STUDIES ON THE SALIVA AND SALIVATION OF RUMINANTS II. THE COLLECTION OF MIXED SALIVA FROM THE SHEEP WITH THE USE OF AN INTRAOESOPHAGEAL FUNNEL

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II. THE COLLECTION OF MIXED SALIVA FROM THE SHEEP WITH THE USE OF AN INTRAOESOPHAGEAL FUNNEL

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Introduction

With the recent progress of ruminology, many attentions have been paid to the significance of mixed saliva by which the ruminant nutrition is affected through the rumen fermentation of feeds. Mixed saliva, which flow into the rumen, is one of the most important factors to maintain the microbial activity and to proceed the many biochemical reactions in the rumen. The importance of mixed saliva for ruminants is acknowledged from the ruminological point of view, since a ruminant can not maintain its life with the extensive microbial synthesis and decomposition of feed stuff. It was interesting to study the function of ruminant salivary glands (1) and to perform the quantitative or qualitative analysis of ruminant mixed saliva under biological conditons (3—7). Ruminants have three major salivary glands and most of saliva are secreted by these three glands. However, it is indispensable in the study of ruminology to collect whole mixed saliva completely because saliva is secreted not only from the parotid, submaxillary and sublingual but also from some other small glands. In order to sample or collect the mixed saliva the following methods have been used by some investigators; with the use of the oesophageal fistula (6, 9, 10), the use of a sponge from which the mixed saliva is obtained by squeezing after having it chewed in the oral cavity (3, 11) and the sucking of mixed saliva accumulated on the rubber baloon at the tip of the oesophagus (5). We attempted to utilize the large rumen fistula (8) frequently used for the study of ruminology to collect the whole mixed saliva from the sheep with the use of the intra-oesophageal funnel.

Materials and Methods

1. Structure of intraoesophageal funnel.

Two models of the intra-oesophageal funnel were made for the experiments.

Model A was composed of two parts, as shown in Fig. 1. Part I was an oesophageal catheter about two meters in length and 0.7 cm in external diameter. Part II was a rubber tube about one meter in length and 1.0 cm in external diameter which was closely fixed in a glass funnel to accumulate mixed saliva and to take it out. a) infused channel for the test solution of marking material: b) Oesophageal catheter: c) Rubber stopper plate capable of moving along the catheter and of stopping at any desired place: d) An opening which was 20 cm above the funnel for taking the test solution out: e) The glass tube as a connector between oesophageal catheter and the rubber tube: f) Space between the glass funnel and the rubber tube: g) Glass funnel: the funnel

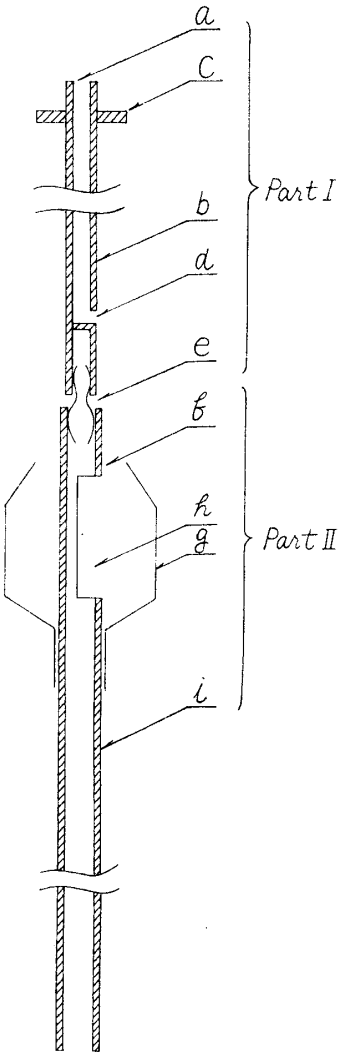

Fig. 1. Structure of model A.

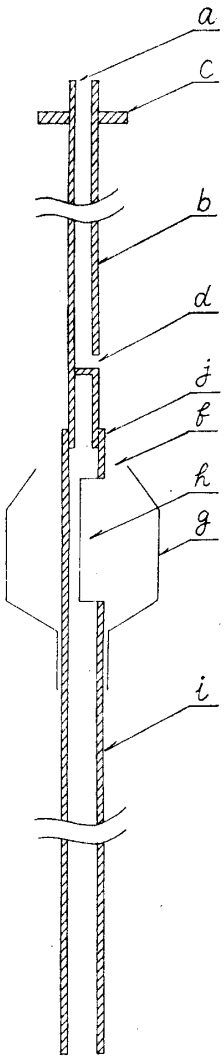

Fig. 2. Structure of model B.

