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Saliva secretion and its relation to feeding in cattle

1. The composition and rate of secretion of parotid saliva in a small steer

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As much as half the dry matter consumed by ruminants may be digested in the reticulo-rumen (Balch, 1958). Digestion in the rumen is brought about by enzymes of bacterial origin. The most efficient utilization of food will depend on the preservation in the rumen of an environment favourable for continuous bacterial activity and the addition of saliva to the rumen is undoubtedly an important means of maintaining such an environment. The high buffering capacity of ruminant saliva (Markoff, 1913; McDougall, 1948; Turner & Hodgetts, 1955) helps to stabilize the pH of the digesta and the fluid itself participates in the continuous-flow system which characterizes the reticulo-rumen and its contents (Adler & Dye, 1957).

The continuous flow of parotid saliva in the ruminant can be ascribed to a small, though essentially constant, basal flow which is not under nervous control (Eckhard, 1867; Babichev, Perstnev & Kulesco, 1930; Coats, Denton, Goding & Wright, 1956; Kay, 1958*a*) and a reflex secretion mediated through the parasympathetic nerve supply (Moussu, 1890; Kay, 1958*a*). The afferent stimuli for this reflex flow are derived from two main areas; these are the mouth on the one hand and parts of the reticulum, rumen, omasum and thoracic oesophagus on the other. The sensory stimuli which act in the mouth appear to be similar to those in other species (Krasusky, Krynskaya & Kotlyarevskaya, 1940; Coats *et al.* 1956; Somers, 1957; Kay, 1958*a*), and in the reticulum and rumen the effective stimuli are either touch or stretch applied to various regions of these organs or to the oesophagus (Clark & Weiss, 1952; Coats *et al.* 1956; Comline & Titchen, 1957; Ash & Kay, 1959; Kay, 1958*a*; Kay & Phillipson, 1959; Phillipson & Reid, 1958). Rumination, which involves areas in the stomach, oesophagus and mouth, has been shown to be particularly effective in the stimulation of parotid secretion (Babichev *et al.* 1930).

The recognition of many of these stimuli and the analyses of the reflex arcs involved have, of necessity, been carried out on anaesthetized animals or decerebrate preparations. The purpose of this paper is to supply information on the rates of secretion and the composition of parotid saliva from the conscious animal, in this instance a steer, in which one of the parotid papillae had been exteriorized. These measurements give the final result of the interplay of many factors upon the parotid gland.

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METHODS

The right parotid papilla was exteriorized in a 450 lb Friesian steer by the technique described by Denton (1957*a*). The animal was given 16 lb of good-quality hay daily in two meals and 100 g of sodium bicarbonate in its drinking water. A salt lick was provided at all times. The sodium bicarbonate was necessary to make good the loss of sodium by the animal. The experiments were conducted about 6 weeks after the operation, though the animal appeared normal and ate well from about the 1st week thereafter. The saliva flowed continuously from the papilla but the rate of secretion was slower during the 1st week after the operation than in the succeeding weeks.

Collection of saliva. Saliva was collected in a rubber funnel-shaped apparatus held lightly under the lower jaw and over the right cheek of the animal by two rubber straps, one over the muzzle and the other behind the ears (Pl. 1). Jaw movements were unhindered when the apparatus was in place. Fluid drained from the papilla into the collecting apparatus and was liberated from the lower end by releasing the pinch-clamp.

The rate of secretion of saliva by the parotid gland was measured over 2-min periods approximately every 5 min during two daytime periods. The periods began immediately after the morning meal and ended immediately after the evening meal. Collections were made by C.B.B. and every attempt was made to avoid conditioned salivary responses to his presence. For this purpose he spent some time with the animal, at irregular intervals during several days before the experiments except at the times when the animal was fed.

