Saliva secretion and its relation to feeding in cattle

2.* The composition and rate of secretion of mixed saliva in the cow during rest

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Bailey & Balch (1961*b*) described the secretory characteristics of the right parotid gland of a small steer. Parotid saliva is one of several salivary secretions produced by ruminants (Dukes, 1955) and the saliva entering the rumen at any given time is a mixture of the individual fluids. Although little is known of the relative amounts of the different fluids secreted under different conditions, information on the activities of some of the glands is available. Both the parotid and the sublingual glands secrete continuously but, whereas parotid saliva predominates in the mixed fluid (McDougall, 1948; Hydén, 1958), the amounts of sublingual saliva are relatively very small (Colin, 1886). The submaxillary glands are quiescent except during eating, when they may secrete almost as much fluid as the parotid glands (Ellenberger & Hofmeister, 1887). In the opinion of Scheunert, Krzywanek & Zimmermann (1929–30), mixed saliva may also contain considerable quantities of the secretions of the ventral buccal glands.

Alterations of the rate of secretion of mixed saliva and of the chemical properties of the secretions can occur as a result of changes in the relative amounts of the component fluids. Such alterations could have an important bearing on the control which mixed saliva exercises on the environment within the reticulo-ruminal sac and indirectly on the activities of the micro-organisms responsible for the digestion of food in the reticulo-rumen. Different diets will tend to vary the environment in the reticulo-rumen, and it is a matter of considerable interest to know what effect the changes might have on the rate of secretion and composition of mixed saliva.

The present communication describes several experiments with cows in which the rate of secretion and chemical properties of mixed saliva were measured at various times relative to the ingestion of different diets. All measurements were made during periods of rest, when the animals were standing but neither eating nor ruminating. A preliminary report of some of this work has been given (Bailey, 1959).

EXPERIMENTAL

Measurement of the rate of secretion of mixed saliva. Mixed saliva was collected quantitatively as it descended into the rumen through the cardia from five dry, Shorthorn cows with long-established rumen fistulas. When saliva secretion was to be

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measured, the bung closing the rumen fistula (Balch & Johnson, 1948) was removed and the rumen was emptied by hand to the point where free access of the hand to the cardia was possible. The level of the contents in the anterior sac of the rumen was lowered to approximately the level of the relaxed anterior pillar. It was necessary to remove 40–70 lb digesta, depending on the diet and the time of day. The reticulum and the ventral sac of the rumen remained full at all times. The animals generally showed little interest in the emptying procedure and rarely interrupted their activities (e.g. drinking, licking, ruminating) while it took place.

Tactile stimulation of certain areas of the reticulum has been shown to augment the secretion of parotid saliva in sheep (Coats, Denton, Goding & Wright, 1956; Comline & Titchen, 1957; Ash & Kay, 1959). Particular care was exercised, accordingly, to prevent stimulation of the sensitive areas during the emptying procedure and, as an added precaution, about 5 min were allowed after emptying before saliva collection began.

For the collection of saliva, a small rubber bag (Pl. 1) was held in the hand and poised near the cardia. When the animal was seen to swallow, the collecting bag was quickly but lightly placed over the cardia, whence the saliva descended into the bag. The bag was then rapidly withdrawn, the volume of saliva was measured and the bag again held in readiness for the next swallow. The 20–40 sec which elapsed between each swallow was a sufficient time for the removal and measurement of the saliva before the next swallow. The bag was held against the cardia for about 3 sec at each swallow and it was assumed that it stimulated no excess flow of saliva. During each of the regular reticulum contractions the level of the fluid in the reticulum rose high enough to submerge the cardia, which did not upset the collecting routine since of about 8000 swallows collected only five or six were during a reticulum contraction. The swallowing of the saliva was not especially related to any other phase of the contraction cycle in the reticulo-rumen.

Two groups of ten swallows each were collected for each estimation. Although large differences were frequently encountered between the secretion rates measured during the first and the second group of ten swallows, the overall mean values calculated from the values for all the cows were very similar: 58 ml/min for the first and 59 ml/min for the second collection. In all instances where individual secretion rates are recorded in this paper they are the means of the first and second collections.

It was frequently observed that during several minutes before rumination began an increase in the rate of secretion of saliva occurred, and, further, that a period of decreased rate occurred immediately after rumination. All saliva collections were made at least 5 min after a period of rumination, and if rumination occurred within 5 min of the completion of a period of saliva collection the results were discarded and the collection was repeated. The collection of saliva was frequently interrupted by a period of attempted rumination, especially when the animals consumed diets high in fibre. The rumens were emptied to a level that tended to prevent the regurgitation of digesta. In some animals, however, the attempts were exceedingly persistent and the collection of saliva was then terminated and resumed at least 5 min after the cessation of the attempted rumination.

Collection of samples for chemical analysis. A representative number, usually ten, of the twenty saliva swallows collected at each measurement of the rate of secretion were mixed and retained for chemical analysis, the remainder being returned to the rumen. Two small subsamples were separated from the main sample and placed one in each of two test-tubes immediately after the main sample was obtained. The tubes were closed with rubber stoppers in such a way that no air spaces appeared between the menisci and the bottoms of the stoppers, which prevented the further loss of CO_2. One of the subsamples was used for the measurement of pH and the other for the determination of CO_2 and urea. When the analyses were delayed longer than about 30 min, the rubber bung was removed and a thin layer of liquid paraffin was poured on to the surface of the saliva.

The main saliva sample was centrifuged at 2900 *g* in an angle-head for 30 min to remove fine particles of debris. The centrifuged samples were clear and occasionally faintly coloured. This colour was due to very slight contamination with rumen fluid, and its possible effect on the composition of the sample has been ignored. The samples were kept frozen until ready for analysis. They were analysed for sodium, potassium, chloride, phosphorus (expressed as HPO_4^{2-}), carbon dioxide (expressed as HCO_3^-), total nitrogen, urea nitrogen, dry matter and ash. The pH of saliva and rumen fluid was also determined each time the secretion of saliva was measured. The pH of the rumen fluid was obtained to give a rough indication of the level of the fermentative reactions occurring in the rumen at any given time. The concentrations of total volatile fatty acids (TVFA) were determined in some of the samples of rumen fluid. The sampling procedure for the rumen fluid has already been described (Bailey & Balch, 1961*a*). The chemical methods were the same as those previously described (Bailey & Balch, 1961*b*).

Table 1. *Expt* 1. *Mean daily intake of sodium, potassium, chloride and phosphorus by cows receiving the five diets*

	Daily intake (g)			
Diet	Sodium	Potassium	Chloride	Phosphorus
Silage	14·2	177	74	24·6
Hay	4·7	92	33	7·5
Hay and dairy cubes	16·0	105	44	40·1
Hay, flaked maize and groundnut cake	6·6	38	7	12·4
Grass	4·7	244	124	24·8

About sixty samples of jugular venous blood were withdrawn from four cows in Expts 1 and 5 within 15 min of the collection of the saliva samples. The urea content of plasma from these samples was determined by the technique of Conway (1957).

Feeding procedure. In all experiments the cows were given food twice daily, at 07.30 h and 17.00 h, and received half their daily quota of food at each meal. They received water *ad lib.* from drinking bowls. Sodium chloride was supplied *ad lib.* to the animals in Expt 4 and one massive dose was offered to the animals in Expt 5. Otherwise, no salt supplements were given to the animals. The amounts of Na, K, chloride and P supplied daily in each diet in Expt 1 are shown in Table 1.