was closely fixed wth the rubber tube without any sort of paste, by means of inserting the rubber tube (1.0 cm in external diameter) into the leg (1.0 cm in internal diameter) of the glass funnel. Since the rubber lose its elasticity and increased its hardness with age, the funnel will become easy to separate from the rubber tube as time goes on. h) Through an oblong opening of the rbber tube, the mixed saliva inflowed into the funnel and then flowed down into the rubber tube: i) A rubber tube 1.0 cm in external diameter and 0.7 cm in internal diameter was fixed to the glass funnel

Model B. The collection of mixed saliva with the use of the model A contrived in the preliminary experiments did not show satisfactory function due to the loss of saliva at the glass junction (e) which connected part I and part II. Namely, the glass junction (e) and oesophageal catheter (b) which were closely connected in appearance, were occasionally free from the joint because of the infiltration of greasy mixed saliva into the junction (e) after the funnel was installed. Model B was an improved type of model A. As shown in Fig. 2, the glass connector of the model A was removed, and the outer wall of the oesophageal catheter (0.7 cm in external diameter) and inner wall of the rubber tube (0.7 cm in internal diameter) were directly connected with gum arabic(j). Therefore, the installation procedure of model B was different from that of model A.

2. Installation of the intra-oesophageal funnel to the animal.

Model A. The oesophageal catheter (b) was inserted into the oesophagus from the nostril through the nasal cavity. Though the distance from the nostril to the pharynx varies with the size of the animal, the tip of the oesophageal catheter can reach the pharynx after feeling of a soft resistance at a distance of about 21 to 24 cm from the nostril. If the catheter stopped at a distance of about 15 to 16 cm from the nostril, we had to take every possible care to avoid the damage of the animal's tissues. In the second step, we had to pull up and push down the catheter very quietly for several times in order to insert it into oesophagus, keeping the animal body in normal position. If the animal neck was too streched, the catheter could easily come into the trachea. After the tip of the catheter went down through the oesophagus, the large rumen fistula was opened and the catheter was pulled out from the animal body through the rumen fistula with the hands. The glass funnel (g) was inserted into the oesophagus through the oesophageal orifice of the rumen side after connecting the oesophageal catheter (b) with the glass junction (e) fixed at the end of Part II. When the diameter of the glass funnel (g) was so large that it was difficult to insert it into the oesophagus, the animal would feel discomfortable, and displayed uneasiness under this condition. In additon, we might not obtain the true physiological observations because of excessive stimulation to the animal. When the

glass funnel (g) was so small that it could be inserted too easily, mixed saliva secreted by the animal might not be completely collected because the mixed saliva oozed out through the aperture between the wall of the oesophagus and the outer wall of the glass funnel (g). Therefore, before proceeding the experiments for salivation, it was necessary to choose one glass funnel just fit for a respective animal. Whether the glass funnel chosen was sufficient to collect the saliva almost completely could be checked with the recovery test of polyethylene glycole. A glass funnel suitable for an animal's oesophagus could be chosen after several trials of the recovery tests, but, as we were accustomed to the experiments, it became possible for us to select a suitable funnel without recovery test. The most suitable position to hold the glass funnel was about two-thirds below from the upper end of the oesophagus and the lower end of the funnel was about five cm upside from the oesophageal orifice of the rumen. However, the colletion of mixed saliva from the sheep could be completely proceeded even if the lower part of the glass funnel came out slightly into the rumen. When it was desirable to collect the mixed saliva for the purpose of the quantitative analysis of its contents, the glass funnel had to be inserted into the oesophagus after the rumen contents were removed through the large rumen fistula and the exposed oesophageal orifice of the rumen was washed with warm water of proper quantity, because of taking off the rumen contents which covered the orifice. Thus, the pure mixed saliva which did not contaminate with rumen contents was collected. The removed contents should be quickly returned to the rumen after insertion of the funnel. To prevent the funnel from being swallowed, a rubber stopper plate was inserted in the oesophageal catheter (b) from the free end and was placed on the animal's nostril. When the oesophageal catheter was too stretched, namely, when the distance of the catheter from the glass funnel to the rubber stopper plate was too short, we had to take care of the animal's discomfortabl feeling. The position of the stopper plate had to be placed about at the nostril under the condition of stretching posture of the neck and to be located at the place of a proper distance from the nostril under a proper posture. The proper graduation on the catheter was recomendable to correct the aberation from the position of the stopper plate. In actual experiment, the glass funnel and stopper plate did not hardly move if they were placed once in proper postition. The position of plate was about 50 cm from the funnel. At the center of the rubber disc with a thickness of 0.5 cm and with a diameter of 5 cm, a round piece was cut out by a corkborer as narrow as the oesophageal catheter which barely passed through the hole. The rubber stopper plate could be moved by our hands, though not be done by forces of swallowing of the animal. The movable rubber plate seemed to be convenient because it could be located at any place of the rubber tube depending on the animal size. The free end of the rubber tube which was led from the rumen

through the large rumen fistula, was connected to a vessel. The large cannula for rumen fistula consisted of two plastic plates with two holes and three rubber sponge plates. The one hole of the cannula was used for the rubber tube (i) for taking out the saliva and the other one for the returning the saliva into the rumen.