During two consecutive 24 h periods, complete collections of saliva were made by allowing saliva to drip from the animal's cheek on to an inclined metal trough and so into a collecting bucket. Inevitably some food spilled on to the trough from the feeding box at meal times. The amount of saliva which soaked into this food was estimated by drying samples of food and of spilled food to constant weight. No account was taken of evaporative losses of fluid from the collecting bucket. The animal was undisturbed throughout the collection period except at feeding times and when, once between each meal, the collecting bucket was emptied.

Composition of saliva. A random selection of samples secreted at various times relative to feeding time and including samples secreted during eating, rumination, and rest was collected on 4 different days. These samples were analysed for sodium, potassium, chloride, phosphorus, bicarbonate, total nitrogen, urea nitrogen, dry matter and ash. For several days, before the 4th day of collection, the sodium-chloride lick was removed and the sodium-bicarbonate supplement was withheld in order to observe the effects of a mild sodium deficiency.

The samples of saliva were clear and limpid. Immediately after collection, a small subsample was separated and stored under liquid paraffin. This sample was used for the determination of total CO_2. All samples were stored at 0° before analysis.

Chemical methods. Na was estimated by the method of Kramer & Gittleman (1924); K by flame photometry in an EEL (Evans Electroselenium Ltd) model flame photometer. The total CO_2 was estimated by the method of Conway (1957) and the results

were expressed as bicarbonate. P was estimated by the method of Allen (1940) and the results were expressed as HPO_4^{2-}. The method of Sendroy (1937) was used for the chloride determinations. Because of the small quantities of chloride in saliva, it was necessary to adjust the relative amounts of saliva and reagent to give final titration figures of suitable magnitude. These adjustments involved mixing larger proportions of sample with smaller proportions of acid reagent, the strength of which was increased. It was also necessary to add extra acid to neutralize the bicarbonate of the saliva. The total N content of the samples was determined by the micro-Kjeldahl technique. Urea was determined by the method of Conway (1957). The dry-matter content of the samples was measured by drying 10 ml portions to constant weight in porcelain

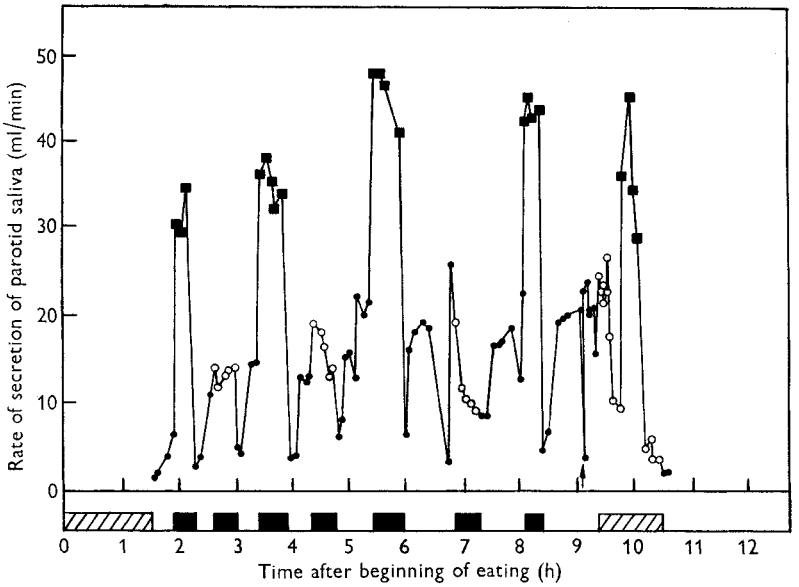

Fig. 1. Rate of secretion of saliva from the right parotid gland of the steer throughout a single daytime cycle beginning immediately after the morning meal and ending soon after the evening meal. Solid bars, periods of rumination; hatched bars, periods of eating; ■, periods when chewing occurred on the operated side of the mouth; ○, periods when chewing occurred on the unoperated side of the mouth; ●, periods when there was no chewing; ↑, teasing with hay.

crucibles. The dried residue was subsequently ashed in a muffle furnace at 550°. All determinations were performed in duplicate and repeated if the replicates differed by more than ±2%.

