Saliva secretion and its relation to feeding in cattle

4.* The relationship between the concentrations of sodium, potassium, choride and inorganic phosphate in mixed saliva and rumen fluid

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Differences in the properties of rumen fluid may influence the activities of the rumen microflora and, consequently, the efficiency of digestion. For example, rumen digestion studies in vitro have shown that potassium, calcium, magnesium and sulphate ions must be present for maximum cellulose digestion (Hubbert, Cheng & Burroughs, 1958*a*, *b*). In addition, Watson (1933) suggested that phosphate may be important for the activity of rumen bacteria. Moreover, the experiments of Balch (1950) and Balch & Johnson (1950) showed that cellulose digestion was favoured by a low dry-matter content in the reticulo-ruminal digesta.

The saliva of ruminants contributes largely to the maintenance of the inorganic composition and the fluid volume of the rumen contents (Annison & Lewis, 1959). It was considered to be a matter of some interest, therefore, to know whether changes in the chemical composition of saliva would give rise to noticeable changes in the composition of the fluid of the reticulo-rumen. The present communication describes the relationship between the concentrations of Na, K, chloride and phosphate ions in mixed saliva and rumen fluid in four cows receiving five different diets. Previous papers in this series presented information on the effects of these diets on the rate of secretion and composition of mixed saliva (Bailey & Balch, 1961*c*; Bailey, 1961).

EXPERIMENTAL

Samples of saliva and rumen fluid were collected from four dry Shorthorn cows which were given diets of (1) lucerne silage, (2) rye-grass–clover hay, (3) hay and dairy cubes, (4) hay, flaked maize, groundnut cake, and (5) a freshly cut mixture of cocksfoot and rye-grass as detailed in Table 1. Values for at least two cows were obtained with each ration. The food was given in two meals at 07.30 and 17.00 h. Water was available *ad lib.* from drinking bowls but the cows did not receive supplemental salt. The amounts of Na, K, chloride and P supplied daily in each diet are also shown in Table 1.

Samples of mixed saliva and rumen fluid were taken four times daily. The first sample was taken about ½ h before the morning meal (14 h after the meal of the

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previous evening) and the other three 1, 4 and 8 h after the beginning of the morning meal. Collections were repeated on 3 consecutive days. It has been assumed that the values measured in saliva and rumen fluid 14 h after the evening meal were similar to those which might have been obtained 14 h after the morning meal.

Saliva was collected at the cardia, and rumen fluid from the ventral rumen sac by the technique already described (Bailey & Balch, 1961*a*). The concentrations of Na, K, chloride and inorganic phosphate ions were determined in both mixed saliva and rumen fluid. The concentration of inorganic phosphate in rumen fluid was estimated by the method of Allen (1940) in a solution prepared by filtering a mixture of 1 part of rumen fluid and 4 parts of 10% (w/v) trichloroacetic acid. The estimation of inorganic phosphate in saliva was done directly in a mixture of 1 part saliva and 4 parts distilled water. The other chemical methods used have been described earlier (Bailey & Balch, 1961*a*).

Table 1. *Daily intake of sodium, potassium, chloride and phosphorus by the cows consuming five diets*

Daily diet	Daily intake* (g)			
	Sodium	Potassium	Chloride	Phosphorus
40 lb lucerne silage	14·2	177	74	24·6
14 lb hay	4·7	92	33	7·5
8 lb hay and 12 lb dairy cubes	16·0	105	44	40·1
2 lb hay, 12 lb flaked maize and 2 lb groundnut cake	6·6	38	7	12·4
Grass to supply 12 lb dry matter	4·7	244	124	24·8

* Total of each element present in the diets.

RESULTS

Composition of mixed saliva. The composition of the samples of mixed saliva is summarized in Table 2. While a cow was receiving a given diet variations in the concentration of the ions in mixed saliva were usually small. However, large differences appeared between cows and between diets in concentrations of Na and K and, to a lesser extent, chloride ions.