Model B. To set model B to the animal, the free tip of it from which the rubber stopper plate was removed, had to be firstly inserted into the oesophagus from the oesophageal orifice of the rumen in contrast to model A, since the glass funnel was fixed previously in the middle of two succeeded rubber tubes. It stopped once at a distance of about 70 cm upside from the oesophageal orifice where the pharynx was located. The catheter was easy to insert into the oral cavity under the normal posture of the body. It was a knack for the easiness of insertion to stretch up the neck as posible in this moment. When the animal showed the chewing movement owing to the misleading of the catheter into the oral cavity, it should be drawn back to the rumen side. By pushing up or drawing down the catheter several times, the tip of the catheter would be entered into the nasal cavity and come out through the nostril. The rubber stopper was put on the catheter and the rubber tube was pulled forward until the glass funnel was placed into the oesophagus. Subsequent operation of model B was same as model A.

Experiments, Results and Discussions

1. Recovery test of polyethylene glycole in the collection of whole mixed saliva from the sheep with use of an intra-oesophageal funnel.

An intra-oesophageal funnel was inserted to an adult sheep of 32.5 kg in body weight according to the way described above to know whether the funnel could collect the whole mixed saliva. The external diameter of the glass funnel used 3.5 cm. The test was started about one hour after feeding with 3.5 kg of orchard and red clover mixed grass. The polyethylene glycole as the marking material, 3500g in molecular weight, was diluted to the solution of 2 g/*dl*. This solution was infused into the channel (a) at the rate of about one *ml*/min from a 50 *ml* burette. As shown by Hydén (5), mixed saliva secreted immediately after the insertion was so viscous that it was doubtful whether mixed saliva and test solution mixed each other completely. For excluding the posibility that the sample for the determination of concentration of polyethylene glycole might not represent the fluid in each period, the sample and the remaining fluid were not returned into the rumen, and the whole fluid in each period was left for about 24 hours to counteract the viscosity of mixed saliva. After shaking the fluid to whip, polyethylene glycole was analyzed turbidimetrically by Hydén's method (12). Though the animal lost mixed saliva for about two hours for the recovery test of polyethylene glycole precisely, it was desirable to proceed

the experiment under physiological normal conditions in other cases.

Table. 1. Recovery of polyethylene glycole in the collection of mixed saliva from the sheep by the use of the intraoesophageal funnel.

Time in min	Period No.	Infusion of PEG		Recovery of PEG		Amount of Fluid *ml*			Output of Saliva			
									Calculated		Measured	
		ml	mg	mg	mg/*dl*	Collected	Calculated	Difference	*ml*/period	*ml*/hr	*ml*/period	*ml*/hr
0		Fistula opened										
11		Rumen contents partially removed										
17		Oesophageal catheter inserted										
23		Funnel inserted, rumen contents returned, infusion started										
43	I	23.5	470	167	0.74	225	633	−408	609	1827	201	603
63	II	20.0	400	414	1.61	257	248	+9	228	684	237	711
83	III	20.3	406	391	1.65	237	245	−8	225	675	217	651
103	IV	20.6	412	409	1.93	212	213	−1	192	576	191	573
123	V	20.6	412	392	2.00	196	206	−10	185	555	175	525
143	VI	20.8	416	421	2.60	162	160	+2	139	417	141	423
163	VII	20.1	402	378	2.28	166	176	−10	156	468	146	438
166	VIII	0	0	255	Infusion stopped, remained PEG was washed out							
Total			2918	2849								

As shown in Table 1, polyethylene glycole infused during experimental periods was 2.918 g and the collected amount was 2.849 g. The recovery rate of polyethylene glycole was 97.6 percent. This value means that polyethylene glycole was almost completely recollected if the experimental error was in consideration. Therefore, the quantitative collection method of mixed saliva from the sheep with the use of an intra-oesophageal funnel reported here satisfied our experimental purpose. Although there was apparently the difference of 408 *ml* between collected and calculated in period I, this value was not caused by the loss of mixed saliva through the aperture of the funnel and oesophagus, but polyethylene glycole solution of 23.5 *ml* infused for the first 20 minutes did not completely mixed with saliva in this period. This was proved by the fact that the maximum difference between collected and calculated fluid from period II to VII was only ± 10 *ml* and total collected fluid of 1230 *ml* was 98.5 percent of total calculated fluid of 1248 *ml* during this six periods. This value might also prove that the collection of mixed saliva from the sheep with this method was almost quantitative. In period VIII, the infusion of test solution was ceased and the remaining solution in the channel was washed out with a known amount of distilled water.