RESULTS

Rate of secretion of parotid saliva. The results of the serial measurements of the salivary secretion on the 2nd day of experiment are depicted in Fig. 1. They were similar in every essential detail to those recorded on the 1st day.

The rate of secretion of parotid saliva varied from 48 ml/min to 1·2 ml/min, and this wide range was characteristic of a well-defined sequence of changes occurring throughout the period of measurement. The rate of secretion immediately after a

meal was very low. Thereafter, during periods of rumination, the rate of secretion increased markedly, especially when mastication occurred on the operated side of the mouth. When mastication occurred on the other side, the rate of secretion was not very different from the mean rates noted during periods of rest.

There were sharp transient declines in the rate of secretion immediately after periods of rumination, regardless of the side on which the animal had been chewing in the preceding period. Secretion increased abruptly thereafter to high rates immediately before the next rumination period. There was, in addition, a consistent rise in the mean rates of secretion during rest periods from about 5 ml/min during the rest period immediately after eating to about 20 ml/min during the rest period immediately before the next meal.

At the beginning of each meal, when chewing was confined to the unoperated side of the mouth, the rate of secretion was somewhat greater than during the previous resting period. Thereafter, secretion declined precipitously to about 5 ml/min on day 1 and 9 ml/min on day 2. Secretion increased markedly while the animal chewed on the operated side of the mouth (Fig. 1), but with subsequent chewing on the other side secretion was only slightly greater than immediately after the end of eating. Similar declines throughout the meal were noted on several other occasions. During eating, when mastication occurred on the operated side of the mouth, secretion was generally less than during periods of rumination in which mastication occurred on the operated side.

It is noteworthy that the side on which mastication occurred never altered during a period of rumination and that mastication on one side was invariably followed by mastication on the other side during the next period of rumination.

The fact that mastication usually began on the unoperated side of the mouth and then switched to the operated side later in the meal was the only indication that the unoperated side was favoured. In sheep, both Scheunert & Trautmann (1921) and Denton (1957*a*) observed that animals tended to masticate more on the unoperated side during rumination and eating.

At the afternoon meal time on each day of the experiment the animal was 'teased' with hay for several minutes before it was fed, which caused parotid secretion to decrease temporarily from about 20 to about 4 ml/min for a short interval, followed by a small transient increase shortly before the animal was given its food.

The mean rates of parotid secretion during periods of eating, rumination, and rest were about 20, 25 and 10 ml/min, respectively, including periods of chewing on each side of the mouth. The total volumes of saliva collected in 24 h on the two occasions were 21·9 and 23·8 l. If it is assumed that both parotid glands of the experimental animal were equally active, together they would have secreted approximately 40, 50 and 20 ml/min during periods of eating, rumination and rest, respectively, and the total secretion would have exceeded 40 l. in 24 h.

Composition of saliva. Large variations were noted in the concentrations of the various constituents of parotid saliva. These variations were not associated with the time of day or with the activity (e.g. eating, rumination, rest) of the animal when they were collected. Differences in the composition of certain of the samples were due to

the withdrawal of the sodium chloride and sodium bicarbonate usually available. The ensuing body deficit of Na led to a decrease in the concentration of Na in the saliva with a commensurate increase in the concentration of K; at equivalent rates of secretion, the Na concentrations in the saliva were 20–30% lower than those in saliva collected when the animal was receiving adequate Na.