Composition of rumen fluid. The composition of the samples of rumen fluid is summarized in Table 3. The concentrations of Na were fairly consistent while a cow was consuming any one diet, but differences between animals and diets were very marked. Comparison of Tables 2 and 3 shows that the concentration of Na in rumen fluid was related to the concentration in mixed saliva, the concentration in rumen fluid being somewhat lower in most instances. For all the pairs of observations the mean value for rumen contents was 17% below the mean value for saliva. This relationship is illustrated in Fig. 1 with Na concentrations in saliva ranging from 28 to 168 m-equiv./l. The results from several other experiments (Bailey, 1959, unpublished) were used in addition to those from the present experiment in preparing Fig. 1; the observations in which the Na concentrations in rumen fluid exceeded 145 m-equiv./l. were made mainly on cows receiving sodium chloride *ad lib.*

Except with the diet of hay, flaked maize and groundnut cake both K and chloride concentrations in rumen fluid were highest shortly after eating and declined steadily thereafter to low values 14 h after eating, or immediately before the next meal. This behaviour is shown in Figs. 2 and 3. Results from a previous experiment in which hay was given through the fistula (Bailey & Balch, 1961 *a*) have been included in order to show that giving hay by this route did not alter the pattern of decline in concentra-

Table 2. *Influence of diet on the concentration of sodium, potassium, chloride and phosphate ions in mixed saliva* 1, 4, 8 *and* 14 *h after the beginning of eating*

(Each value is the mean for the three samples collected on consecutive days)

		Na^+ (m-equiv./l.)				K^+ (m-equiv./l.)				Cl^- (m-equiv./l.)				HPO_4^{2-} (m-equiv./l.)			
Diet*	Cow	1	4	8	14	1	4	8	14	1	4	8	14	1	4	8	14
Silage	B	92	114	114	114	70	44	40	42	5·2	5·3	5·0	5·3	41	28	30	29
	D	153	159	152	160	12	8	10	7	15·4	8·7	8·2	7·6	37	26	24	31
Hay	A	74	93	93	95	75	66	56	56	3·0	3·0	3·0	3·0	25	23	20	18
	B	157	159	159	160	5	5	5	4	9·0	8·5	8·4	7·5	23	23	22	22
	C	153	158	151	152	11	9	14	8	8·9	7·5	7·2	6·9	27	20	21	20
	D	98	106	116	105	54	44	40	46	4·1	4·2	4·7	3·8	28	22	21	22
Hay and dairy cubes	A	166	163	163	163	4	4	4	4	8·6	8·3	7·9	7·7	33	26	27	29
	C	166	164	164	163	4	4	4	4	6·6	6·1	6·5	6·1	31	24	26	30
Hay, flaked maize and groundnut cake	A	140	144	138	143	26	21	22	23	6·5	7·0	6·6	6·3	32	28	28	28
	D	157	157	153	156	9	7	10	10	8·3	9·7	9·2	8·7	39	31	35	37
Grass	A	148	149	150	151	14	14	14	12	7·2	8·6	8·3	8·9	24	22	23	19
	B	153	150	150	156	14	15	15	12	7·0	7·3	7·0	6·7	31	31	31	39
	C	124	124	125	128	43	39	39	36	4·4	4·5	4·7	4·6	27	23	22	23

* Amounts of each food given daily are shown in Table 1.

Table 3. *Influence of diet on the concentration of sodium, potassium, chloride and phosphate ions in rumen fluid* 1, 4, 8 *and* 14 *h after the beginning of eating*

(Each value is the mean for the three samples collected on consecutive days)

		Na^+ (m-equiv./l.)				K^+ (m-equiv./l.)				Cl^- (m-equiv./l.)				HPO_4^{2-} (m-equiv./l.)			
Diet*	Cow	1	4	8	14	1	4	8	14	1	4	8	14	1	4	8	14
Silage	B	87	74	83	93	79	64	66	61	28	22	18	14	5	3	6	12
	D	105	107	123	129	46	35	30	26	25	19	15	10	4	4	6	6
Hay	A	83	77	95	82	85	75	73	75	15	11	10	9	13	15	14	16
	B	110	120	121	128	33	27	24	18	18	16	13	11	16	14	18	16
	C	133	145	159	157	37	26	23	17	18	12	11	9	15	14	16	20
	D	91	90	94	92	65	61	58	58	18	12	9	7	14	16	17	17
Hay and dairy cubes	A	136	133	132	137	36	29	21	17	26	20	15	14	26	34	38	37
	C	147	157	148	145	32	24	20	18	28	14	11	11	36	37	39	45
Hay, flaked maize and groundnut cake	A	130	128	126	127	34	31	29	33	15	11	11	14	42	38	33	40
	D	134	141	140	150	24	25	23	23	12	9	9	10	36	38	33	35
Grass	A	113	114	125	133	57	48	41	32	32	27	22	16	29	26	27	27
	B	128	135	145	137	56	51	43	34	27	24	18	16	34	35	38	39
	C	94	95	109	113	71	66	60	54	29	22	15	12	19	19	26	25

* Amounts of each food given daily are shown in Table 1.

tions of K and chloride in rumen fluid. The low values for both K and chloride in rumen fluid 14 h after feeding were invariably higher than the mean concentrations of these ions in mixed saliva at this time.