Expt 1. The rate of secretion of mixed saliva was measured when at least two of the four cows (A, B, C and D) were receiving one of the following diets: 40 lb of good-quality lucerne silage; 14 lb of medium-quality hay; 8 lb of hay and 12 lb of dairy cubes; 12 lb of flaked maize, 2 lb of groundnut cake and 2 lb of hay; a freshly cut mixture of cocksfoot and rye-grass supplying 12 lb of dry matter daily. Four measurements were made daily with each animal and were repeated on 3 consecutive days. The first measurement was made before the morning meal (14 h after the previous meal) and the other three were made at 1, 4 and 8 h after the morning meal. It has been assumed that the measurement made 14 h after the evening meal was similar in all respects to that which might have been made 14 h after the morning meal. Time was always measured from the beginning of eating. The water consumption was measured daily.

Expt 2. To ascertain the effects on saliva secretion of low pH values in the reticulo-ruminal contents, the pH of the contents in four cows (A, C, D and E) was adjusted toward the lower extreme of the normal range by the addition of acetic acid. This adjustment was accomplished by removing about 50 lb of contents, mixing into them 300 ml of glacial acetic acid (diluted several times with water) and returning the mixture to the rumen, and was done once with each cow. Saliva secretion was measured $\frac{1}{2}$ h before the addition of acid, and $\frac{1}{2}$ h, $1\frac{1}{2}$ h and 3 h after the addition. The experiment was confined to the latter half of the day to avoid that period when secretion rate changed rapidly (see p. 387).

Expt 3. This experiment was undertaken to investigate whether the act of eating *per se* had an important influence on the nature of the pattern of secretion. The act of eating was replaced in three cows (A, B and C) by stuffing the daily allowance of 14 lb hay into the ventral sac of the rumen through the fistula. The hay was chopped to approximately the same particle size as that attained during normal mastication of the same hay offered in the long state, and was placed in the ventral sac of the rumen to ensure that wetting should be as rapid as possible. The procedure of placing hay in the rumen has been described in detail elsewhere (Bailey & Balch, 1961*a*). About 2 weeks after the beginning of this experiment, the secretion rate of mixed saliva was measured on 2 consecutive days with each cow at 1, 4, 8 and 14 h after feeding.

Expt 4. The diets given in Expt 1 supplied different amounts of dry matter, and it is possible that the level of intake may have affected the rate of secretion of mixed saliva. To test this possibility and at the same time to exclude any complications that might result from the use of different diets, three cows (A, D and E) were given hay at three levels of intake and the rate of secretion of mixed saliva was measured 2 and 8 h after the beginning of feeding on 2 consecutive days. The levels of feeding were 6, 12 and 18 lb hay daily. Six days were allowed for the change from one level to the next.

Since the Na content of bovine parotid saliva was reduced with Na depletion (Bailey & Balch, 1961*b*) and as most plant feeding materials have a low Na content, it is probable that the Na intake of the animals in the experiments described here would be sufficiently low with most rations (see Table 1) to make the animal sensitive to Na loss. Accordingly, sodium chloride was supplied *ad lib.* throughout Expt 4 in order that the chemical composition of the saliva samples could be used as a standard against which to compare the chemical composition of the samples collected in the other experiments.

Expt 5. The results of Expt 1 were obtained over a period of more than a year. Had long-term trends occurred in the rate of salivary secretion they could have invalidated the differences in measurements with given animals on different diets when the measurements were made at intervals of several months. To determine whether long-term trends existed, the rate of salivary secretion was measured approximately twice weekly for more than 2 months in two cows (A and C) 4 h after they had eaten 7 lb hay. The results of the chemical analysis of the saliva samples were also examined for long-term trends. Toward the end of the experiment each animal was given 500 g sodium chloride in the manger on one day 2 h before saliva collection began. The salt was readily consumed. This supplement was added to observe the effect of salt on the composition of the saliva.

RESULTS

Rate of secretion

Expt 1. Mean values for the measurements of the rate of salivary secretion are shown in Fig. 1.

A most striking uniformity was apparent in the mean daily pattern which was independent of the diet. The lowest rates of secretion were invariably found 1 h after the beginning of eating (10–30 min after the end of eating). Thereafter, they increased at 4 and 8 h to the high values measured 14 h after eating. The grass diet invariably evoked the highest rate of secretion of saliva, and silage the lowest, with intermediate values for the other diets.

Comparison of Table 2 and Fig. 1 shows that there was no consistent relationship between the rate of secretion of saliva and the pH of the reticulo-ruminal contents.

Expt 2. Table 3 lists the rates of secretion of mixed saliva, the pH of the rumen fluid and the concentration of TVFA in the rumen fluid from each cow. The mean pH of the rumen fluid was lowered from 6·8 to 5·4 and the mean TVFA concentration was increased from 93 to 167 m-equiv./l. by the addition of acetic acid to the rumen contents. The rates of secretion of mixed saliva were not related to either the pH or the concentration of TVFA in the rumen and the results obtained with cows A, D and E showed no consistent variations over the experimental period of $3\frac{1}{2}$ h. The mean rates of secretion in these three animals were 56, 52, 56 and 54 ml/min at the four times of collection. Animal C, on the other hand, showed a progressive drop in the rate of secretion, which accounts for the slow decline observed in the mean values for all four cows in Table 3.

Expt 3. The rate of secretion of mixed saliva 1 h after the animals had been given hay through the fistula was notably higher, relative to the rates measured at the succeeding times, than it was 1 h after normal feeding. Table 4 lists the mean rates of secretion measured in this experiment together with values for the same three cows when they consumed equal amounts of the same hay in Expt 1. It has already been shown that, compared with normal feeding, administering foods through the fistula caused no change in the pH or chemical composition of rumen fluid (Bailey & Balch, 1961*a*), and it follows that the altered pattern of secretion measured in Expt 3 was independent of the chemical characteristics of the reticulo-ruminal digesta.

Expt 4. Table 5 gives the mean rates of secretion of mixed saliva in the three cows used in this experiment together with the pH and TVFA concentration of rumen fluid. In cows A and E there was a tendency for the rate of secretion of saliva to be smaller with increased intakes of hay. In cow D this tendency was apparent 2 h after feeding but not 8 h after feeding. The rate of secretion 8 h after the consumption of 18 lb hay was greater than that 8 h after the consumption of either 6 or 12 lb of hay. At the two lower levels of intake in cow D, the rate of secretion 2 h after the beginning

