub>2</sub> level and the increase of total VFA level. The marked increase in the ketone bodies, glucose and VFA level and the decrease of CO<sub>2</sub> content were observed during the butyric acid infusion.

4) It is assumed that the stimulative effect of acetate on the parotid gland or on its secretory center is due to the direct action through the humoral pathway but that the inhibitory effect of butyrate introduced into the rumen is due to nervous action through the nerve endings in the rumen wall.

It was well established that ruminant saliva is an important factor in neutralizing the rumen environment. On the contrary, it was not known whether pH or the volatile fatty acids (VFA) concentration in the rumen fluid may influence the rate of the saliva secretion. On this point, Obara et al. (1, 2) hitherto observed the changes of the salivary flow from the unilateral parotid gland of sheep while the ruminal pH was maintained at 5 by VFA administration into the reticulo-rumen. They concluded that the secretion rate was depressed especially by butyric acid administration. As for the regulatory mechanism of VFA introduced into the

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rumen on the rate of parotid secretion, two mechanisms were considered. One of them is a neural pathway in which the introduced VFA acts on salivary gland through the nervous system and the other is a humoral pathway in which the absorbed VFA from the rumen into the blood may influence the salivary flow.

The present investigation was designed to inquire into the humoral pathway of secretion in which the change of salivary flow was observed when the total VFA concentration of carotid arterial blood was increased to about 2 mM by the intravenous infusion of VFA.

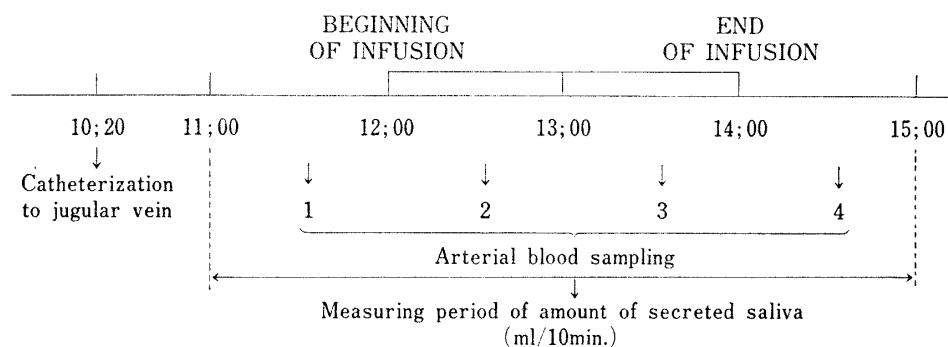
### Materials and Methods

Six sheep weighing 35–50 kg were used in this experiment. All the sheep were prepared with unilateral parotid fistulae and ipsilateral carotid loop. At 11 a.m. they were fed 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 the fistula.

The experiments were always started at 24 hours after feeding in order to make the ruminal fluid as constant as possible.

The experiment was carried out in the following manner. The left jugular vein of the sheep was cannulated with a 1 mm (i.d.) polyethylene tube. The catheter was filled with heparin saline to prevent blood coagulation. A motor-driven syringe which was controlled to move at a constant rate of 3.33 ml/min. was used for the infusion of VFA solution.

The procedure is shown diagrammatically;



The experiments were started 40 min. after insertion of the catheter to the left jugular vein. The parotid saliva flowing out from the fistula was collected every 10 minutes through a stainless funnel into a 100 ml beaker to measure the volume. The control flow rate was checked for one hour before the start of infusion. One of the following acids (warmed to 39°C), 0.5 M acetic, or butyric acid was infused into the blood through the catheter for 2 hours at the rate of 0.04 mmole per minute per kg of body weight. In the control experiment, Ringer solution was infused at a constant rate of 3.33 ml/min. for 2 hours. The experiment was finished at 1 hour after the end of the acid infusion. The blood sample was taken