Induced Na depletion did not have a measurable effect on the concentrations of bicarbonate and phosphate in the saliva. All the values for the concentrations of

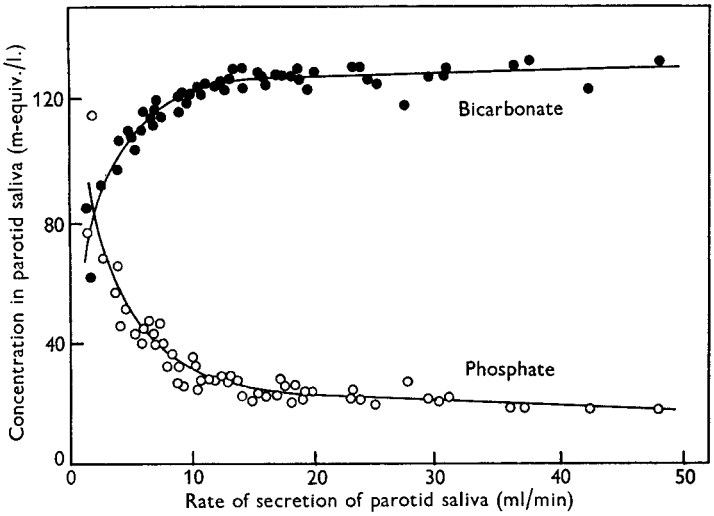

Fig. 2. Concentrations of bicarbonate and phosphate in parotid saliva of the steer at different rates of flow. The positions of the best-fit lines were judged by eye.

Table 1. *Concentrations of sodium, potassium, chloride, bicarbonate and phosphate in parotid saliva secreted at rates exceeding 12 ml/min by the steer when receiving enough sodium chloride. Values are also shown for changes in the concentration in saliva with changes in secretion rate* and for the plasma concentration*

Ion	Concentration in saliva secreted at rates exceeding 12 ml/min (m-equiv./l.)	Estimated composition of saliva secreted in excess of 1·2 ml/min* (*a*) (m-equiv./l.)	Plasma concentration (*b*) (m-equiv./l.)	*a*/*b*
Sodium	157	164	139†	1·18
Potassium	7·0	4·0	4·7†	0·85
Chloride	7·4	5·1	105†	0·05
Bicarbonate	127	131	28‡	4·68
Phosphate	23	15	2·9§	5·17
Total cations	164	168	—	—
Total anions	157	151	—	—

* Values taken from the slope of the regressions in Figs. 3 and 4 (see pp. 378, 379).
† Bailey (1958, unpublished).
‡ Fisher (1959).
§ Blackwood & Wishart (1936).

these two ions in parotid saliva have, for this reason, been used in calculating the effects of secretion rate on saliva composition. The concentrations of chloride were reduced slightly during Na depletion and, accordingly, only the samples collected when Na salts were available have been used in deriving the relationship between the secretion rate and the chloride concentration.

The variations in the concentrations of Na, K, chloride, bicarbonate and phosphate which were not attributable to loss of Na were strongly associated with the rate of secretion of the saliva. Increased secretion was accompanied by increased concentrations of bicarbonate and Na and by decreased concentrations of K, chloride and phosphate. The relationship between the rate of secretion and the concentrations of bicarbonate and phosphate is shown in Fig. 2. Above a secretion rate of about 12 ml/min, the concentrations of these ions changed only slightly as the secretion rate increased, but had not reached steady values at the highest rate recorded. Table 1 shows the mean composition of the saliva samples secreted at rates in excess of 12 ml/min.

There was no indication that the rate of parotid secretion influenced the concentration of total N or urea N in the saliva, although considerable variations occurred. The percentage of the total N represented by urea was very consistent regardless of the rate of secretion or of the concentration of total N in the saliva. The mean values for the various nitrogenous fractions were as follows:

N fraction	Concentration in saliva (mg/100 ml)	Percentage of total N
Urea N	6·28	82·8
Non-urea N	1·29	17·2
Total N	7·57	—

Thus urea N accounted for more than 80% of the total N. These values are higher than those for sheep reported by Somers (1957) in which urea accounted for 65–70% of the total salivary N.

The mean dry-matter and ash contents of the saliva were 1·05% ± 0·07 and 0·91% ± 0·06, respectively.

DISCUSSION

The observations made in the course of the experiment invite speculation on the factors controlling the rate of secretion of parotid saliva and the composition of the parotid saliva produced at various times.