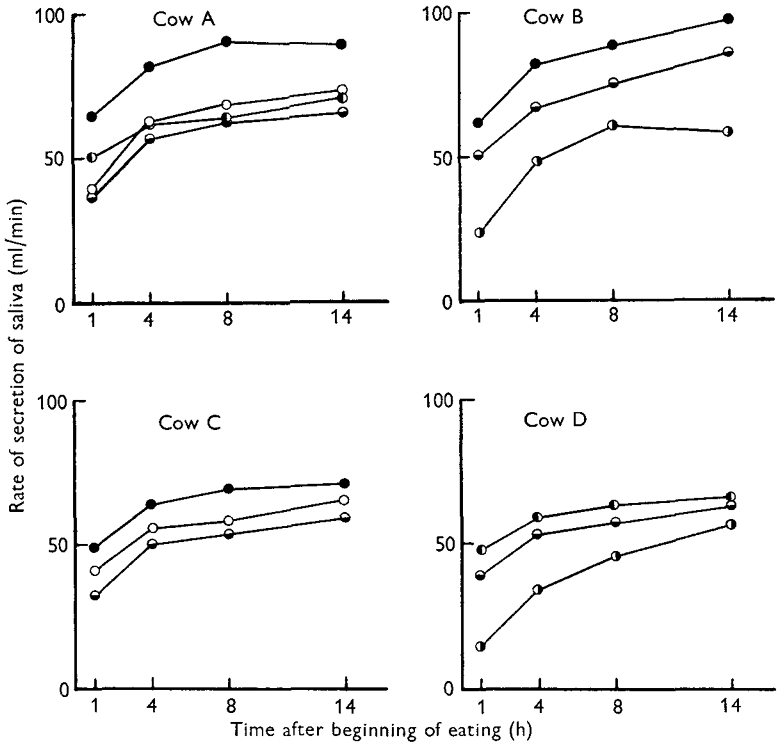

Fig. 1. Expt 1. Mean rates of secretion of mixed saliva in the four cows at rest 1, 4, 8 and 14 h after the beginning of eating. Values are shown for diets of 40 lb silage, ◑ ; 14 lb hay, ◒ ; 8 lb hay and 12 lb dairy cubes, ○ ; 2 lb hay, 12 lb flaked maize and 2 lb groundnut cake, ◐ ; and freshly cut grass, ●.

of feeding was greater than that 8 h after feeding. This variation is contrary to the invariable pattern of secretion observed in this as in all the animals of Expt 1. There is, perhaps, some justification for assuming that the rates of secretion in cow D 8 h after the consumption of 6 and 12 lb of hay were anomalous, although no explanation of this situation can be offered.

Expt 5. The rates of secretion of saliva over the total period of the experiment are shown in Fig. 2. The values for cow A show no trends over the 100 days during which measurements were made. Moreover, the variability was remarkably low. Although no trends were apparent in cow C also, the difference between individual measurements was large. Cow C was a nervous animal and her reaction to the taking of a blood sample shortly before the saliva sample may have contributed to this difference. Unfamiliar handling procedures are known to influence the rate of secretion by the parotid gland in sheep (Denton, 1957).

The exceedingly low rates of secretion noted on two occasions (Fig. 2), one for each animal, were probably due to the administration of 500 g of sodium chloride.

Table 2. *Expt.* 1. *Effect, in cows A–D, of diet on the pH of rumen fluid* 1, 4, 8 *and* 14 *h after the beginning of feeding, and on changes in the rate of secretion of mixed saliva during rest after feeding*

(Values are means for 3 days)

Diet	Cow	pH of rumen fluid after 1 h	4 h	8 h	14 h	Increase in secretion rate from the 1st to the 14th h after beginning of feeding (ml/min)	Ratio of the mean rate of saliva secretion to the rate with hay diet
Silage	B	6·70	6·77	6·78	6·91	35	0·69
	D	6·84	6·95	7·07	7·22	43	0·70
	Mean					—	0·70
Hay	A	6·61	6·77	6·76	6·84	29	1·00
	B	6·58	6·68	6·76	6·78	35	1·00
	C	6·86	7·02	7·16	7·18	27	1·00
	D	6·64	6·82	6·93	6·88	24	1·00
	Mean					—	1·00
Hay and dairy cubes	A	6·16	6·19	6·45	6·74	33	1·09
	C	6·43	6·44	6·96	7·03	25	1·15
	Mean					—	1·12
Hay, flaked maize and groundnut cake	A	5·92	5·72	6·53	7·28	20	1·09
	D	6·59	5·84	6·42	6·90	18	1·11
	Mean					—	1·10
Grass	A	6·73	6·81	6·80	6·99	25	1·46
	B	6·85	6·80	6·83	7·03	35	1·20
	C	6·88	6·91	6·98	7·13	23	1·36
	Mean					—	1·34

Saliva composition

Inorganic constituents. Tables 6 and 7 show the composition of the saliva samples collected in Expts 1–3. In an animal subjected to any particular treatment, variations in the concentrations of the individual constituents were usually small. Except for the two cows consuming the diet of hay and dairy cubes, the mean concentrations of Na, K and chloride showed considerable differences which were not consistently related to any particular animal or treatment. When cows A and C were given hay and dairy cubes the compositions of their saliva were virtually identical. The mean concentrations of bicarbonate and phosphate in the saliva samples were more uniform for all animals and all treatments than those of Na, K or chloride. The composition of the samples of mixed saliva collected from the four cows in Expt 2 was affected little or not at all by the addition of acetic acid to the rumen (Table 7).

Table 8 shows the composition of mixed saliva from the three animals in Expt 4. When the cows received 6 lb of hay daily the concentrations of phosphate in the saliva were somewhat greater than at the higher levels of feeding; this was the only indication that the level of feeding influenced saliva composition. In general, the concentrations of all the constituents were very uniform and no large differences between animals were apparent. Bicarbonate was not determined in these samples.

Table 3. *Expt 2. Effect of adding* 300 *ml glacial acetic acid to the rumen contents on the rate of secretion of mixed saliva, the pH of the rumen fluid and its total volatile fatty-acid (TVFA) content in cows A, C, D and E at rest*

Time after addition of acid (h)	Cow	Rate of secretion of saliva (ml/min)	pH	TVFA (m-equiv./l.)
−½	A	68	6·78	88
	C	56	7·24	73
	D	49	6·47	109
	E	50	6·69	101
	Mean	56	6·80	93
½	A	51	5·50	150
	C	41	5·37	188
	D	53	5·42	158
	E	51	5·38	171
	Mean	49	5·42	167
1½	A	66	5·90	121
	C	30	5·97	132
	D	53	5·89	140
	E	48	6·28	137
	Mean	49	6·01	133
3	A	61	6·53	112
	C	21	6·60	124
	D	41	6·37	113
	E	60	6·61	114
	Mean	46	6·53	116

Table 4. *Expt* 3. *Rates of secretion, in cows A–C, of mixed saliva (ml/min) during rest at* 1, 4, 8 *and* 14 *h after the beginning of normal feeding and after the administration of hay through the fistula*

	Rate of secretion of mixed saliva (ml/min)							
	Hay eaten				Hay placed in rumen			
Cow	1 h	4 h	8 h	14 h	1 h	4 h	8 h	14 h
A	37	57	64	66	61	66	70	78
B	52	68	76	87	69	60	82	80
C	32	50	53	59	44	39	38	42
Mean	40	58	64	71	58	55	63	67