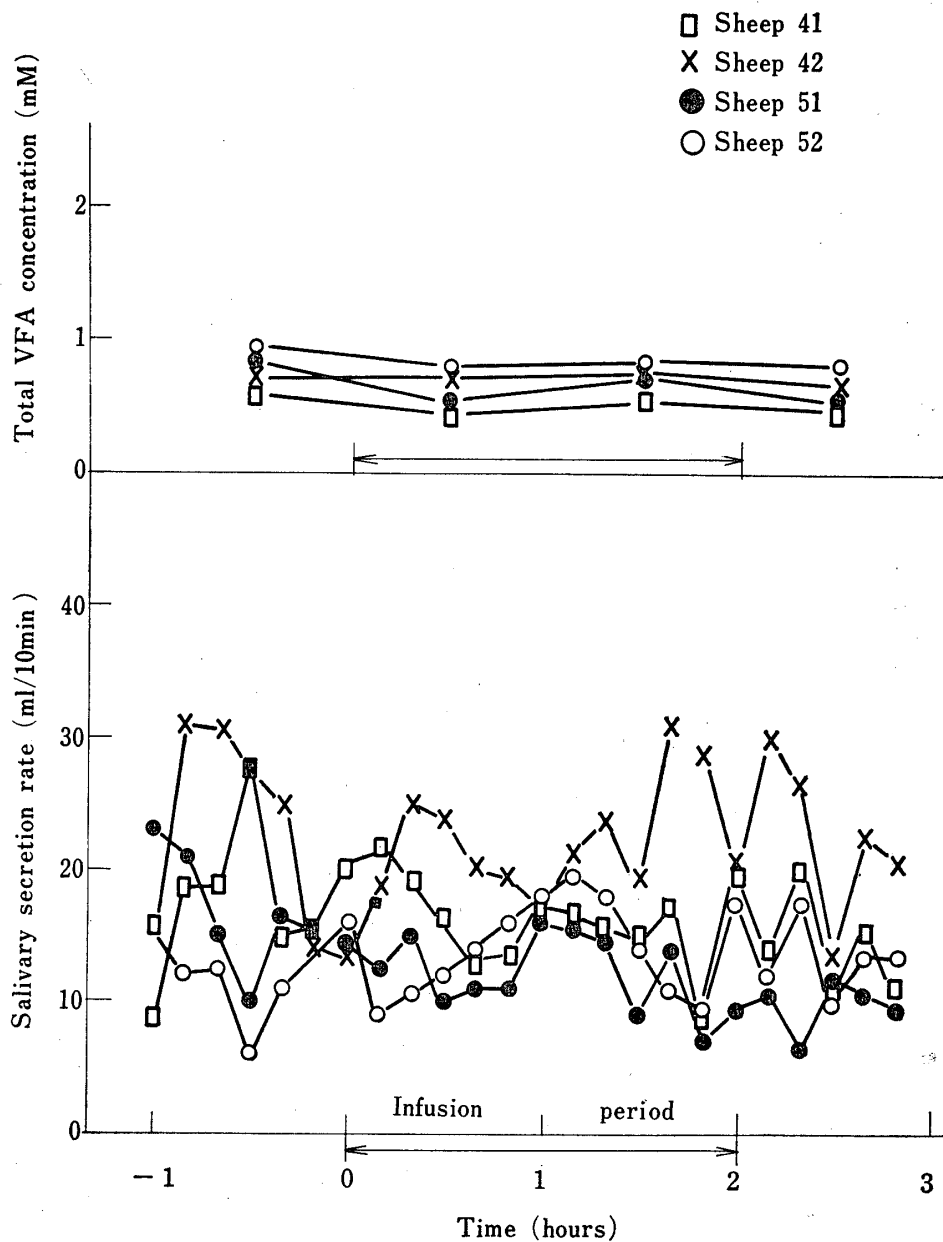


FIG. 1. Changes of the rate of the saliva secretion and the total VFA concentration in carotid arterial blood during the intravenous infusion of Ringer solution.

every 60 minutes after the first one was taken through the carotid loop at thirty minutes after the experiment started. The blood VFA concentration was measured by Friedman method, modified by Sasaki (3). Blood CO<sub>2</sub> contents were determined by Conway's microdiffusion technique (4). Blood ketone bodies were determined by the method of Thin and Robertson (5). Blood glucose concentration was determined by glucose oxidase method of Hugget and Nixon (6). Lactic acid was determined by modification of Barker and Summerson's method (7).

