EFFECTS OF PAROTID SALIVA REMOVAL ON THE RUMEN FERMENTATION AND BODY CONSTITUENTS OF SHEEP

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By

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Introduction

The saliva of the ruminant is one of the most important factors to maintain the microbial activity, by which many biochemical reactions in the rumen proceed. It is well known that the saliva has its origin in the parotid glands, submaxillary glands, sublingual glands and small salivary glands. It has been demonstrated that the parotid glands are the major source of the saliva in the ruminant (1).

The sheep with unilateral parotid fistula can be maintained with good health indefinitely, provided that sufficient sodium bicarbonate is added to the dietary ration in order to compensate for the loss of sodium which is contained in the parotid saliva (2).

The purpose of this investigation was to determine if an elimination or a lack of parotid saliva is accompanied by changes in the ruminal fermentation and also in some physiological status of the sheep.

Material and Method

Procedure of parotid fistula preparation: A female sheep was anesthetized by injection with sodium pentobarbital. The aboral incision from the oral angle (*Anguls oris*) was made on the cheek widely enough so that the orifice of the parotid duct could be easily identified. A small area of the mucosa, usually 2–3 cm in diameter, was stripped from the muscle layer so that the orifice of the parotid duct was located in the central part. The parotid duct of a few centimeters in length was carefully separated from the surrounding muscles. The epidermis of the cheek was detached in circular shape 2–3 cm in diameter to expose the subcutaneous tissue. The buccal mucosa containing the orifice of a parotid duct was drawn

through a small hall of the buccal wall outside the cheek surface, and then secured on the subcutaneous tissues with the suture. Attention should be paid to avoid the twisting of the parotid duct in this procedure. Finally, the incised area of the cheek was closed with the suture. Swelling around the mouth was observed following the operation, but it disappeared at two or three days after the operation. To prevent the odious infection, 6×10^5I.U. of penicillin was given intramuscularly daily for two weeks.

Animal: The parotid fistulated sheep was fed in a metabolic cage and the urine was collected separately from the feces. The feed was given at 10: 00 a.m. each day. One day's intake of the diet was 400 g of orchard hay and 400 g of the commercial concentrate for dairy cattle. This diet was estimated to contain 70mEq sodium per day.

Water was given ad libitum. The amount of sodium bicarbonate given was 50 g per day for the unilaterally fistulated sheep, and 100 g per day for the bilaterally fistulated sheep. The sodium bicarbonate was fed, mixed with the concentrate. In the experiment with sodium bicarbonate and salt block supplementation, the former was given to fill the requirement for sodium and bicarbonate ions, and the latter for chloride ion.

In the first experiment, the sheep with unilateral fistula was fed with a basal diet containing sodium bicarbonate and salt block for 222 days. After the bilateral fistulae were operated on this sheep, she was placed on the same diet for 29 days. In the second experiment, the same sheep as was used in the first experiment, was placed on a basal diet alone. In these experiments the changes of the body weight, several blood constituents and the activity of ruminal fermentation were measured.

Method for analysis: The concentration of the individual volatile fatty acid (VFA) in the rumen was estimated by the method of Belasco (3), and the concentration of the total VFA was expressed as the sum of the individual acids. Blood for analysis was drawn from a juglar vein. The serum concentration of sodium and potassium were estimated by a flame-photometric method (Lange Flame photometer. Model 2A). The serum was diluted with redistilled water 25 times for the estimation of potassium and 275 times for sodium. The saliva, rumen fluid and urine were diluted with redistilled water so that the concentration of sodium and potassium in these samples would be approximately 20ppm.

The carbon dioxide content in the whole blood was estimated by the microdiffusion technique of Conway (4). To obtain the dry matter content in the blood, 1–2 ml of blood was placed in a desiccator under vacuum for two hours and then evaporated to dryness in an electric oven at 100°C. Aceton bodies in urine were estimated by 'Shino-test No. 3.'. Serum protein content was determined by the hand protein refractometer (Hitachi Co.).

To check the developement of proteinuria, the sulfosalycylic acid test, and heat

and acid test were employed.

Results and Discussion

Experiment 1.

Changes in the sheep with unilateral and bilateral parotid fistula with the supplement of sodium bicarbonate and salt block to the basal diet.

The results are shown in Tabel 1. The removal of saliva via bilateral parotid fistulae resulted in a slight decrease in body weight followed by an increase in water intake by 0.71kg per day. During the experimental period, the sheep consumed all of the diet given.

Wilson (5) has demonstrated an increase in the secretion of salivary glands other than parotid glands, when the saliva from the bilateral parotid glands was not allowed to enter the rumen. He also found that this increase was accompanied by an increase in water intake under such an experimental condition.
These findings may suggest that the secretory mechanisms of the salivary glands are under a homeostatic control.