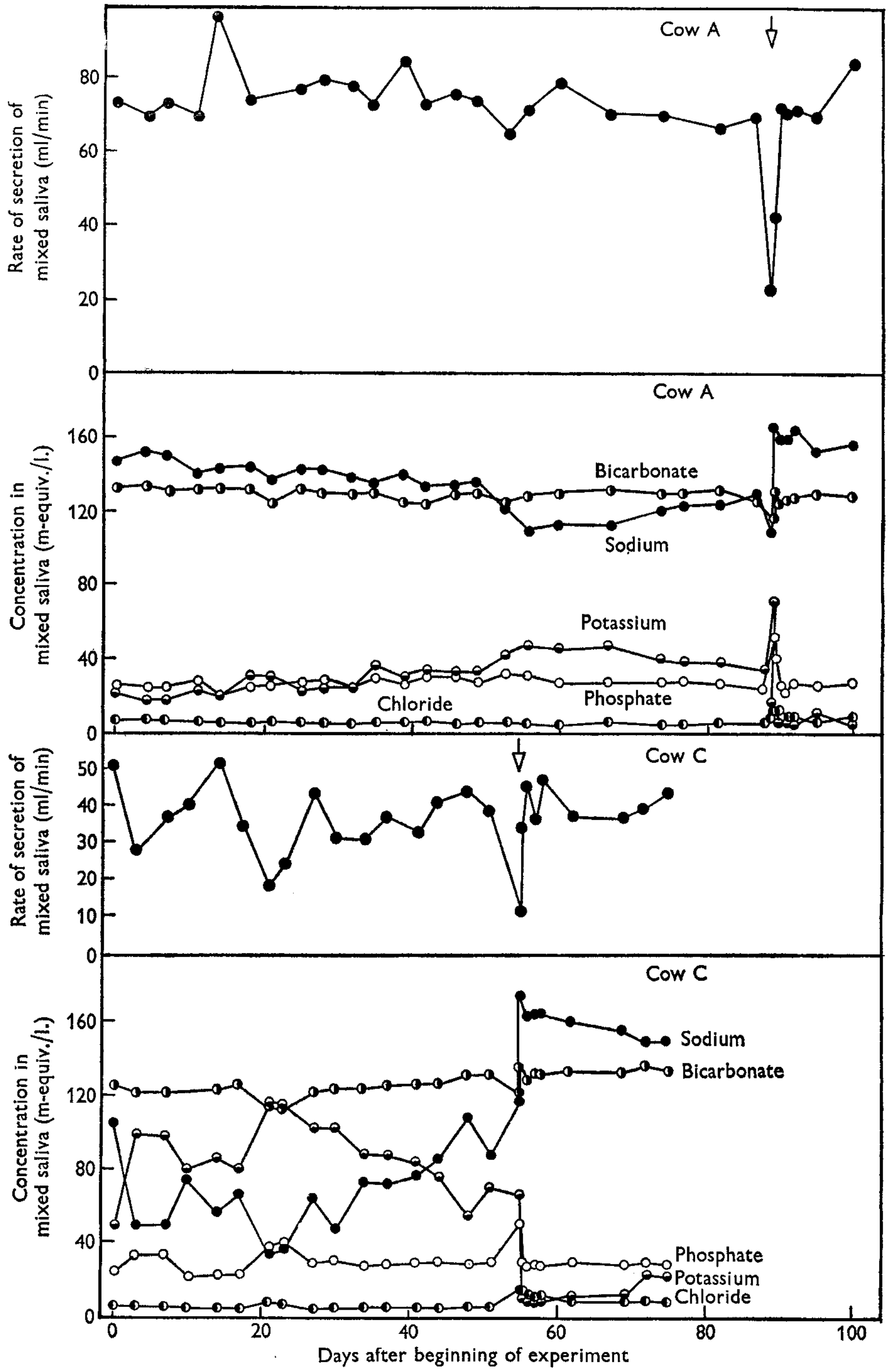

Fig. 2. Expt 5. Rates of secretion, in two cows, of mixed saliva collected 4 h after the beginning of feeding, and the concentrations of sodium, potassium, chloride, bicarbonate and phosphate in the saliva. The arrows show the single occasion when 500 g of sodium chloride were given.

Table 5. *Expt 4. Effect, in cows A, D and E, of the level of hay intake on the mean rate of secretion of mixed saliva, the pH of rumen fluid and its total volatile fatty-acid (TVFA) content during rest at 2 and 8 h after the beginning of feeding*

Daily hay intake (lb)	Cow	Saliva secretion rate (ml/min) 2 h	8 h	Mean	Rumen fluid: pH 2 h	8 h	Mean	TVFA (m-equiv./l.) 2 h	8 h	Mean
6	A	75	95	85	7·1	7·3	7·2	68	65	67
	D	64	48	56	7·2	7·3	7·3	49	46	48
	E	51	58	54	7·3	7·2	7·3	58	55	57
	Mean	63	67	65	7·2	7·3	7·3	58	55	57
12	A	51	67	59	7·0	6·9	7·0	76	87	82
	D	53	43	48	6·9	7·0	7·0	86	80	83
	E	46	53	49	7·1	7·1	7·1	81	68	75
	Mean	50	54	52	7·0	7·0	7·0	81	78	80
18	A	51	64	57	7·0	7·0	7·0	96	94	95
	D	49	61	55	6·8	6·7	6·8	91	91	91
	E	34	50	42	6·9	6·9	6·9	89	101	95
	Mean	45	58	51	6·9	6·9	6·9	92	95	94

Table 6. *Expts 1 and 3. Effect of diet on the sodium, potassium, chloride, bicarbonate, phosphate, total nitrogen, urea nitrogen, dry-matter and ash contents and the pH of mixed saliva from cows A–D at rest*

(Values are means for determinations at 1, 4, 8 and 14 h after the beginning of feeding on each of 3 days)

Diet	Cow	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)	Chloride (m-equiv./l.)	Bicarbonate (m-equiv./l.)	Phosphate (m-equiv./l.)	Total nitrogen (mg/100 ml)	Urea nitrogen (mg/100 ml)	Dry matter (%)	Ash (%)	pH
Silage*	B	109	49	5·2	120	32	11·6	9·8	1·05	0·90	8·4
	D	157	9	10·0	121	29	14·4	12·3	1·00	0·86	8·4
	Mean	133	29	7·6	121	31	13·0	11·1	1·03	0·88	8·4
Hay (given normally)*	A	89	63	3·0	122	21	6·0	4·4	1·07	0·89	8·4
	B	158	5	8·4	126	23	—	3·2	0·97	0·86	8·5
	C	153	11	7·6	127	22	4·5	2·7	0·97	0·86	8·5
	D	106	46	4·2	126	23	3·3	2·1	1·04	0·89	8·3
	Mean	126	31	5·8	125	22	—	3·1	1·01	0·88	8·4
Hay (given through fistula)†	A	107	61	3·8	123	20	—	—	—	—	8·4
	B	133	36	6·0	121	23	—	—	—	—	8·4
	C	75	89	3·9	116	27	—	—	—	—	8·4
	Mean	105	62	4·6	120	23	—	—	—	—	8·4
Hay and dairy cubes*	A	164	4	8·1	124	30	12·0	10·6	1·01	0·88	8·5
	C	164	4	6·3	125	28	12·4	10·4	1·04	0·89	8·4
	Mean	164	4	7·2	125	29	12·2	10·5	1·03	0·89	8·5
Hay, flaked maize and groundnut cake*	A	140	23	6·6	122	29	11·8	9·0	1·04	0·92	8·4
	D	156	9	9·0	121	28	9·1	7·6	1·00	0·88	8·4
	Mean	148	16	7·8	122	29	10·5	8·3	1·02	0·90	8·4
Grass*	A	150	14	8·3	126	22	—	7·3	0·98	0·87	8·5
	B	153	14	7·0	121	30	—	6·8	1·02	0·90	8·3
	C	125	39	4·6	125	24	—	5·0	1·04	0·93	8·4
	Mean	143	22	6·6	124	25	—	6·7	1·01	0·90	8·4

* Expt 1. † Expt 3.

