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# Determination of parotid sulfate secretion in sheep by means of ultrasonic flow probes<sup>1</sup>

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**ABSTRACT:** The bilateral output of sulfate in parotid saliva, the relationship with its plasma level and with parotid flow, and its variation according to feeding behavior were determined in ad libitum, normal-sulfate (0.28% DM)-fed sheep (n = 6) using a transit time ultrasonic flow meter system to measure salivary flow. Ultrasonic flow meter probes were bilaterally implanted, under general anesthesia, around parotid ducts previously fitted through their oral ends with nonobstructive sampling catheters. Salivary flows were continuously recorded during 24 h, and saliva and blood samples for sulfate determinations were obtained hourly. Jaw movements were monitored with the submandibular balloon technique. The sulfate concentration in parotid saliva (mean of the group =  $4.9 \pm 3.7$   $\mu\text{g/mL}$ ) showed high variability between sheep (individual means from  $0.4 \pm 0.3$  to  $9.3 \pm 5.9$   $\mu\text{g/mL}$ ) and averaged 12.3% of the more stable plasma level ( $41.2 \pm 8.1$   $\mu\text{g/mL}$ ). Pronounced intraindividual variations were also evident (0.1 to 26.3  $\mu\text{g}$  of sulphate/mL of parotid saliva), in strong association with the fluctuations of salivary output. In 4 sheep, a decreasing exponential relationship was observed between parotid sulfate concentration

and salivary secretion rate ( $r^2 = 0.36$ ,  $P < 0.01$ ). This fact and the absence of a relationship between sulfate levels in plasma and in saliva suggest a sulfate secretory process during the passage of primary saliva through the ductal tree of the gland. The greatest rates of bilateral salivary sulfate output were observed during feeding ( $14.1 \pm 14.0$   $\mu\text{g/min}$ ) and rumination ( $12.7 \pm 11.0$   $\mu\text{g/min}$ ). Nevertheless, 49% of the sulfate output in parotid saliva was present during rest, as a result of the length of the resting times. The contribution of parotid sulfate to the ruminal S pool was highly variable and averaged 13.2 mg/d, representing less than 1% of the S intake. In conclusion, the accurate, reliable, non-obstructive, and bilateral salivary flow monitoring, using a previously characterized ultrasonic flow meter technique, allowed a detailed determination of the secretory dynamics of sulfate in parotid saliva, without disturbing the animal's routine or altering the physiological regulation of salivary output. The results indicated that, in the absence of S deficiency, the recycling of sulfate via saliva seems not to be a major factor in sheep nutrition.

**Key words:** feeding behavior, parotid saliva, sheep, sulfate secretion, ultrasonic device

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## INTRODUCTION

Inorganic sulfate is essential for the digestion of roughage and as a substrate for de novo synthesis of S amino acids by ruminal microorganisms. Sulfate entry into the forestomachs is provided by the diet (exoge-

nous) and by recycling from the blood (endogenous) through the ruminal wall and with saliva (reviewed by Kandyliis, 1983). Ruminal bacteria can obtain a significant proportion of their S from endogenous sources, mainly when the exogenous supply of S is restricted (Kennedy and Milligan, 1978). Estimations of endogenous sulfate input into the forestomachs of sheep vary considerably (0.1 to 30  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ , reviewed by Kandyliis, 1983). Divergence in data can be attributed to differences in type and S content of diets, methodological approaches, and measurement techniques.

Because the secretion of saliva is voluminous and continuous, this pathway may provide significant amounts of sulfate to the forestomachs. Reported values of the sulfate content of sheep saliva varied widely between 0.3 and 18.6  $\mu\text{g/mL}$  (Bray and Hemsley, 1969;

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Doyle et al., 1982), depending on diets and experimental conditions. Few investigations on the amount of sulfate flowing into the rumen via the saliva, or on the factors influencing its secretion, are available (reviewed by Kandyliis, 1983). Kennedy and Milligan (1978) estimated that about 87% of the recycled endogenous sulfate entered the rumen with salivary secretions. Unfortunately, these data should be considered with caution as precise determinations were difficult to obtain, owing to the shortcomings and specific problems related to cannulation techniques estimating salivary flow (reviewed by Méot et al., 1997).