Rate of secretion of parotid saliva. Wide variations occurred in the rate of secretion of parotid saliva throughout the experimental period and the rates were related to the activity of the animal at the time of measurement. Thus, the secretion was least immediately after periods of eating and periods of rumination and greatest during mastication, but only when it occurred on the operated side of the mouth. The rate of secretion was intermediate during mastication on the unoperated side of the mouth or during periods of rest.

Coats & Wright (1957) found a mean basal secretion rate of 0·21 ml/min from denervated parotid glands of sheep. On the basis of equal basal secretion rate per

unit body-weight, a basal secretion rate of 1·0 ml/min might be expected from the exteriorized papilla of our animal. The rate of secretion found immediately after eating (1·2 ml/min) may have been very close to the basal rate. Because stimulation of tactile and stretch receptors by the contents of the reticulo-rumen would tend to provide a continuous excitation of parotid secretion superimposed on the essentially constant basal secretion, the resting secretion rates noted in one animal would suggest that, at these times, the efficacy of the background stimulation was reduced. This effect may have been due to a reflex inhibition caused by sympathetic vasoconstriction (Kay, 1958*b*) or to an inhibition of central origin. It is unlikely that it was due to exhaustion of the gland or its secretory nerves since Moussu (1890) showed that the parotid gland of a cow continued to secrete for 90 min at a high rate without any apparent histological change in the structure of the gland.

It is not clear what factors were responsible for the increased parotid secretion during mastication on the operated side of the mouth. Conceivably, massage of the gland due to the masticatory movements could have produced this effect. On the other hand, the observation of Kay (1958*a*) that the rate of parotid secretion increased in sheep after tactile stimulation of the mouth in the area between the molar teeth and tongue might suggest that the greater flow during mastication was a reflex response to stimulation of receptors in the mouth. Lack of parotid ensalivation of boluses chewed on the operated side could have been responsible for their dryness and have led to an increased afferent stimulation of the parotid gland and a consequently exaggerated secretion. It remains to be shown, however, that the sensitive areas of the mouth are stimulated more on the side on which mastication is taking place than on the opposite side and that the degree of stimulation is greater during ruminating than during eating. It is also difficult to envisage a sufficiently one-sided stimulation of chemoreceptors to suggest their involvement even in an augmentory role. For a similar reason it seems unlikely that receptors in the oesophagus and reticulo-rumen were directly involved to any great extent.

The gradual increase of mean resting rates of secretion throughout the interval between meals occurred on both days of the experiment and corroborated a similar increase observed in the secretion rate of mixed saliva in cows (Bailey, 1959). There was also, between meals, a gradual increase in the mean rates of parotid-saliva secretion during rumination with chewing on the operated side of the mouth. In the interval between meals the mean rate of secretion increased by about 13 ml/min during such rumination and 15 ml/min during rest. This finding implies that, if the low rate of secretion after eating was due to an inhibition of reflex stimulation, this inhibition was effective at the markedly different intensities of reflex stimulation displayed during rumination and resting. It is possible that an inhibitory response was initiated by the act of eating *per se*.

The observation of a sharp decline in secretion while the animal was teased with its food is at variance with the results of Denton (1957*b*) and Somers (1957) with sheep. These authors showed that the sight or smell of food or the noise of food being handled or eaten usually evoked increases in the rate of secretion of parotid saliva. On the other hand, Colin (1886), Scheunert & Trautmann (1921) and Scheunert,

Krzywanek & Zimmermann (1929–30) could find no clear evidence for psychic stimulation of the parotid glands. No explanation can be offered for these differences.