The concentration of chloride in rumen fluid and the pattern of the decline over the interval between meals varied little from cow to cow with the same diet. With K, however, the pattern of the decline was similar for different cows receiving the same diet, but the concentrations varied considerably. The mean concentration of K in rumen fluid was proportional to its concentration in saliva (Fig. 4).

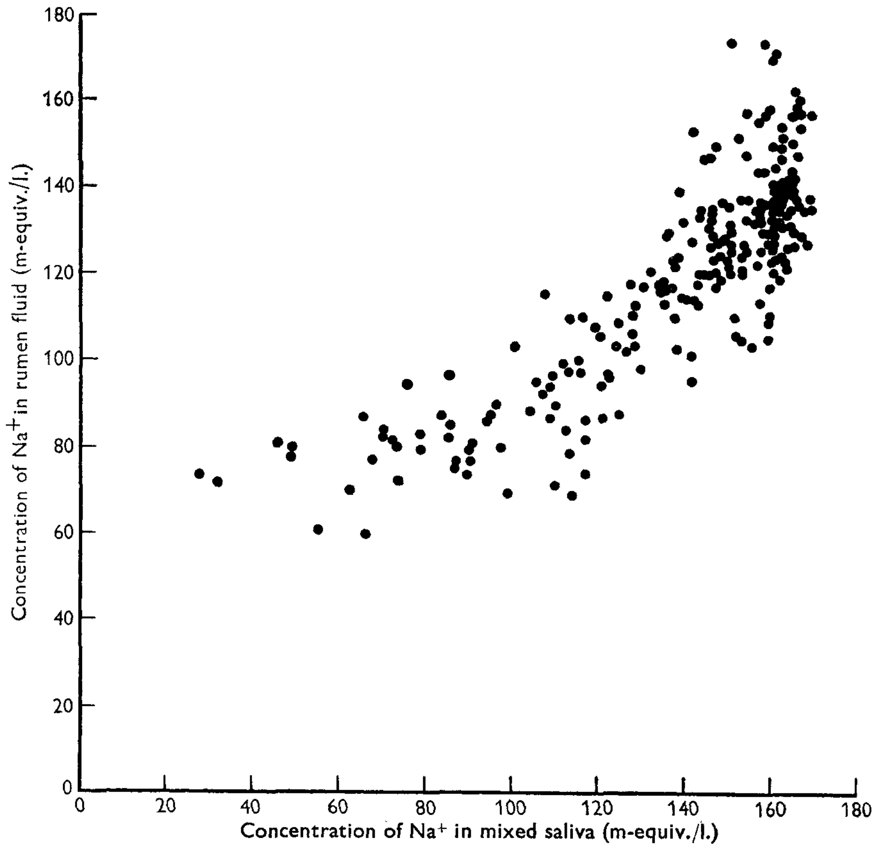

Fig. 1. Relationship between the concentrations of sodium ions in samples of mixed saliva and in rumen fluid collected at the same time from the cows.

The concentrations of chloride and K in rumen fluid from the two cows consuming hay and flaked maize were low and, with the exception of a small decline in chloride concentration from 1 to 4 h after feeding, unrelated to the time of collection.

The concentrations of phosphate in saliva were similar for all cows and all diets. The concentrations of phosphate in the rumen fluid were similar for the different cows consuming any one diet, but there were large differences between diets. In the cows consuming silage, phosphate concentrations were low in rumen fluid (mean 5·7 m-equiv./l.) and only about one-quarter those in saliva from the same cows. In the cows receiving hay, phosphate concentrations in rumen fluid (mean 15·7 m-equiv./l.) were also somewhat lower than in saliva. In the cows receiving the other three diets phosphate concentrations in rumen fluid were the same as, or somewhat higher than, in saliva.