The aims of this work were to determine the bilateral sulfate output in parotid saliva, the relationship with its plasma level and with parotid flow and its variation according to feeding behavior in sheep, using a previously characterized (Méot et al., 1997) ultrasonic flow meter system to measure salivary flow.

## MATERIALS AND METHODS

### *Animals and Diets*

Six adult Préalpes du Sud ewes (38 to 50 kg of BW), housed in individual pens (1 × 1.5 m) in a temperature-controlled room (20°C) with a natural lighting cycle, were fed ad libitum on a diet with a normal S level (0.28% on a DM basis, Alves de Oliveira et al., 1996). Diet composition was 60% alfalfa pellets (DM 89%; OM 893, CP 186, and crude fiber 276 g/kg of DM) and 40% hay (DM 90%; OM 923, CP 186, and crude fiber 295 g/kg of DM). Water was provided ad libitum. Considering the gregarious nature of feeding behavior and salivary reflexes (Denton, 1957), to promote social interaction sheep were always grouped 2 or 3 to a room. They were acclimated to these conditions for 10 d before surgery. Animals were cared for in accordance with international ethical guidelines, and the animal care procedures were approved by the Ethical Committee of the Ecole Nationale Vétérinaire de Lyon.

### *Surgical Preparation and Recording*

To measure bilateral parotid flow, transit time, ultrasonic flow meter probes (3 mm, R-series; Transonic Systems, Ithaca, NY) were implanted around both parotid ducts at the cheeks. For saliva sampling, nonobstructive polyvinyl catheters (i.d. 0.58 mm; o.d. 0.96 mm, Biotrol Pharma, Paris, France) were fitted into parotid ducts through their oral papilla, the free end exteriorized through the cheek and fixed to the skin on the top of the head. Both surgical procedures, previously described in detail (Méot et al., 1997), were performed under general anesthesia induced with intravenous sodium thiopentone (15 mg/kg of BW, Nesdonal, Specia) and maintained with halothane (0.5 to 2%) in O<sub>2</sub> (6 L/min). At the end of the surgery, 1 jugular vein was catheterized for blood sampling. Healing was without complications, and no specific postsurgical care was necessary.

Salivary flows were recorded by connecting the flow probes to a dual-channel flow meter (model T208; Transonic Systems). Mean parotid flows were recorded by connecting the mean output (0.1 Hz) of the flow meter to a physiograph (Narco Biosystems Inc., Houston, TX). To monitor feeding behavior, jaw movements were recorded by the submandibular balloon technique connected to the physiograph through a pressure transducer. Averaged parotid flows over 1-min periods were calculated and recorded by a computer coupled to the instantaneous output (10 Hz) of the flow meter.

### *Experimental Protocol*

Eight days after surgery, which allowed for full fibrous encapsulation of the probes as required for optimal ultrasonic signals, bilateral parotid flow and jaw movements were recorded during 24 h for each sheep. Samples of saliva (2 mL for each gland, by free flow or gentle aspiration) and blood (5 mL into tubes containing EDTA) were obtained hourly, with as little disruption as possible to the daily routine of the sheep. Blood samples were immediately centrifuged (5 min, 2,000 × g), and both plasma and saliva samples were frozen until analyzed. Probes were calibrated for zero and full-scale salivary flows at the beginning of the recording period. At the end of the experiments, the probes were validated for zero flow (ligatures of ducts proximally to the probes) and calibrated in situ by downward perfusion of the ducts at known rates with a syringe pump, as previously described (Méot et al., 1997).

### *Analysis and Calculations*

Plasma sulfate was determined by the turbidimetric method of Sörbo (1987). Because the sensitivity of this method is insufficient to measure extremely low concentrations of sulfate, in saliva samples sulphate was determined by controlled-flow anion chromatography according to the method of Cole and Landry (1985). The unilateral sulfate secretion (mg/min) was calculated as the product of the flow rate of each gland (mL/min) and the concentration of sulfate in the corresponding sample (mg/mL) over a 2 to 5 min interval. The bilateral sulfate secretion during sampling was calculated by addition of the left and right sulfate secretions. The mean concentration of sulfate in parotid saliva from both glands ( $[SO_4]_{sal}$ ) was calculated as the bilateral sulfate secretion divided by the total (left + right) flow rate during sampling. The sulfate clearance from blood to saliva during sampling was calculated as the bilateral sulfate secretion divided by the plasma sulfate concentration ( $[SO_4]_p$ ). The total amount of sulfate secreted during the experimental day was calculated from the computer-calculated parotid flow rate and the salivary sulfate concentration by linear extrapolation applied to the different chewing activities (eating, rumination, and rest). Feed intake was recorded daily to calculate the total S intake.