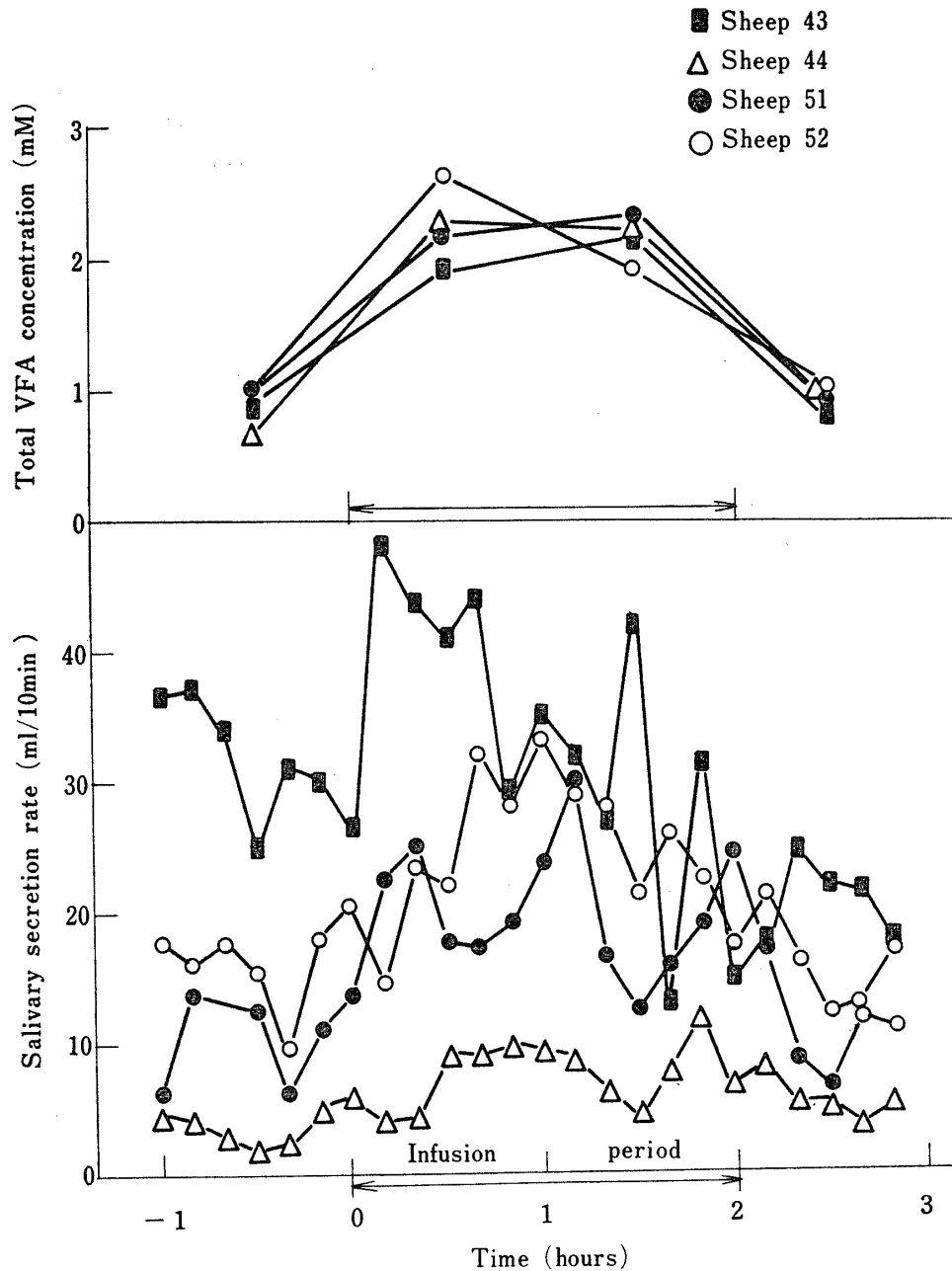


FIG. 2. Changes of the rate of the saliva secretion and the total VFA concentration in carotid arterial blood during the intravenous infusion of acetic acid.

### Results

The changes of the rate of saliva secretion during the intravenous infusion of Ringer solution was shown in Fig. 1. Total VFA concentration in arterial blood did not change with this treatment. The mean value of four experimental animals was  $0.7 \pm 0.1$  mM ( $\pm$ S.D.). The infusion of Ringer solution showed no obvious effect on the rate of saliva secretion in all four animals. The average of salivary flow rate were  $16.0 \pm 3.4$ ,  $22.0 \pm 4.8$ ,  $12.5 \pm 2.9$  and  $14.0 \pm 3.6$  ml/10 min. in sheep 41, 42, 51 and 52, respectively, and mean value of the four animals was  $16.1 \pm 2.4$