Composition of parotid saliva. Coats & Wright (1957) have shown that the tonicity of the parotid saliva secreted at the basal rate in sheep remained as high as that of saliva secreted at faster rates; the concentrations of the ions in basal saliva, however, differed from those in saliva secreted at faster rates. With increases in the rate of

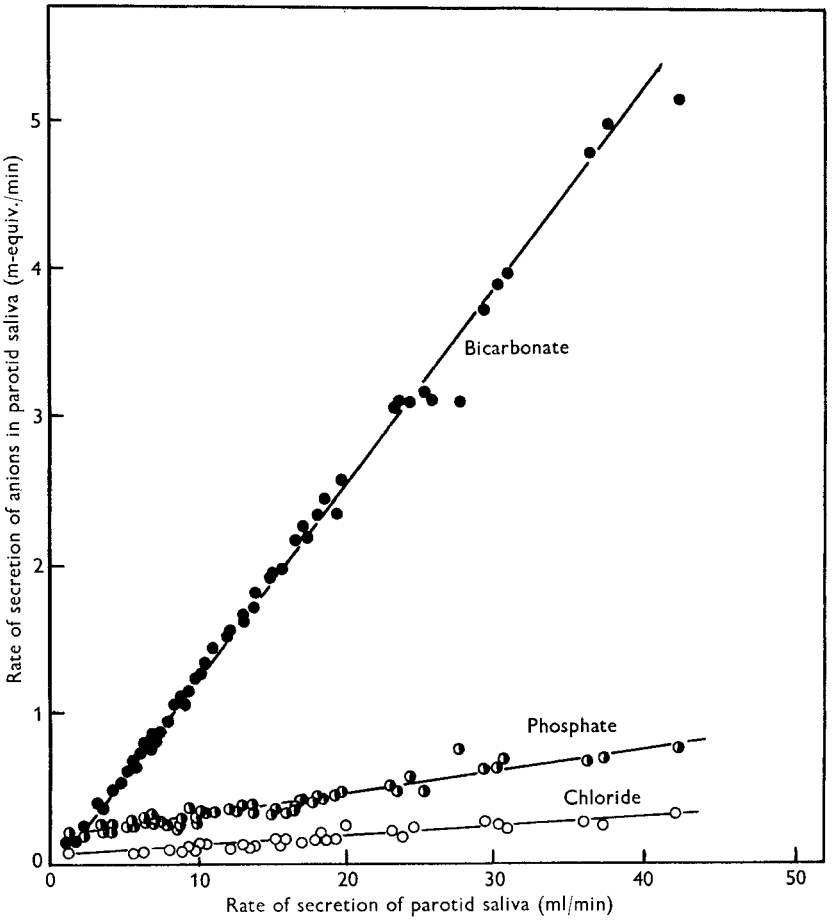

Fig. 3. Secretion rates of the anions in parotid saliva of the steer at different rates of flow.

secretion the K and phosphate concentrations tended to decrease and the Na and bicarbonate concentrations tended to increase. The chloride concentrations either did not change or decreased. These relationships were essentially similar to those described here for the parotid saliva of the steer, in spite of the fact that in the sheep the rate of secretion was varied by electrical stimulation of the motor nerve at various frequencies.

It seems reasonable to assume that the relationships shown in Figs. 2–4 are valid expressions of the secretory characteristics of the parotid gland of this steer despite

the fact that there may have been small fluctuations in rate of secretion while each sample was being collected. The composition of the samples secreted at very low rates of flow suggested that basal saliva, of the kind examined by Coats & Wright (1957) in sheep, would contain in the steer about equal concentrations of Na and K and of bicarbonate and phosphate.

The relationship between the rate of secretion of parotid saliva (as ml/min) and the rate of secretion of the ions (as m-equiv./min) reveals certain characteristics of