**Table 1.** Mean  $\pm$  SD salivary ( $[\text{SO}_4]_{\text{sal}}$ ) and plasma ( $[\text{SO}_4]_{\text{p}}$ ) sulfate concentrations, total (left + right) parotid flow,  $\text{SO}_4$  clearance, and bilateral sulfate secretion by the parotid glands during the experimental day (n = 24 samples)

Sheep	$[\text{SO}_4]_{\text{sal}}$ , $\mu\text{g/mL}$	$[\text{SO}_4]_{\text{p}}$ , $\mu\text{g/mL}$	Parotid flow, <sup>1</sup> L/d	$\text{SO}_4$ clearance, mL/min	$\text{SO}_4$ secretion, mg/d
1	5.0 $\pm$ 5.3	39.9 $\pm$ 2.6	3.53	0.15 $\pm$ 0.11	10.5
2	0.4 $\pm$ 0.3	40.7 $\pm$ 3.2	4.28	0.03 $\pm$ 0.02	1.1
3	5.2 $\pm$ 3.3	50.9 $\pm$ 6.7	4.72	0.25 $\pm$ 0.10	14.9
4	9.3 $\pm$ 5.9	49.9 $\pm$ 2.1	4.98	0.29 $\pm$ 0.13	29.3
5	8.6 $\pm$ 6.0	30.0 $\pm$ 1.2	3.62	0.58 $\pm$ 0.33	20.5
6	1.1 $\pm$ 0.8	35.9 $\pm$ 3.9	3.76	0.07 $\pm$ 0.06	2.6
Mean $\pm$ SD	4.9 $\pm$ 3.7*	41.2 $\pm$ 8.1	4.15 $\pm$ 0.61	0.23 $\pm$ 0.20	13.2 $\pm$ 10.8

<sup>1</sup>Values obtained by the addition of 1,440 1-min data.

\* $P < 0.01$  vs.  $[\text{SO}_4]_{\text{p}}$ .

## Statistics

Results were expressed as means  $\pm$  SD. Differences between plasma and salivary sulfate concentrations (Table 1) were evaluated by the Student's paired *t*-test. Significance for data in Table 2 was evaluated by 2-way (sheep and feeding behavior) ANOVA, and the means were compared using Fisher's test (Statview, SAS Inst., Inc., Cary, NC). Probability values less than 0.05 were considered statistically significant. An exponential regression analysis was performed to describe the relationships between  $[\text{SO}_4]_{\text{sal}}$  and parotid flow.

## RESULTS

Probe calibration showed good accuracy in the range of flow rates observed during experiments (0 to 10 mL/min). The slope of the linear regression analysis of implant-measured flow rate plotted against true flow rate (pump infusion rate) was not different from the line of identity based on 95% confidence limits.

Main results are shown in Table 1. Sulfate concentration in saliva averaged 12.3% of the plasma values (range 1.0 to 28.7%). A high degree of variability was observed in the mean salivary sulfate concentration

between sheep. Pronounced intraindividual variations of parotid sulfate level were also evident (values ranged from 0.1 to 26.3  $\mu\text{g/mL}$ ) in strong association with the fluctuations of salivary secretion rate (Figure 1). In sheep 1, 3, 4, and 5, parotid sulfate concentration was markedly flow-rate dependent, and an inverse exponential relationship between both variables was observed (Figure 2). In sheep 2 and 6, with extremely low sulfate level in saliva, the pattern observed in Figure 1 was less evident, and their parotid sulfate concentration showed no flow rate dependency. No statistical correlations were observed in any sheep between the amount of sulfate secreted and salivary flow rate, between sulfate concentrations in plasma and saliva, and between sulfate salivary clearance and parotid flow (data not shown).