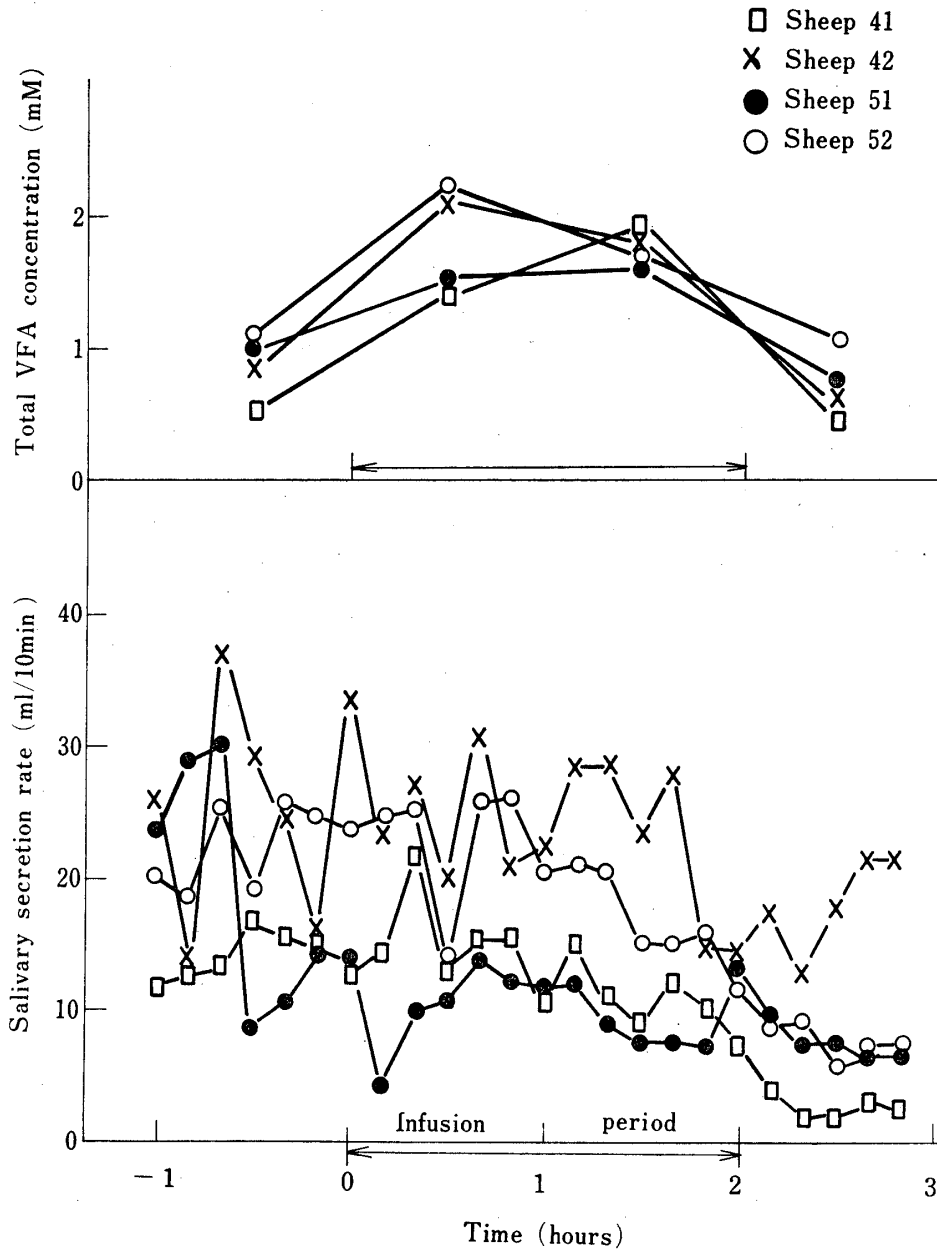


FIG. 3. Changes of the rate of the saliva secretion and the total VFA concentration in carotid arterial blood during the intravenous infusion of butyric acid.

ml/10 min.

The changes of the salivary flow and the total VFA concentration in arterial blood accompanied with the intravenous infusion of acetic acid are shown in Fig. 2. Total VFA concentration remarkably increased from  $0.9 \pm 0.1$  mM to  $2.3 \pm 0.3$  mM, at 30 minutes after the start of infusion. The VFA concentration returned to the control level 30 minutes after the end of the infusion. The parotid salivary flow showed a tendency to increase during the infusion period in all experimental animals. There is a difference in the absolute volume of saliva secreted between each animal, however, no difference was observed in the secreting pattern

TABLE 1. *Changes of Arterial Blood Properties during the Period of Intravenous Infusion of Ringer, Acetate and Butyrate Solutions*