The composition of the mixed saliva collected from cows A and C in Expt 5 is shown in Fig. 2. Large fluctuations in the Na and K concentrations were revealed, especially in cow C. In cow A there was a tendency for the Na and chloride concentrations to fall and the K concentration to increase throughout the period of the experiment before the administration of 500 g of sodium chloride. The administration

Table 7. *Expt 2. Effect of adding 300 ml glacial acetic acid to the rumen contents on the sodium, potassium, chloride, bicarbonate, phosphate, total nitrogen and urea nitrogen contents and the pH of mixed saliva from cows A, C, D and E at rest*

Time after addition of acid (h)	Cow	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)	Chloride (m-equiv./l.)	Bicarbonate (m-equiv./l.)	Phosphate (m-equiv./l.)	Total nitrogen (mg/100 ml)	Urea nitrogen (mg/100 ml)	pH
$-\frac{1}{2}$	A	137	25	6·3	128	34	3·2	1·4	8·4
	C	146	12	6·7	134	18	3·4	2·1	8·4
	D	73	74	3·8	120	25	3·9	2·1	8·3
	E	160	7	6·7	129	23	3·7	2·1	8·4
	Mean	129	29	5·9	128	25	3·6	1·9	8·4
$\frac{1}{2}$	A	137	26	6·3	128	23	3·4	1·3	8·3
	C	147	15	6·6	128	22	3·9	2·0	8·3
	D	68	78	3·7	114	23	3·8	2·2	8·1
	E	158	5	7·0	128	19	3·6	2·5	8·3
	Mean	128	31	5·9	125	22	3·7	2·0	8·3
$1\frac{1}{2}$	A	138	22	7·0	126	22	3·0	1·4	8·4
	C	150	13	10·4	130	20	4·6	2·5	8·3
	D	86	64	5·0	121	21	3·5	2·1	8·2
	E	160	6	8·2	129	19	4·0	2·4	8·4
	Mean	134	26	7·7	127	21	3·8	2·1	8·3
3	A	135	29	7·2	125	25	3·8	1·4	8·4
	C	138	23	7·1	125	20	4·9	2·6	8·4
	D	70	84	3·5	116	30	4·9	3·0	8·2
	E	160	6	6·9	131	24	3·6	2·6	8·4
	Mean	126	35	6·2	124	25	4·3	2·4	8·4

Table 8. *Expt 4. Effect of the level of hay intake on the sodium, potassium, chloride, phosphate, total nitrogen, urea nitrogen, dry-matter and ash contents and the pH of mixed saliva in cows A, D and E at rest*

(Values are means for samples collected during rest at 2 and 8 h after the beginning of feeding)

Daily hay intake (lb)	Cow	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)	Chloride (m-equiv./l.)	Phosphate (m-equiv./l.)	Total nitrogen (mg/100 ml)	Urea nitrogen (mg/100 ml)	Dry matter (%)	Ash (%)	pH
6	A	164	4·1	7·2	32	4·4	2·8	1·00	0·89	8·4
	D	166	4·9	8·0	33	5·8	4·4	1·04	0·89	8·3
	E	162	6·2	6·7	29	6·8	5·0	1·02	0·87	8·4
	Mean	164	5·1	7·3	31	5·7	4·1	1·02	0·88	8·4
12	A	156	8·6	7·4	23	4·7	2·9	0·97	0·85	8·5
	D	153	10·2	8·6	22	7·0	4·8	0·99	0·86	8·4
	E	159	5·0	7·4	28	5·1	3·6	0·98	0·85	8·4
	Mean	156	7·6	7·4	24	5·6	3·8	0·98	0·85	8·4
18	A	162	5·6	6·5	22	4·5	3·2	0·99	0·87	8·4
	D	164	5·4	7·1	24	6·3	4·5	0·98	0·86	8·5
	E	161	5·7	6·4	25	5·7	3·8	1·00	0·87	8·5
	Mean	163	5·6	6·7	24	5·5	3·8	0·99	0·87	8·5

of the sodium chloride led to rapid and profound adjustments in the concentrations of the ions, especially of Na, K and chloride. After the adjustment the concentrations of all the ions resembled very closely those in the saliva collected from the three cows in Expt 4 (Table 8).

Nitrogenous constituents. The contents of both total N and urea N in saliva were dependent to a large extent on the nature of the diet, values being low when the animals consumed hay, intermediate when they consumed grass or hay and flaked maize, and high when they consumed silage or hay and dairy cubes. Urea N constituted

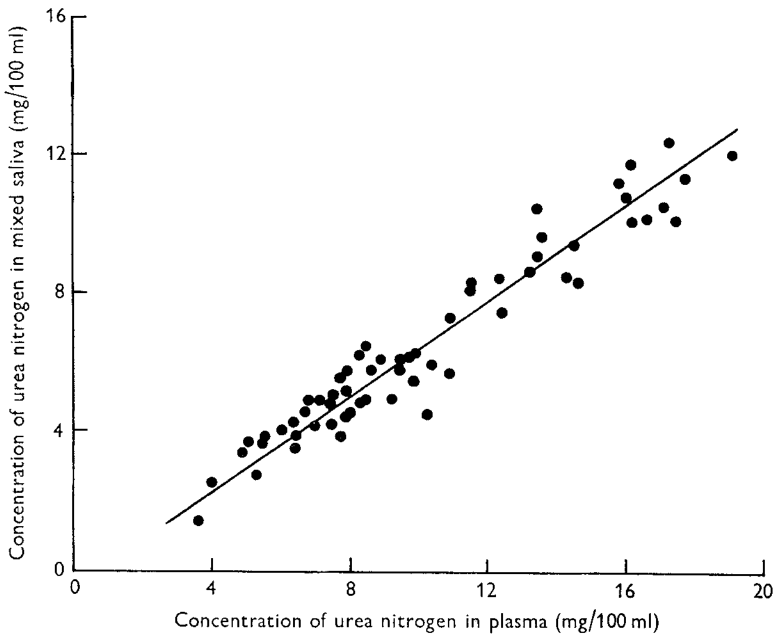

Fig. 3. Relationship, in four cows, between the concentrations of urea N in the mixed saliva and in blood plasma collected at the same (± 15 min) time.

a large proportion of the total N in saliva. In Expt 1, the urea N accounted for 85·5, 62·9, 86·8 and 79·6 % of the total N in the mixed saliva of the cows consuming the silage, hay, hay and dairy cubes, and hay and flaked-maize diets, respectively. The overall mean value for all cows and rations was 77·2 % of the total N. The differences in values with the different diets were due to increases in the urea content of saliva which were not accompanied by commensurate increases in non-urea N.

The plasma from the samples of jugular venous blood taken in Expts 1 and 5 was analysed for urea and the relationship between the values so obtained and the urea concentrations in the saliva samples collected at the same time is shown in Fig. 3. The urea content of saliva was about 65 % of that in the plasma, with plasma values ranging from 4 to 19 mg urea N/100 ml plasma.