Bilateral parotid sulfate secretion was studied during feeding, rumination, and rest periods (Table 2). Despite the reduced salivary sulfate concentrations due to greater salivation, the greatest rates of bilateral sulfate secretion were observed during feeding and rumination (although mean values were not significantly different from resting mean values). Nevertheless, the total amount of secreted sulfate was larger ( $P < 0.05$ ) during rest (49% of the daily amount), as a result of longer

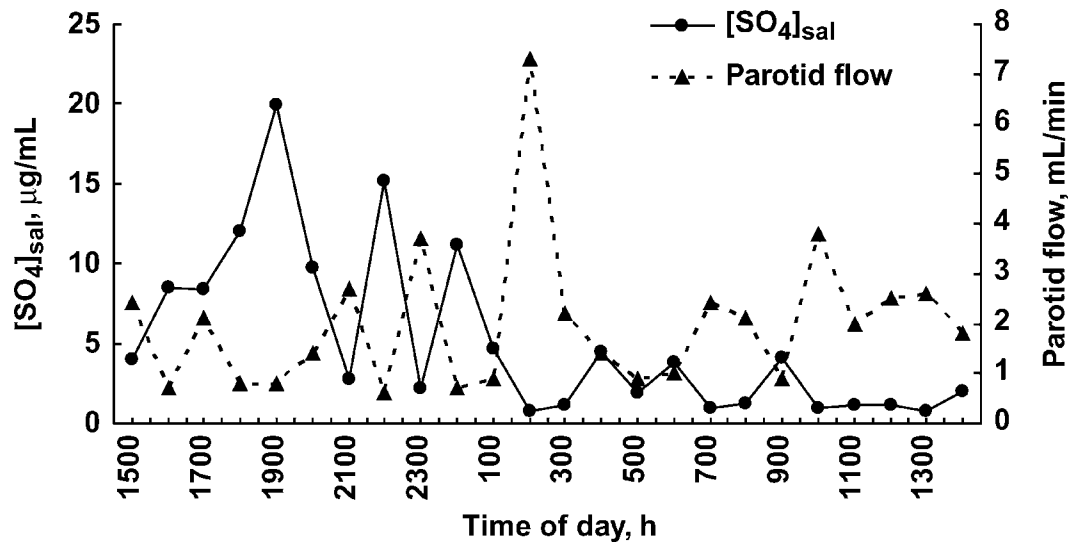
**Table 2.** Total (left + right) parotid sulfate secretion during eating, rumination, and rest during the experimental day<sup>1</sup>

Sheep	Bilateral sulfate secretion								
	Feeding			Rumination			Rest		
	$\mu\text{g/min}$	mg/d	% <sup>2</sup>	$\mu\text{g/min}$	mg/d	% <sup>2</sup>	$\mu\text{g/min}$	mg/d	% <sup>2</sup>
1	15.6	2.31	22	6.2	1.87	18	6.7	6.30	60
2	1.0	0.17	15	2.0	0.38	34	0.7	0.59	51
3	10.5	3.67	25	13.4	4.80	32	9.3	6.42	43
4	39.3	6.32	22	30.0	9.02	31	15.3	13.92	47
5	16.4	6.48	32	21.0	5.82	28	11.6	8.18	40
6	1.6	0.48	18	3.6	0.77	30	1.5	1.38	52
Mean	14.1 <sup>x</sup>	3.24 <sup>y</sup>	22 <sup>z</sup>	12.7 <sup>x</sup>	3.78 <sup>y</sup>	29 <sup>z</sup>	7.5 <sup>x</sup>	6.13 <sup>x</sup>	49 <sup>x</sup>
SD	14.0	2.76	6	11.0	3.37	6	5.7	4.87	7

<sup>x-z</sup>Between behaviors, values with different superscripts differ,  $P < 0.05$ .

<sup>1</sup>Values obtained by extrapolation of the data to 1,440 min on the basis of 1 min of observation.

<sup>2</sup>Percentage of the total amount of bilateral parotid sulfate secreted during the experimental day.