Infusion solution	Arterial blood constituents	Before infusion period	Infusion period		After infusion period
Ringer	VFA (mM)	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1
	CO <sub>2</sub> (Vol%)	50.7 ± 1.7	48.4 ± 3.0	48.4 ± 2.9	48.6 ± 3.4
	Ketone bodies (mg/dl)	1.8 ± 0.6	1.6 ± 0.3	1.4 ± 0.4	1.4 ± 0.5
	Glucose (mg/dl)	57.0 ± 12.6	55.8 ± 11.4	55.3 ± 9.1	53.3 ± 13.5
	Lactic acid (mg/dl)	11.6 ± 1.9	13.5 ± 3.3	11.9 ± 2.7	11.5 ± 4.6
Acetic acid	VFA (mM)	0.9 ± 0.1	2.3** ± 0.3	2.1** ± 0.2	0.9 ± 0.1
	CO <sub>2</sub> (Vol%)	54.2 ± 2.1	48.5* ± 4.0	41.5** ± 2.9	53.0 ± 3.5
	Ketone bodies (mg/dl)	2.3 ± 0.8	2.0 ± 1.2	2.0 ± 1.6	1.9 ± 1.1
	Glucose (mg/dl)	62.9 ± 2.3	63.0 ± 5.3	68.8 ± 11.8	63.1 ± 7.4
	Lactic acid (mg/dl)	11.6 ± 4.4	12.8 ± 3.4	11.3 ± 1.9	10.8 ± 0.9
Butyric acid	VFA (mM)	0.9 ± 0.3	1.8* ± 0.4	1.8** ± 0.1	0.9 ± 0.1
	CO <sub>2</sub> (Vol%)	50.5 ± 1.9	37.0** ± 5.0	38.1** ± 5.6	44.9 ± 8.0
	Ketone bodies (mg/dl)	1.7 ± 0.8	4.5* ± 1.3	5.5** ± 2.0	2.4 ± 0.3
	Glucose (mg/dl)	43.8 ± 4.8	134.7** ± 43.2	87.4* ± 32.4	59.8 ± 21.8
	Lactic acid (mg/dl)	16.2 ± 2.4	21.8 ± 5.3	13.3 ± 2.4	14.2 ± 4.7