Dry matter and ash. The dry-matter and ash contents of mixed saliva showed only small variations and no relationship was discerned between these values and any treatment, animal or experiment. The mean values for all the samples were 1·02 % dry matter and 0·89 % ash.

DISCUSSION

The experiments have provided information about the rate of secretion and composition of mixed saliva, collected at the cardia, in resting cattle. In discussing the values obtained our primary concern is the extent to which either the rate of secretion or the composition appeared to vary in response to changing conditions in the reticulo-rumen.

Rate of secretion of mixed saliva

During the collection of a swallow of saliva the small rubber bag was applied for only a few seconds to the area surrounding the cardia. Care was taken to make the application brief and light since preliminary experiments (Bailey, 1957, unpublished results) had shown that the rates of secretion in two cows were increased from 61 and 63 ml saliva/min with this technique to 111 and 116 ml saliva/min respectively when a large bag with a rigid rim was held firmly against the cardia for 5–10 min.

Care was also taken to leave undisturbed the contents of the reticulum, and it is thought that the prior removal of part of the digesta from the rumen, in order to expose the cardia, had little effect on the rate at which saliva was secreted. It certainly seems likely that any abnormality of flow so induced would be less than might be expected to result from techniques using oesophageal cannulas (Colin, 1886) and the aspiration of saliva trapped in the oesophagus by inflated catheters, as has been used in both cattle (Cunningham, 1958) and sheep (Hydén, 1958). It has been shown that stimulation of tactile receptors in the oesophagus influences the rate of secretion of parotid saliva (Clark & Weiss, 1952; Ash & Kay, 1959). We attach considerable importance to the fact that our results were obtained with cows that, apart from cow C, were placid, comfortable and undisturbed.

The rate of secretion of mixed saliva was invariably least shortly after a meal and increased with time after the meal. There was, therefore, a remarkable similarity between this pattern of secretion of mixed saliva and the pattern of secretion of parotid saliva (Bailey & Balch, 1961*b*). This finding suggests that the factors that gave rise to the pattern of secretion in the parotid were also involved in establishing it in the mixed saliva, and confirms the probability that the mixed saliva largely contains parotid saliva or saliva from other glands with similar secretory properties (Kay, 1960).

The lowest rates of secretion of mixed saliva occurred at times when chemical and physical stimulation in the reticulo-rumen would be maximal, or nearly so, as a result of the ingestion and fermentation of the food. Further when, in Expt 2, the mean pH of the rumen fluid was lowered from 6·8 to 5·4 and the TVFA concentration was raised from 93 to 167 m-equiv./l. as a result of adding acetic acid to the rumen contents, no consistent changes resulted in the secretion of the mixed saliva. These observations were unexpected since it was logical to suppose that tactile receptors located in the reticulum would be stimulated and the rate of secretion of saliva increased after a meal. Indeed, Weiss (1953) suggested that saliva secretion was enhanced after the ingestion of food, owing to the tactile stimulation of receptors in the stomach. Further, in view of the importance of buffers in maintaining a fairly stable rumen pH (McManus, 1959) it seemed reasonable to suppose that alterations in the acidity of the rumen contents would influence the secretion of saliva.

The effect on salivary secretion of tactile stimulation of the sensitive areas of the oesophagus and reticulum has been established beyond reasonable doubt. It is a matter of conjecture as to why measurable increases of salivary secretion were not observed after the ingestion of food in the experiments described here. Possibly the effectiveness of tactile stimulation shortly after a meal was inhibited by some factors whose nature is not known. Both in these experiments and in those concerned with the secretion of parotid saliva (Bailey & Balch, 1961*b*) the experimental animals were fed twice daily and the inhibition of the effects of tactile stimulation may have been initiated by the act of eating *per se*. This possibility is supported by the results of Expt 3, in which three cows received all their food through the fistula for 2½ weeks. When salivary secretion was measured towards the end of that period it was found that the rate did not fall as low after a meal of hay given through the fistula as it did in the same cows after normal feeding (Table 4). This finding suggests that the degree of inhibition is not related to the amount of digesta in the rumen but, as discussed previously (Bailey & Balch, 1961*b*), does not preclude the possibility that during eating the sensory nerve endings tire. Whatever causes were responsible for establishing the pattern of secretion of mixed saliva during periods of rest, it seems clear that this pattern was an integral part of the response of the animal to the feeding routine.

Table 9. *Rate of secretion of mixed saliva collected by means of a large rubber bag during rest at 2 and 17 h after the beginning of feeding in cows A and B (Bailey, 1957)*

Diet	Cow	Rate of secretion of saliva 2 h after beginning of feeding (ml/min)	Increase in secretion rate from 2 to 17 h after beginning of feeding (ml/min)
Hay	A	86	30
Hay and dairy cubes	A	116	30
Hay and dairy cubes	B	119	22
Grass	B	125	36

With different diets the cows secreted different amounts of mixed saliva while they were resting but, in Expt 1, the increase in the rate of secretion of saliva from the 1st to the 14th h after feeding was remarkably constant in each cow, irrespective of diet (Table 2). Further, in the preliminary experiment (Bailey, 1957, unpublished) referred to above, saliva was collected in a large bag held firmly over the cardia, and the rates of secretion (Table 9), although faster than with the usual method of collection, increased by the same increments over the period from the 2nd to the 17th h after feeding. Accordingly it appears that the characteristics of the diets which evoked the differences in the rate of salivary secretion in Expt 1 were simply superimposed on a pattern of decreasing inhibition during the periods of rest after a meal.

It is clear from Tables 2 and 9 that the grass diet invariably evoked the highest rate of secretion of saliva, silage evoked the lowest rate and values for the other diets were intermediate. It would be of particular interest to know what characteristics of the various diets led to these differences in the secretion of saliva. The amount of digesta seems unlikely to have been responsible, because the emptying of the rumen before collection of the saliva will have eliminated many differences. The fibrous and abrasive properties of the digesta in the reticulo-rumen were clearly not responsible, because the highest rates of secretion were observed with grass, which produced the softest and most spongy digesta. Moreover, differences in the acidity of the rumen contents had no consistent relationship to the rate of secretion of mixed saliva.

These experiments did not reveal, therefore, the dietary characteristics responsible for the differences in rate of saliva secretion. The possible effect of the degree of fill and nature of the contents of the lower gut should not be overlooked, however, especially in view of the results of Expt 4.