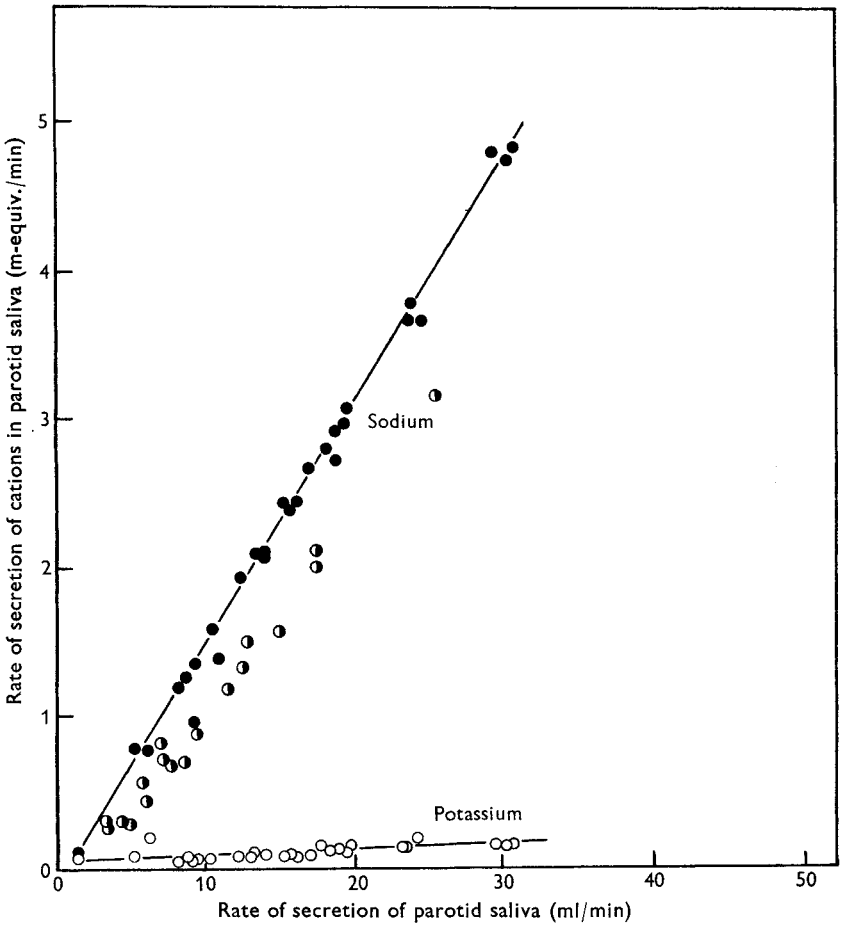

Fig. 4. Secretion rates of the cations in parotid saliva of the steer at different rates of flow. ◑, the rate of sodium secretion in saliva produced when the animal was in a state of mild Na deficiency.

the transfer of water and of the ions from plasma to saliva, as illustrated in Fig. 3 for the anions and in Fig. 4 for the cations. When the rate of secretion of parotid saliva exceeded 1·2 ml/min the rate of transfer of each of the ions was linearly related to the rate of transfer of water. Thus, although the amount of saliva secreted per min in excess of 1·2 ml might vary, the ratio of the additional amount of each ion to the additional amount of water secreted remained constant. These ratios are given by the

slopes of the lines in Figs. 3 and 4. The values of the slopes are given in Table 1. At high rates of parotid secretion the composition of the fluid approached that given by the slopes of the lines in Figs. 3 and 4 and the composition was not markedly different from these values above a secretion rate of about 12 ml/min.

Thaysen, Thorn & Schwartz (1954) showed that the concentration of Na but not of K changed with increasing rates of parotid-saliva secretion in man. This result was interpreted as evidence for a process of limited reabsorption of Na from a hypothetical precursor solution. A similar type of mechanism could also explain the changes in the composition of the saliva in our animal which accompanied changes in the rate of secretion. However, considerably more complex shifts of ions and water would be required in order to preserve the uniform tonicities which were noted in the saliva at all rates of secretion.