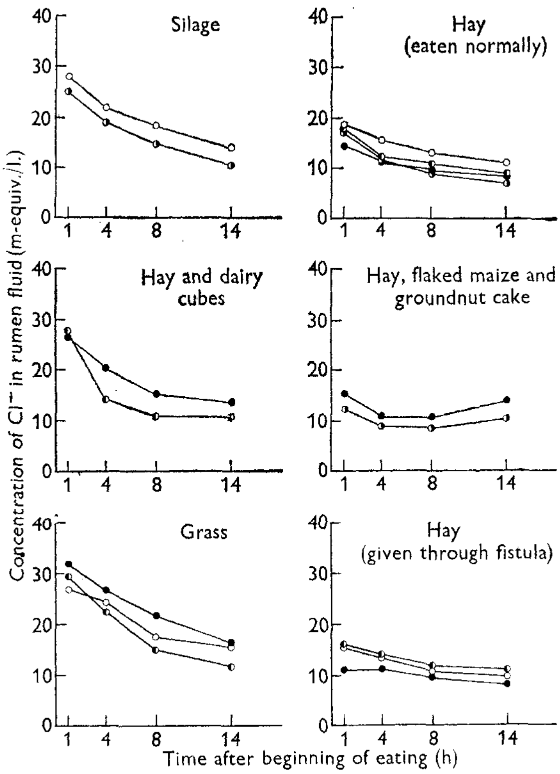

Fig. 2. Concentration of chloride ions in the rumen fluid of the cows 1, 4, 8 and 14 h after the beginning of a meal. Values are shown for cows A, ●, B, ○, C, ◐, and D, ◑, receiving various diets. Each cow was given three or four diets, but one of the diets was given in two ways.

DISCUSSION

The relationship between concentrations in saliva and rumen fluid varied with the several ions studied. Consideration of the reasons for these variations underlines the importance of saliva in ruminant digestion.

Sodium. The diets used in these experiments supplied 4–16 g Na daily. The Na concentration in the saliva rarely fell below 100 m-equiv./l., yet at this concentration 100 l. of saliva (Bailey, 1961) would supply 230 g Na daily. As would be expected, therefore, the concentration of Na in the rumen contents was parallel to, but lower than, the concentration in saliva. The consistency of this relationship was high, which suggests that when food was eaten, and salivary flow increased, absorption of Na and the voluntary water intake during the meal was sufficient to prevent any rise in Na concentration in the rumen.

At any one time the concentration of Na in the saliva was clearly the dominant factor determining the concentration found in the rumen. From the results given in previous papers (Bailey & Balch, 1961 *b*, *c*) it would appear that at least some of the diets were deficient in Na. Over a long period with a Na-deficient diet the amount of Na supplied by the diet could have an effect on Na concentration in rumen fluid owing to a progressive depletion of body Na and a consequent decline in salivary Na concentration (Bailey & Balch, 1961 *b*). Thus, differences in the Na concentrations in saliva and rumen fluid between cows consuming the same diets probably reflect the varying lengths of time the cows had been consuming the diets.

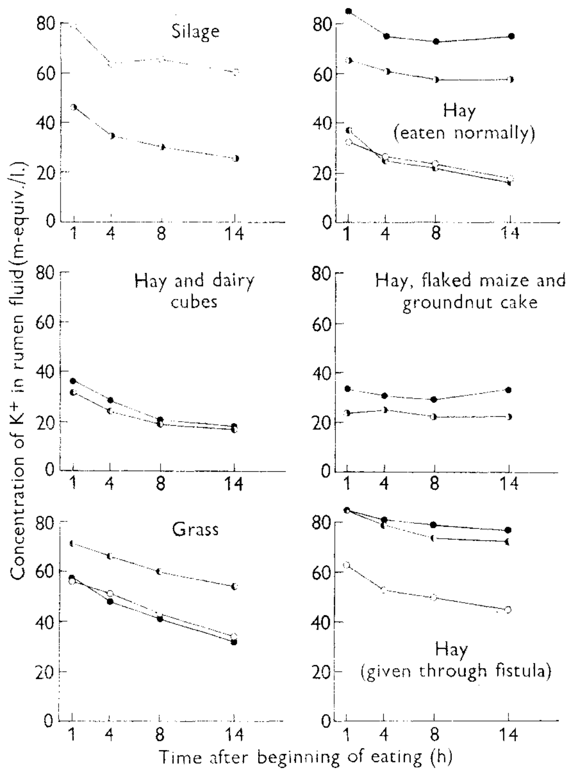

Fig. 3. Concentrations of potassium ions in the rumen fluid of the cows 1, 4, 8 and 14 h after the beginning of a meal. Values are shown for cows A, ●, B, ○, C, ◐, and D, ◑, receiving various diets. Each cow was given three or four diets, but one of the diets was given in two ways.