Composition of mixed saliva

Inorganic constituents. Values obtained by analysing some 284 samples of mixed saliva produced at rates of secretion ranging from 12 to 110 ml/min are summarized in Tables 6–8 and in Fig. 2. When saliva was secreted at rates greater than about 30 ml/min the concentrations of both bicarbonate and phosphate in the fluids showed only very small fluctuations; the mean concentrations were similar for each cow (Table 10) and no consistent effects of the treatments could be discerned. With decrease of secretion rate below 30 ml/min, the bicarbonate concentrations decreased and the phosphate concentrations increased. These changes were essentially like those that occurred in the bicarbonate and phosphate concentrations of parotid saliva secreted at low rates of flow in sheep (Coats & Wright, 1957) and in a steer (Bailey & Balch, 1961*b*). With the steer there was a direct linear relationship between the rate of secretion of parotid saliva (as ml/min) and the rate of secretion of the ions (as m-equiv./min). The observations with the steer suggested that the composition of additional amounts of saliva, secreted in a given time at rates greater than the lowest rate measured, were of constant composition if it is assumed that the basal flow is secreted at a standard speed and composition. The same conclusion may be reached for the bicarbonate and phosphate ions in these experiments, for the secretion rates of these two ions in mixed saliva were linearly related to the rate of salivary secretion. For the bicarbonate ion, the value of the slope was 130 m-equiv./l., which corresponds

Table 10. *Mean values with their standard errors (m-equiv./l.) for concentrations of bicarbonate and phosphate in mixed saliva secreted at flow rates greater than* 30 *ml/min in cows A–E at rest*

Cow	No. of samples	Bicarbonate	Phosphate
A	97	125 ± 0.43	25 ± 0.45
B	41	123 ± 0.52	26 ± 0.66
C	66	125 ± 0.61	27 ± 0.68
D	48	123 ± 1.03	26 ± 0.67
E	16	129 ± 0.65	25 ± 0.82
Mean		125	26

very closely to the value of the slope for the same ion in bovine parotid saliva (131 m-equiv./l., Bailey & Balch, 1961 *b*). This observation suggests that the secreting structures which elaborated each type of fluid were very similar and the same conclusion follows from a consideration of the secretory characteristics of the phosphate ion. It is reasonable to conclude, therefore, that the samples of mixed saliva comprised largely parotid (or parotid-like) components.

Na and K together constitute about 98% of the cations of the mixed saliva of ruminants (McDougall, 1948; Phillipson & Mangan, 1959) and a decrease or increase

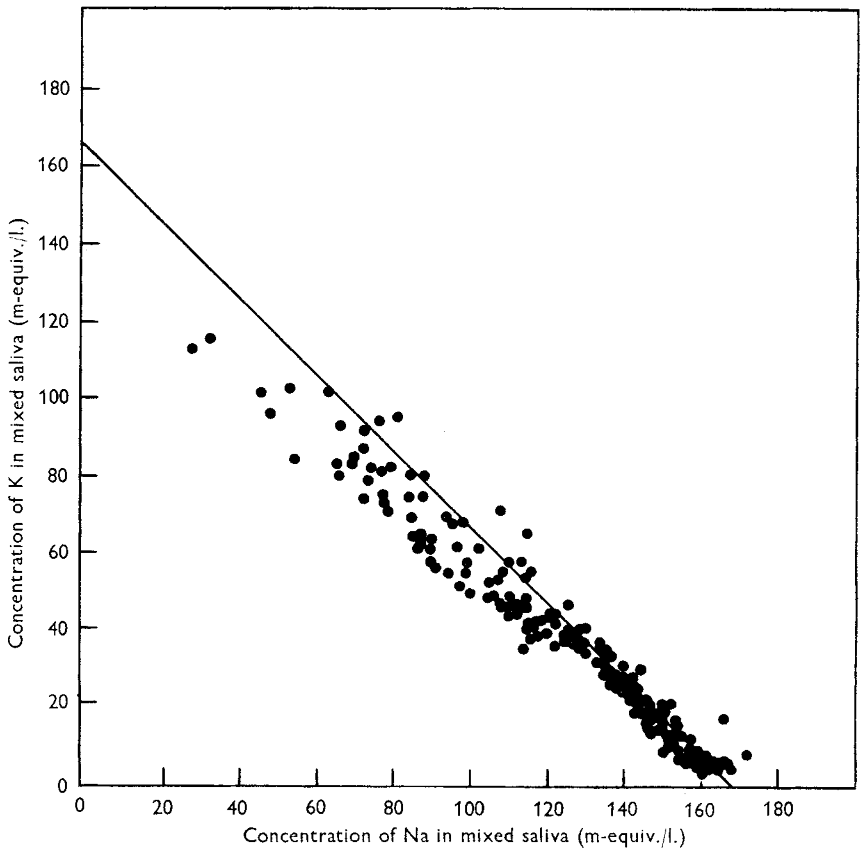

Fig. 4. Relationship, in five cows, between the concentrations of sodium and potassium in mixed saliva. The straight line joins points at which the sum of the Na and K concentrations was 166 m-equiv./l.

in Na content is likely to be accompanied by a commensurate increase or decrease in that of K. In the experiments described here the total concentrations of Na and K in saliva tended to be about 166 m-equiv./l., decreasing by a small extent when the Na concentration fell below about 120 m-equiv./l. (Fig. 4). Moreover when the Na concentration increased above about 164 m-equiv./l., the K concentration did not decrease below the mean plasma K concentration, and the sum of the concentrations of Na and K increased accordingly above 166 m-equiv./l. The salivary glands were apparently incapable, at this level, of transferring water to the saliva without K. Within values for any animal receiving a given diet in Expt 1, variations in the content of Na, K and chloride were small (Table 6), but the contents of these ions varied considerably when the same animal received different diets. For example, at equivalent rates of secretion (62 ml/min) the mean Na concentrations in the mixed saliva of cow A were 87 m-equiv./l. with hay and 163 m-equiv./l. with hay and dairy cubes; the corresponding concentrations of K were 62 and 4 m-equiv./l. respectively. It is not clear, however, why these variations occurred, although with the vegetable diets used, a shortage of Na seems more likely than a shortage of K. A shortage of dietary Na would probably lead to lowering of Na concentrations in the parotid saliva just as they are lowered when (Denton, 1956) the Na lost in the fluid from a parotid fistula is not replaced.

It is probable that in Expt 1 Na-retaining mechanisms were induced in the animals to a greater or less degree with all diets except that of hay and dairy cubes. With sodium chloride added *ad lib.* to a diet of hay (Expt 4) the mean concentration of Na in the mixed saliva was high (161 m-equiv./l.) and differences between animals were small, which suggests that a concentration of about 160 m-equiv./l. may be taken as characteristic of that in the saliva of cows receiving adequate salt.

On the supposition that the Na content of the mixed saliva of normal cows accurately reflects the state of Na nourishment, an estimate of Na requirement could be obtained by determining the minimum amount of Na needed to maintain the maximum Na concentration in mixed saliva. An estimate of the requirement for Na of the cows may be obtained from the results of Expt 1. Maximum concentrations of Na in saliva appeared in the cows consuming hay and dairy cubes; the levels in the saliva of the cows consuming lucerne silage were lower. The daily Na intakes of the cows consuming these rations were 16·0 and 14·2 g/day, respectively (Table 1), and it follows that the requirement must lie somewhere between these two values. The Na needs of milking cows would, of course, be considerably greater than of the dry cows used in these experiments.

When the cows received adequate Na the mixed saliva accordingly consisted primarily of solutions of sodium bicarbonate and sodium phosphate with smaller amounts of K and chloride. This observation supports those of Phillipson & Mangan (1959) and Emery, Smith, Grimes, Huffman & Duncan (1960) for cattle and of McDougall (1948) and Hydén (1958) for sheep. The similar mean concentrations of the five inorganic ions in mixed saliva and (Bailey & Balch, 1961*b*) in parotid saliva, both at high rates of secretion (Table 11), again suggest that the mixed saliva is largely composed of parotid or parotid-like components.