2. Measurements of total amount of mixed saliva in the sheep.

After demonstration of accuracy on the use of this method for the collection of the mixed saliva noted above, the amount of mixed saliva secretion was measured in seven sheeps. The results obtained are shown in Table 2. In this experiment, mixed saliva collected for each 20 minutes was returned into the rumen through the rumen fistula after the measurement of the secreted amount. Though there was little evidence to prove that the calculation of the daily amount of secretion of mixed saliva with the value of short time measurement was regarded correct, it seems valuable to calculate such value to know the

Table 2. Output of saliva from the sheep.

Animal	Period of collection	Total	Per hr	Per dey	Per day/kg B.W.	Body weights	Remarks
	min	*ml*	*ml*	*l*	*ml*	kg	
Sheep I	120	1107	554	13.3	400	32.5	immediately after feeding
II	100	493	296	7.1	230	30.5	four hours after feeding
III	120	635	318	7.7	280	27.5	immediately after feeding
IV	360	1160	193	4.6	250	17.5	before feeding
	180	513	171	4.1	230	17.5	before feeding
V	360	1565	261	6.3	160	40.0	before feeding
	200	1413	424	10.2	250	40.0	before feeding.
VI	1440	8016	334	8.0	230	35.0	immediately after feeding and following 24 hour's fasting.
VII	200	661	198	4.8	160	30.0	before feeding
	110	392	213	5.1	170	30.0	before feeding
	145	1046	432	10.4	350	30.0	before feeding

Sheep I to V fed orchard-red clover grass and sheep VI and VII fed orchard hay.

ability of the daily amount of mixed saliva secretion in a sheep. As shown in Table 2, the calculated data showed that the amount of mixed saliva secreted a day was about seven to eight liters and about 250 *ml*/day/kg of body weight. Since the oesophagus of animal was filled with the funnel in this method, mixed saliva secretion during mastication and rumination was unknown, the value was calculated as that the secretion continued with constant rate throughout a day.

We could use the intra-oesophageal funnel not only for the measurement of the amount of the mixed saliva secretion but for the some ruminological experiment (2) and for the mixed saliva secretion under different experimental conditions.

Summary

An intra-oesophageal funnel method was devised for the total collection of mixed saliva secreted by a sheep. The polyethylene glycole recovery test for the

determination of the accuracy of this method quite satisfactory. A calculation based on the data obtained relatively short time measurements was made to know the ability of daily amount of mixed saliva secretion. The result calculated showed that a sheep secreted about seven to eight liters of mixed saliva a day.

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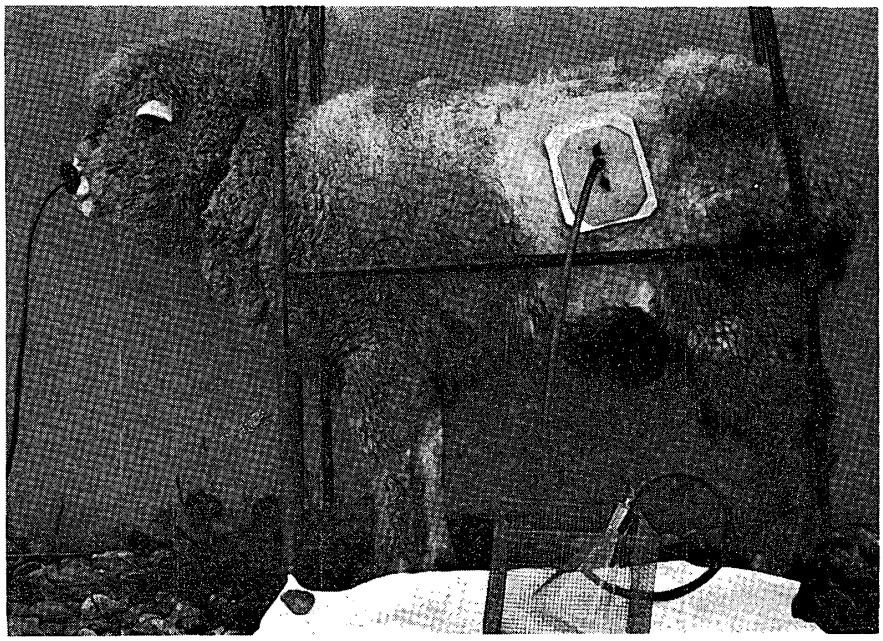

Plate 1. Collection of mixed saliva from the sheep.