The composition of saliva secreted at the basal rate in our animal is not known but, as was suggested above, it was probably similar to that of the saliva collected at the lowest rate of secretion (1·2 ml/min). It was undoubtedly very different from the composition of increments of saliva secreted in excess of the basal rate. The secretion of basal saliva is not under nervous control (Coats *et al.* 1956; Kay, 1958*a*) and the mechanism of this secretion may well be different from and independent of the secretory mechanisms initiated by nervous excitation of the gland. If so, an alternative scheme to that of Thaysen *et al.* (1954) may explain the changes in saliva composition resulting from changes in the rate of secretion. Thus, if the basal flow were small and fairly uniform and the magnitude of the increments of flow above basal varied greatly, the ultimate composition of any sample of saliva would depend on the relative sizes of the basal flow and of the flow in excess of the basal flow, that is to say on the rate of secretion itself. In this scheme, the greater the flow in excess of basal, the more the composition of the total saliva would tend to the composition given by the slopes of the lines in Figs. 3 and 4. This is exactly the result depicted in Fig. 2 for bicarbonate and phosphate.

Na and K constitute about 98% of the cations of saliva (McDougall, 1948) and gross Na depletion in sheep causes much of the Na in saliva to be replaced by K without concurrent changes in the tonicity of the fluid (Denton, 1956). The extent of this change depends on the severity of the Na deficit. When Na loss was not replaced in our experiments, this ion was replaced by K to the extent of about 20–30% of the Na in saliva collected from the adequately nourished animal. Although the 'extra-basal' secretion rate of the Na ion was reduced relative to that of water, the ratio of these two constituents of saliva was nevertheless fairly constant over the range of values recorded in Fig. 4. The slope of the line for the Na-deficient saliva samples was 136 m-equiv./l. compared to 164 m-equiv./l. for the samples collected from the steer when given enough Na. This may have been a response to lowered plasma concentrations of Na, although evidence from the experiments of Denton & McDonald (1957) shows that the effect of variations of concentrations of Na and K in the saliva are mediated by mineralocorticoids of the adrenal cortex. The results reported in this paper suggest that there is no simple relationship between the behaviour of the animal and the rate of secretion or the composition of the parotid saliva.

SUMMARY

1. The rate of secretion of saliva from the right parotid gland of a small steer in which the right papilla was exteriorized was measured over 2-min periods approximately every 5 min during the period beginning immediately after the morning meal. In addition, saliva samples secreted at rates of 1·2–48·0 ml/min were collected at random and examined for their concentrations of sodium, potassium, chloride, bicarbonate, phosphate, urea, total nitrogen, dry matter and ash. A few of these samples were obtained when the animal suffered from a mild Na depletion.

2. Chewing on the operated side of the mouth was the most effective stimulus to saliva secretion. When chewing occurred on the opposite side the rate of secretion was raised little or not at all above that found during periods of rest.

3. The mean rates of secretion found at the end of each period of rest increased from about 5 ml/min immediately after eating to about 20 ml/min immediately before the next eating period. This pattern of gradual increase throughout the period between meals was also observed in the mean rates of parotid secretion during rumination, when mastication occurred on the operated side of the mouth.

4. The mean rates of parotid secretion (from one gland) during eating, rumination, and periods of rest were about 20, 25 and 10 ml/min, respectively. The total amounts of saliva collected from the exteriorized papilla in two consecutive 24 h periods were 21·9 and 23·8 l.

5. The concentrations of Na and bicarbonate increased, and of phosphate, K and chloride decreased as the rate of parotid-saliva secretion increased. Variations in the concentrations of the ions, however, were not associated with the time of day or with the activity of the animal when they were collected. Na depletion reduced the concentration of Na in saliva with a commensurate increase in the K concentration. The relationship of the rates of secretion of the ions in saliva (as m-equiv./min) to the secretion rate of the saliva itself was linear.

6. There was no relationship between the rate of secretion of saliva and the total N or the urea N concentrations in saliva. Urea N averaged 82·8% of the total N of parotid saliva.

7. The mean dry-matter and ash percentages of the parotid saliva samples were 1·05 and 0·91 respectively.

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EXPLANATION OF PLATE

Head of the steer showing in place the apparatus for collecting saliva. Saliva was liberated by releasing the pinch-clamp.

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