It is possible that in addition to the large fluctuations in the salivary concentrations of Na, K and chloride already discussed, there were smaller effects related to differences in the rate of secretion and analogous to the changes in the concentrations of bicarbonate and phosphate. These smaller effects appeared to be present in Expt 5 when, for example, on the 21st and 23rd days (Fig. 2) the rate of secretion of mixed saliva in cow C was depressed, the concentrations of Na and bicarbonate in the saliva were low and those of phosphate, K and chloride were high relative to the concentrations on other days. It is likely that the variations in the concentrations of Na and K between days 25 and 48 were due in some degree to change in the Na intake. No explanation can be offered for the large transient fall in secretion rate after the administration of sodium chloride, and it is clear that this effect did not persist.

Table 11. *Mean composition of mixed and parotid saliva secreted during high rates of secretion in cows receiving adequate intakes of sodium chloride*

Constituent	Mixed saliva*	Parotid saliva†
Sodium (m-equiv./l.)	161	157
Potassium (m-equiv./l.)	6·2	7·0
Chloride (m-equiv./l.)	7·1	7·4
Bicarbonate (m-equiv./l.)	126	127
Phosphate (m-equiv./l.)	26	23
Sum of cations (m-equiv./l.)	167	164
Sum of anions (m-equiv./l.)	159	157
Dry matter (%)	1·02	1·05
Ash (%)	0·89	0·91

* Means for some forty-eight samples from four cows receiving diets of hay.
† From Bailey & Balch (1961*b*).

Nitrogenous constituents. Urea N constituted a variable, but generally high, proportion of the total N of saliva. The non-urea fraction was relatively constant at 1–2 mg N/100 ml irrespective of the diet.

The salivary urea N content varied from 1·3 to 14·4 mg/100 ml owing to variations in the amount of ammonia produced in the rumen with the different diets and consequent changes in plasma urea levels (Fig. 3). The importance, in the N economy of the animal, of the recycling of dietary N through the saliva as urea was first pointed out by McDonald (1948) and knowledge of the process was extended by Lewis, Hill & Annison (1957) and Lewis (1957).

SUMMARY

1. In cows with rumen fistulas the rate of secretion of mixed saliva was measured by removing part of the digesta from the rumen and collecting the saliva as it emerged from the cardia. Care was taken to prevent stimulation of the region bordering the cardia by contact with the collecting vessel, and it is thought that in consequence the collecting technique did not cause changes in the rate of secretion. The rate of secretion was measured during rest, that is, when the animals were neither eating nor ruminating.

2. Four cows were used to study the rate of secretion 1, 4, 8 and 14 h after the beginning of the first of the two daily meals. The rate of secretion was invariably least shortly after a meal and increased as the next meal time approached. The mean rates of secretion differed between diets, being greatest with grass, least with silage and intermediate with (1) hay, (2) hay and dairy cubes and (3) hay, flaked maize and groundnut cake. Neither the changes within one day nor the effects of diets could be related to any change in the physical or chemical characteristics of the contents of the reticulo-rumen.

3. The addition to the rumen contents of sufficient acetic acid to lower the mean pH from 6·8 to 5·4 and to increase the total volatile fatty acids from 93 to 167 m-equiv./l. had no obvious effect on the rate of secretion of mixed saliva.

4. In cows given their hay through a fistula the rate of secretion after 1 h was higher than 1 h after they had eaten hay, which suggests that the act of eating *per se* contributes to the establishment of the daily pattern of secretion of mixed saliva.

5. The rate of secretion tended to be greater during periods of rest after small meals than during periods of rest after large ones.

6. In mixed saliva the concentrations of bicarbonate decreased and those of phosphate increased when the rate of secretion fell below 30 ml/min. At higher rates the concentrations of these ions were very uniform irrespective of animal, diet or experiment.

7. Variations, below adequate levels, in the intake of sodium and perhaps of chloride were accompanied by large variations in the concentrations of sodium, chloride and potassium in mixed saliva at all rates of secretion. With adequate intakes of sodium chloride the concentrations in saliva secreted at high rates were similar in all the cows. Aside from the effects of low intakes of Na and chloride the diets had no significant effect on the composition of saliva.

8. Saliva secreted at high rates by cows receiving adequate sodium chloride typically contained 161 m-equiv. Na/l., 6·2 m-equiv. K/l., 7·1 m-equiv. chloride/l., 126 m-equiv. bicarbonate/l. and 26 m-equiv. phosphate/l.

9. The experiments suggested that the daily Na requirement of these dry cows to prevent changes in the Na content of the saliva lay between 14·2 and 16·0 g.

10. The concentration of urea in mixed saliva varied with the diets. The mean content of urea nitrogen in the total salivary N was 77 %, and the urea concentration in saliva was consistently above 65 % of the urea concentration of plasma.

11. The mean dry-matter and ash contents of all the samples of saliva were 1·02 and 0·89 % respectively.

12. The results strongly support the suggestion that the resting mixed saliva consists largely of parotid or parotid-like components.

We thank Dr M. J. Head and Dr J. A. F. Rook for much advice and for help with certain of the analyses, Mr V. W. Johnson and his assistants who, throughout this series of experiments, were responsible for the animals, and Miss L. H. M. Birch, Mr D. Millard and Mr A. W. Wagstaff who kindly carried out the analyses of food and some of the TVFA determinations.

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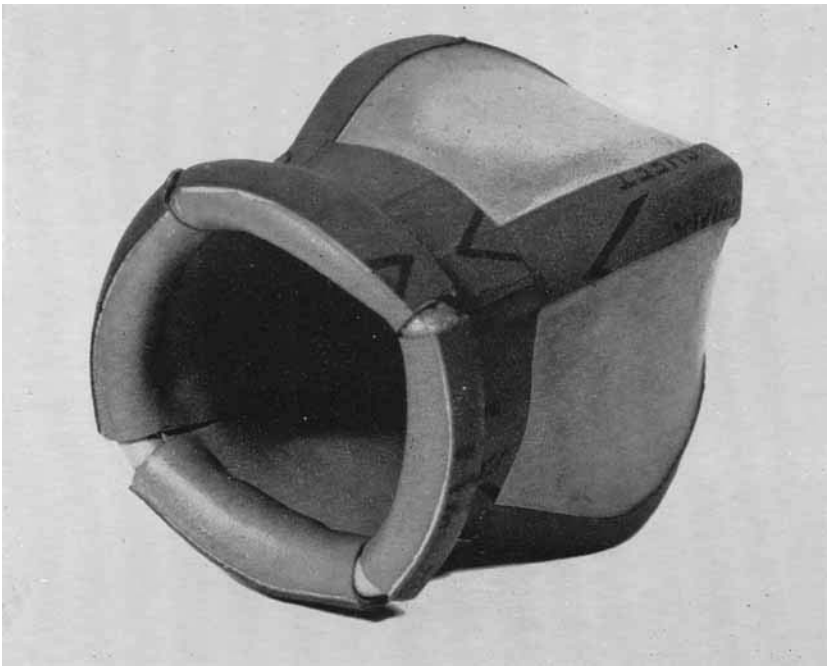
EXPLANATION OF PLATE

Rubber bag used for the collection of saliva from the cows.



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