**Figure 1.** Values of salivary sulfate concentrations ( $[\text{SO}_4]_{\text{sal}}$ ) and total (left + right) parotid flow in 1 sheep during the experimental day.

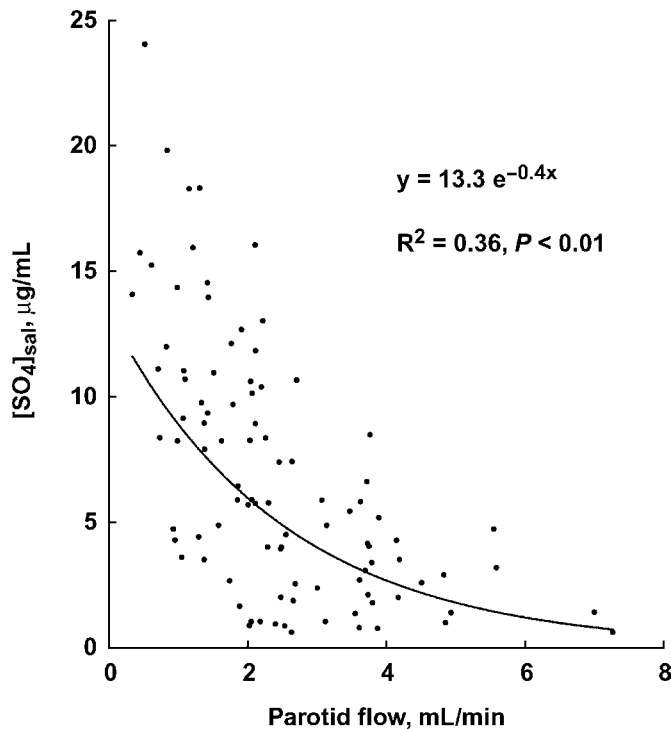
resting time. The total S intake during the experimental day was  $2.81 \pm 0.37$  g. Sulfate-S inputs into the rumen with parotid saliva were highly variable, ranging from 1.1 to 29.3 mg/d (means  $13.2 \pm 10.8$  mg/d and  $0.29 \pm 0.22$  mg/kg of BW). Therefore, the contribution of parotid sulfate-S to the ruminal S pool was  $0.45 \pm$

0.34% of the total S intake. These values were obtained without disturbing eating, rumination, or other routine ruminal functions.

## DISCUSSION

Our blood sulfate concentrations were in the range of values reported for sheep fed normal S diets (20 to 62  $\mu\text{g/mL}$ , reviewed by Bray and Till, 1975). As sheep were fed ad libitum, only weak circadian variations of plasma sulfate were observed. The greatest individual concentration (61  $\mu\text{g/mL}$ ) was similar to the maximum levels recorded for sheep by Bray and Hemsley (1969), who suggested that at this level its maximal tubular reabsorption is reached and the excess is excreted in the urine. Our results on salivary sulfate concentration were also within the range of previously reported values and, as observed in the literature (Bray and Hemsley, 1969; Kennedy et al., 1975; Doyle et al., 1982), were lower than plasma levels. The large variability in the salivary sulfate concentration between sheep (mean values ranging from 0.4 to 9.3  $\mu\text{g/mL}$ ) was also observed in other research. For example, Doyle et al. (1982) reported individual variations up to 5.5  $\mu\text{g/mL}$  from the mean values and Bray and Hemsley (1969) observed a high degree of variability not only between sheep but also with daytime. Concerning the parotid flow, reported values in sheep are extremely variable and generally estimated from only 1 fistulated gland (reviewed by Méot et al., 1997), so critical comparison with our results is difficult. Nevertheless, our data were in agreement with most of the reported daily volumes.

Bray and Hemsley (1969) observed an apparent relationship between the levels of sulfate in blood and in parotid saliva in sheep given different amounts of supplemental sodium sulfate: both levels are low in sheep fed a S-deficient diet and increase on S-rich diets. Other



**Figure 2.** Exponential decline of sulfate concentration in parotid saliva ( $[\text{SO}_4]_{\text{sal}}$ ) with increasing total (left + right) parotid flow in sheep 1, 3, 4, and 5, during the experimental day. Samples were obtained during eating, rumination, and rest.

reports (Kennedy et al., 1975; Kennedy and Milligan, 1978) also suggest that the rate of sulfate recycling to the rumen is related to sulfate concentration in blood. However, like Moir (1970) and Doyle et al. (1982), our data did not support this idea, and the relationship between the concentrations of sulfate in blood and saliva throughout the day suggests that its salivary secretion cannot be explained merely in terms of passive diffusion from blood to saliva.