\*\* P < 0.01 } Significantly different from the value of before infusion period.  
 \* P < 0.05 }

Mean value ± standard deviation

accompanied with the lapse of time. The rate of secretion returned to the control level by stopping the infusion.

In butyric acid infusion, total VFA concentration increased from 0.9 ± 0.3 to 1.8 ± 0.4 mM and maintained its level during the acid infusion period. The infusion of butyric acid had no obvious effect on the rate of salivary secretion (Fig. 3). However, there were differences in the pattern of the changes of salivary flow between the experiments. Namely, in sheep 41 and 52, the butyric acid infusion did not affect the flow rate during the infusion period although the significant decrease was observed after the end of infusion period. On the contrary, the effects of butyric acid infusion on the salivary flow in sheep 42 and 51 were irregular.

Table 1 shows the changes of carotid arterial blood constituents during the intravenous infusion of Ringer, acetic and butyric acid. In Ringer infusion, total VFA, CO<sub>2</sub>, ketone bodies, glucose and lactic acid concentration in blood remained unchanged. In acetic acid infusion, the decrease of CO<sub>2</sub> content was observed but ketone bodies, glucose and lactic acid concentration hardly changed. In butyric acid infusion, the remarkable increases of total VFA, ketone bodies and glucose level were observed but the lactic acid level was unchanged. The CO<sub>2</sub> content of the blood decreased with butyric acid infusion. These changes observed in the intravenous infusion period of acetic and butyric acid were about the same as those of the ruminal administration of them (2).

### Discussion

Sasaki and Umezu (8) previously reported that the salivary gland slices of ruminant utilize more VFA than glucose in oxygen uptake and in amount of consumption and they proved that acetate was utilized best among the three VFA. Sasaki (9) found that the increase of aerobic utilization of acetic acid in the parotid slices agrees with the development of the rate of parotid secretion in the calves and suggested that the positive relation exists between the saliva secretion rate and the acetate concentration in the carotid artery.

In the present investigation, when the total VFA concentration of arterial blood was elevated by intravenous infusion of acetic acid to about 2 mM which was the same level as the observed in intra-ruminal infusion experiment (2), an increase of the salivary flow resulted. It is suggested, therefore, that the acetate in the blood acted directly on the parotid gland and or on the center of saliva secretion to facilitate their activities.

The infusion of butyric acid had no obvious effect on the rate of secretion during the infusion period. These results were different from the results obtained in the intra-ruminal administration experiments (1, 2). The changes of the blood constituents caused by butyric acid administration, however, were the same as in the previous experiments (2). Therefore, the inhibition of parotid secretion induced by intra-ruminal infusion of butyric acid may be mainly depended upon nervous pathways to the nerve endings in the rumen epithelium.

Another possibility considered as a reason for parotid secretion inhibition is that butyric acid or its metabolites paralysed the saliva secretion center in the medulla oblongata. If so, the decreased secretion rate observed during 2 to 3 hour may be partly explained by this mechanism. However, no definite evidence was obtained in the present investigation.

Acidosis was also observed in all cases of intravenous infusion of each VFA. It could be said that the acidosis at the level observed in this investigation did not affect the saliva secretion.

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