The generally lower concentration of Na in rumen fluid compared with mixed saliva implies its absorption from the rumen. Sperber & Hydén (1952) demonstrated that the rumen epithelium of sheep is permeable to Na, and Dobson (1959) found in sheep that movement of Na from the rumen to the blood occurred against both electric and concentration gradients. This mechanism could account for the results obtained in the experiments now described. Some of the values included in Fig. 1 from other experiments would indicate, however, that the concentration of Na in rumen fluid does not usually fall below about 60 m-equiv./l., even though its concentration in the saliva may decrease to as little as 28 m-equiv./l. This finding suggests that when the concentration of Na in saliva decreased to low levels there were alterations in the balance between the addition of water and Na to the rumen and the removal of water and Na from it.

Values, also shown in Fig. 1, from these and other experiments suggest that, whereas with sodium chloride *ad lib.* the concentration of Na in rumen fluid may rise to 180 m-equiv./l., the concentration in saliva does not exceed 168 m-equiv./l.

Chloride. With a concentration in saliva of 8 m-equiv./l., 100 l. of saliva would supply 30 g of chloride. Whereas four of the five diets used in these experiments supplied

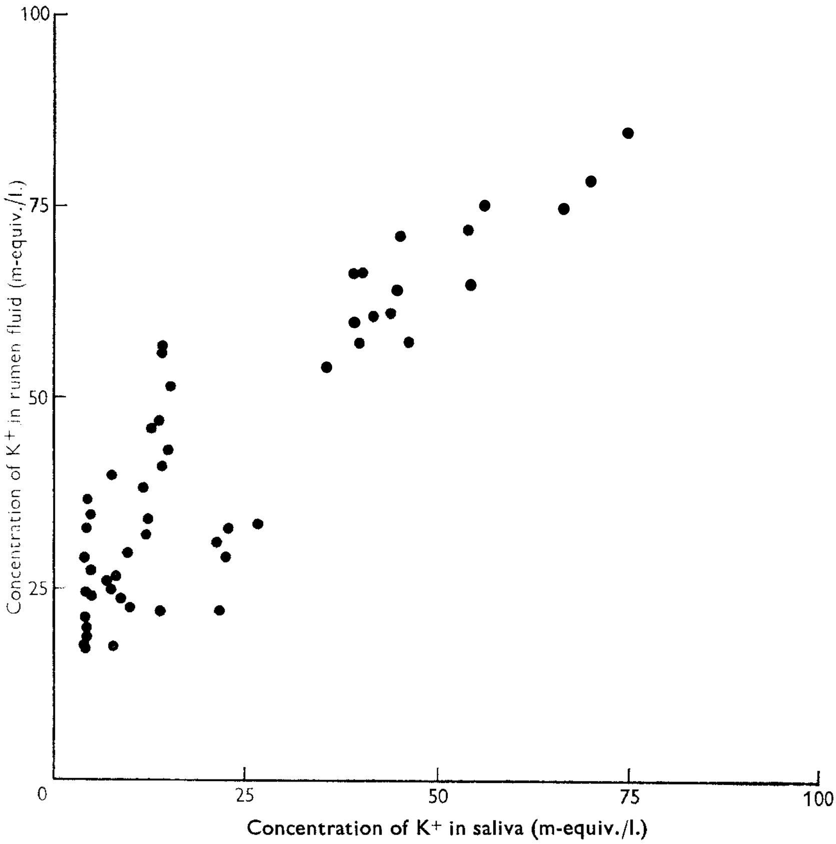

Fig. 4. Relationship between the concentrations of potassium ions in samples of mixed saliva and in rumen fluid collected at the same time from the cows.

more than this amount of chloride daily, the amounts of chloride supplied by all the diets were greater, relative to those in the saliva, than were the amounts of Na. Unlike that of Na, the concentration of chloride in rumen fluid was invariably greater than the concentration in saliva, and the uniformity in the concentration of chloride in the rumen fluid in different cows consuming a given diet (Fig. 2) confirms that the dietary chloride was important in establishing these concentrations. Dobson (1959) demonstrated that chloride is removed from the rumen of sheep by diffusion in the direction of the electrochemical gradient. The highest concentration of chloride in rumen fluid in the experiments now described was less than one-third of that usually found in plasma, and in order for diffusion to have occurred the electric potential gradient must have been high enough to overcome the adverse concentration gradient. The absence of a continuous decline in the concentration of chloride in rumen fluid after a meal of hay, flaked maize and groundnut cake was probably due to the small amount of chloride in this diet and the consequent low concentration immediately after feeding.