Except for the extremely low parotid sulfate levels, a constant secretory pattern (salivary flow peaks corresponding with drops in salivary sulfate concentration and vice versa) was observed. It was expressed by a decreasing exponential relationship between sulfate concentration in saliva and parotid flow, a rather unexpected pattern as the electrolyte content of sheep parotid secretion is considered to be relatively independent of flow rate (reviewed by Cook, 1995). The results of Doyle et al. (1982) also suggested that when parotid saliva production is modified by changes in the coarseness of the roughage fed to sheep, the S concentration in saliva seems to be inversely correlated with the daily total saliva secretion. Nevertheless, the authors did not measure the parotid flow. Several inverse relationships have been described for salivary phosphate in the parotid gland of calves (Kay, 1960; Bailey and Balch, 1961). This secretory pattern may explain why the amount of secreted sulfate was not correlated to the parotid flow.

Both the absence of a relationship between sulfate levels in plasma and in saliva and the decreasing exponential relationship between salivary sulfate concentration and parotid flow observed in our work could be explained by accepting that the sulfate content in acinar saliva is modified during the passage through the ductal tree of the gland. The secretory pattern of sulfates related to salivary flow was similar to that described for potassium and phosphate secretion in the sheep mandibular gland (Kay, 1960) and for potassium secretion in monogastric parotid saliva (reviewed by Schneyer et al., 1972). In all these cases, active ductal secretory processes are involved (Schneyer et al., 1972; Cook, 1995), and at high secretory stimulation the concentrations of these substances in final saliva fall toward their concentrations in primary saliva. This hypothesis would also support why a linear relationship between the salivary clearance of sulfate and parotid flow, as observed for parotid urea secretion (Cirio et al., 2000), was not observed for sulfate.

A satisfactory explanation for the extremely low salivary concentration of sulfate in sheep 2 and 6, leading to a very reduced sulfate clearance and secretion rate, is unavailable. It cannot be attributed to a lower level of plasma sulfate or a greater parotid flow because these variables were not different from those of the other sheep. This particularity was coincident with the relative absence of variations in salivary sulfate level associated with changes in parotid flow and with the lack of a decreasing exponential relationship between both measurements. It is likely that at low sulfate concentra-

tion, its secretory pattern becomes independent of parotid flow rate.

In our ad libitum normal-S fed sheep, the contribution of parotid sulfate-S to the ruminal S pool was very small, less than 1% in any case. Considering that sheep parotid glands produce about 50% of total saliva (reviewed by Kay, 1966), and assuming that sulfate concentration in mixed saliva is similar to that in parotid saliva (Bray, 1969a), it is reasonable to propose that a mean of 26 mg/d of sulfate-S entered into the rumen with saliva. These values are in the range of those reported by Bray (1969b) and Kennedy et al. (1975) and not far from those of Doyle et al. (1982), who estimated this transfer from 33 to 62 (mean values on different diets) and from 5 to 125 mg/d (individual values). Nevertheless, Kennedy and Milligan (1978) reported greater values, showing that 127 to 159 mg of S entered daily into the rumen as endogenous sulfate, 110 to 140 mg of which entered via salivary secretion.

In conclusion, a high degree of variability was observed in the mean salivary sulfate concentration among sheep, and the values averaged 12.3% of its plasma level. It seems that an inverse exponential relationship exists between parotid flow and salivary sulfate concentration. This pattern and the absence of correlation between saliva and plasma sulfate concentrations suggest a ductal secretion of sulfates. The greatest input of salivary sulfates into the rumen was related to chewing activity (eating and rumination). Nevertheless, in the absence of S deficiency, the recycling of sulfate via saliva (less than 1% of the total S intake) seems not to be a major fact in sheep nutrition.

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