Table 1. Changes of the various components in the sheep with parotid fistula, to which basal diet was fed, supplemented by $NaHCO_3$ and salt block.

	Unilateral parotid fistula (222 days after operation)	Bilateral parotid fistulae (29 days after operation)
Body weight	27 kg	26 kg
Water intake	3.23 kg/day	3.94 kg/day
Feed intake	800 g/day	800 g/day
Rumen fluid		
pH	7.6	7.4
VFA*	193 mg/dl	234 mg/dl
Molar proportion acetic acid : propionic acid : butyric acid	69:17:14	81:14:5
Na concentration	109 mEq/l	104 mEq/l
K concentration	25 mEq/l	20 mEq/l
Parotid saliva		
Na concentration	182 mEq/l	182 mEq/l
K concentration	8 mEq/l	11 mEq/l

*VFA concentration are expressed as mg/dl of acetic acid.

Changes of VFA concentration, molar proportion of VFA and pH in the rumen fluid were little. These results suggest that fermentation in the rumen was not greatly influenced by parotid saliva removal, if an appropriate amount of sodium bicarbonate and sodium chloride were provided. There was no significant difference in the sodium and potassium concentration of the parotid saliva and the rumen fluid between the unilateral parotid fistula sheep and the bilateral one.

Experiment 2.

Changes in the sheep with bilateral parotid fistulae fed with basal diet only.

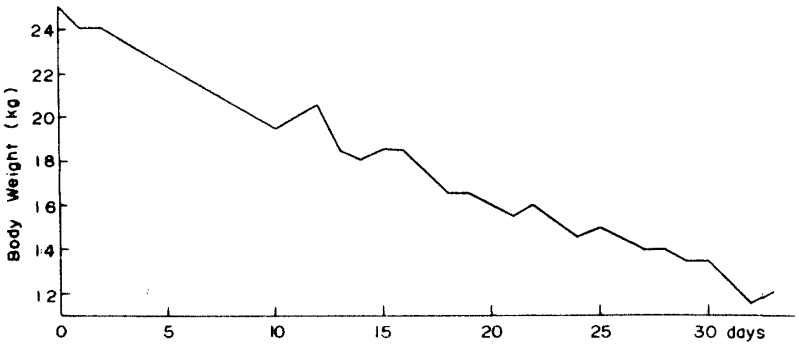

Fig. 1. Changes of the body weight in sheep during the feeding period of basal diet alone.

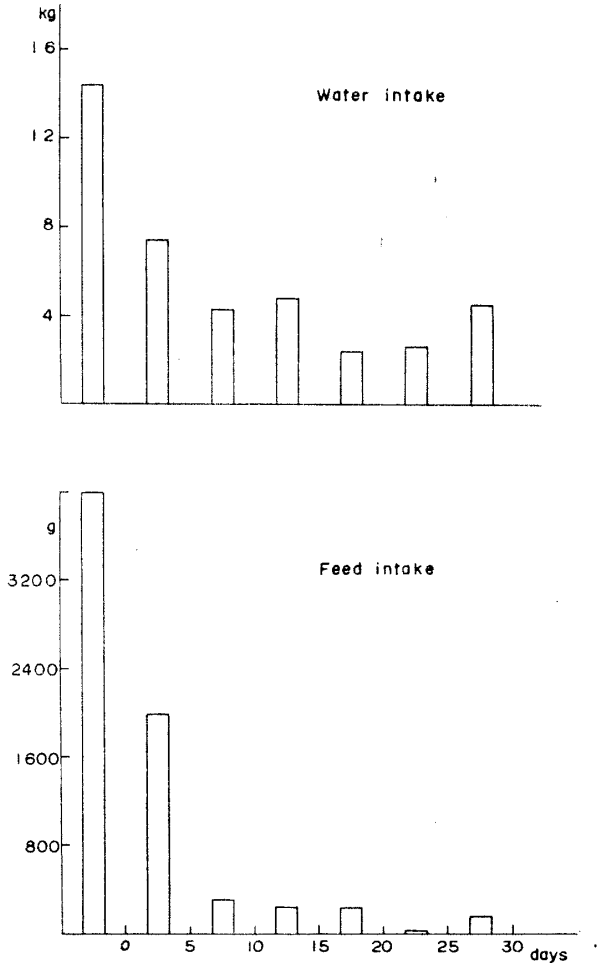

Fig. 2. Changes of the amount of water intake (shown in upper figure) and feed intake (shown in lower figure) in sheep during the period of basal diet alone. Each value was expressed as the sum of five days intake.

Supplement of sodium bicarbonate and salt block to the sheep with bilateral parotid fistulae was withdrawn. On the 33rd day of the salts withdrawal, the sheep was sacrificed, because it seemed impossible to live more.

The dietary intake decreased markedly and the body weight decreased to 48 per cent (Fig. 1). The marked decrease in dietary intake may account for such a great loss of body weight in this sheep.

The ratio of water to dietary intake was found to be increased, although the absolute amount of water intake was rather decreased markedly (Fig. 2).

The urinary excretion of sodium and potassium decreased to less than 1–2 per cent of the initial value. This reflects a possible renal adaptability to the decrease in feed intake. The excretion of urinary aceton bodies showed a marked increase after 16 days of the salts withdrawal. Proteinuria developed at the fifth to 15th day after the salts withdrawal.

There was a little change in the serum sodium concentration; the serum potassium concentration decreased from 4.9mEq/1, to 2.3mEq/1 at the 33rd day.

The carbon dioxide content in the blood was found to be decreased, suggesting the development of acidosis. Protein concentration in the serum and the dry matter content in the blood showed a slight increase (Table 2).

Table 2. Influences of salts withdrawal on the several constituents of blood and urine in the bilaterally fistulated sheep.

	Salts supplement	Salts withdrawal		
		5th day	16th day	32nd day
Serum Na concentration (mEq/l)	142	136	141	145
Serum K concentration (mEq/l)	4.9	3.8	3.8	2.6
Serum protein concentration (%)	6.4	6.3	7.8	8.5
CO_2 content in whole blood (%)	58	—	40	29
Dry matter in blood (%)	17.35	18.08	19.12	18.17
Urinary aceton bodies (mg/dl)	5-10	10-15	more than 100	10-15
Urinary Na excretion (mEq/day)	438.9	95.0	2.4	2.7
Urinary K excretion (mEq/day)	154.9	71.6	3.1	8.4

The rate of parotid saliva secretion was 2.6 l per day when salts were added to the diet, whereas it was significantly decreased when the supplement of salts was withheld. The values were 144ml, 32ml, 32ml per day on the 10, 20, 32 days, respectively.

Sodium concentration of parotid saliva decreased to 28 per cent and potassium concentration increased to 366 per cent on the sixth day (Fig. 3). It was apparent that the sheep was in sodium-depletion. Dobson (6) reported that the sodium concentration in saliva was decreased in the cows grazing on heavily fertilized grass too.

The potassium and sodium concentration of the rumen fluid decreased from

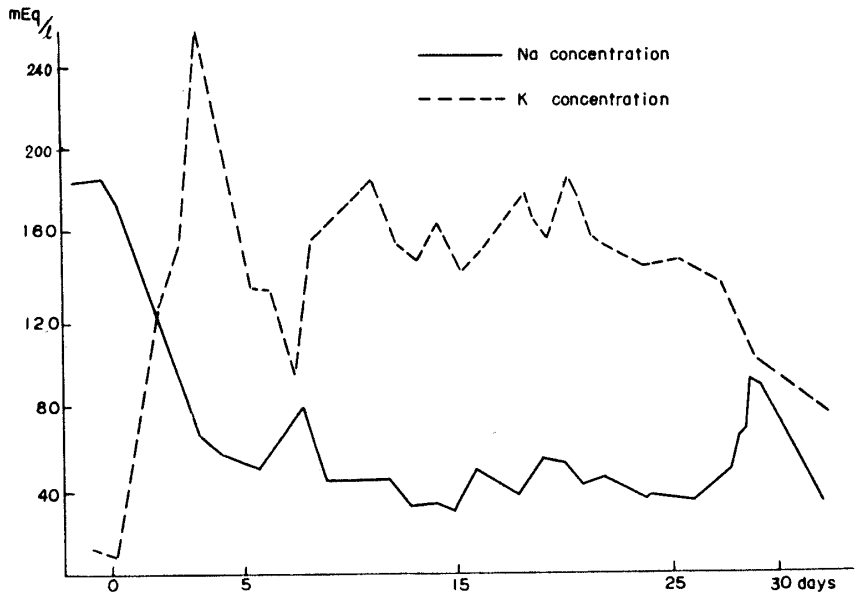

Fig. 3. Changes of Na and K concentration in parotid saliva in sheep during the feeding period of basal diet alone.

25mEq/1 to 18mEq/1 and from 100mEq/1 to 53mEq/1, respectively, on the 32nd day.

Kay (7) observed that when sheep was in sodium-depletion, the secretion rate of mixed saliva decreased, and the sodium concentration of each saliva other than palatine saliva also decreased.

A decrease of the sodium concentration in the rumen fluid may be principally due to the decrease in the sodium influx into the rumen.

The pH in the rumen fluid decreased from 7.05 at salts supplement, to 5.40 on the third day of the salts removal, whereas it increased on slightly thereafter.

From three to six days after the salts removal, the rumen contents bore the stimulative odor of fruits. Since lactic acid was not detected in the rumen fluid in these periods, this particular odor would not originate from lactic acid fermentation. The rumen was filled with feed in this period. The dry matter of rumen content at the fifth day of the salts free diet amounted to 20.5 per cent, whereas it was 6 per cent at the 13th day of the salts removal, and the rumen content almost returned

Table 3. Changes of the rumen fluid in the bilaterally fistulated sheep fed with basal diet only.

	Initial day	3rd day	13th day	32nd day
VFA concentration* (mg/dl)	234	—	197	74
Molar proportion of VFA, acetic acid: propionic acid: butyric acid	81:14:5	71:26:3	81:13:6	60:29:11
Na concentration (mEq/l)	100	32	47	53
K concentration (mEq/l)	25	63	36	18

* Expressed as acetic acid concentration.

to the normal appearance.

The changes of VFA production in the rumen is shown in Table 3. The total VFA concentration in the rumen fluid decreased. This may mainly depend on the decrease of the substrate which produces VFA, accompanied by the decrease of dietary intake. The proportion of acetic acid between VFA decreased slightly on the 32nd day after the salt withdrawal. This proportion (60: 29: 11), however, may not be abnormal when hay and concentrate were fed as the ration. All samples of the rumen fluid were collected 24 hours after the feeding, which is the most stable period of rumen fermentation. However, since VFA in the rumen are absorbed in a proportion corresponding to each concentration of the VFA (8), it seems reasonable to assume that the molar proportions of VFA obtained 24 hours after feeding are identical to those of VFA at the most actively fermentating period.

A decrease of pH on the third day of the salts removal, the occurrence of stimulative odor during the period of three to six days and the decrease of the sodium concentration in the rumen fluid had a little effect on the changes in the molar proportions of VFA in the rumen fluid (Fig. 4).

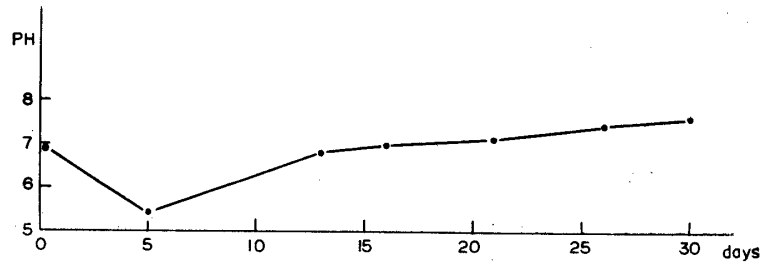

Fig. 4. Variations of pH in the rumen fluid during the feeding period of basal diet alone.

It seems likely that the decrease of sodium concentration in the rumen fluid is due to an absence of the parotid saliva and that changes in the body weight, the urinary excretion of aceton, sodium, potassium and VFA concentration in the rumen might be a result of either parotid saliva depletion itself or the starvation associated with it, or both.

Summary

1. A sheep with parotid fistula was fed orchard hay, concentrate, sodium bicarbonate and salt block. The removal of unilateral and bilateral parotid saliva did not influence the amount of dietary intake, the sodium and potassium concentration of parotid saliva and the condition of the rumen fluid, such as pH, sodium and potassium concentration, VFA concentration and the molar proportions of VFA, as long as sodium bicarbonate and salt block were supplied to the basal diet.

2. The sheep with bilateral parotid fistulae was fed orchard hay and concentrates only. The body weight of this sheep decreased markedly (52%). It seems apparent that this sheep lived no longer than 33 days. The dietary intake decreased and urinary excretion of acetone increased. Serum potassium concentration and carbon dioxide content in the blood decreased. The secretion rates of parotid saliva decreased from 2.6 l per day to 32 ml per day.

The sodium concentration of parotid saliva decreased from 170 mEq/l to 21 mEq/l, while the potassium concentration increased from 36 mEq/l to 66 mEq/l.

Although the molar proportions of the VFA in the rumen fluid did not show significant change, the sodium concentration decreased 47 per cent and the VFA concentration decreased 68 per cent.

On the third day, a reduction of pH accompanied by the appearance of fruit-like stimulative odor in the rumen contents was noted.

3. It seems likely that these changes observed in the rumen content and animal body may be primarily a result of parotid saliva depletion and secondarily of starvation following the prolonged saliva depletion.

Acknowledgement

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