Potassium. The wide variations noted in the saliva concentrations of K were previously shown to be due to a tendency for the animal to maintain a uniform tonicity in the saliva despite large fluctuations in the Na concentration (Bailey & Balch, 1961*c*). With concentrations of 4 and 75 m-equiv. K/l., the lowest and highest noted in the experiments now described, 100 l. of saliva would supply 16 and 293 g K, respectively. As with Na, the concentration of K in rumen fluid reflected these differences in the amounts of K supplied by the saliva (Fig. 4). On the other hand, the concentrations of K in rumen fluid were invariably higher than the concentrations in saliva, which suggests that dietary K was a more important fraction of the K in the rumen than was the dietary Na of the Na in the rumen.

The permeability to K of the rumen wall in sheep has been demonstrated by Dobson (1959), the movement of this ion being in the direction of the electrochemical gradient. The lowest concentration of K in the rumen fluid in the experiments described here was about four times greater than the mean concentration of plasma K, and this difference may well have been sufficient to overcome the adverse electric potential gradient which exists between rumen fluid and blood (Dobson, 1959). The absence of a continuous decline in the concentration of K in rumen fluid after a meal of hay, flaked maize and groundnut cake was probably due to the small amount of K in this diet and the consequent low concentration immediately after feeding.

The high concentrations of K and chloride in rumen fluid shortly after a meal probably resulted from a rapid solution of the dietary K and chloride in the rumen water. The subsequent decline may have been due to absorption from the rumen and to dilution of the rumen fluid by saliva. The continuous secretion of saliva with fairly uniform concentrations of both chloride and K ions (Bailey & Balch, 1961*c*) would tend to ensure that after a meal the concentrations of these ions approach their concentrations in saliva. The rate of approach would depend on the rate of addition of saliva to the rumen and the rate of absorption of the ions from the rumen.

Phosphate. The concentration of soluble phosphate in rumen fluid from the cows in the experiments now described was frequently as high as, or even higher than, the concentration in saliva, but with certain diets, notably hay and silage, the mean concentration in rumen liquor was less than the concentration in saliva. As these differences were not due to markedly different intakes of phosphate from the diet it seemed possible that they resulted from either differences in the rate of absorption of phosphate from the rumen or changes in the solubility of the phosphate. Thus, though Sperber & Hydén (1952) found only very small movements of phosphate across the rumen epithelium of goats from salt solutions added to rumen pouches, Scarisbrick & Ewer (1951) and Parthasarathy, Garton & Phillipson (1952) showed that, in anaesthetized sheep, the rumen epithelium was permeable to phosphate although the net flux of this ion was small. It seems possible therefore that the rate of net absorption may have been high with my diets of hay or silage; the alternative possibility, a lowering of concentration of soluble phosphate due to the formation of insoluble salts or incorporation into bacterial cells, must not be overlooked. Garton (1951) found that after having been used to extract hay, a synthetic saliva contained less phosphate than the original fluid.

Importance of saliva in ruminant digestion. As this is the last paper of the series on saliva secretion in relation to feeding in cattle, it is desirable to assess our present knowledge of the importance of saliva in ruminant digestion.

It has been suggested frequently that the secretion of copious amounts of buffered saliva is a major factor in the maintenance in the rumen of optimum conditions for digestion by micro-organisms (cf. Annison & Lewis, 1959). There can be little doubt that a large part of the water and much of the inorganic content of the rumen digesta comes from the saliva, and there is clearly a distinct tendency for the ionic composition of the rumen fluid to change towards that of saliva as the interval between meals progresses.

The amounts of saliva added while meals of various foods are being eaten, varies somewhat as, with different diets, do the rates of secretion during resting and ruminating, but we have been able to find little evidence of any changes in the amount or composition of saliva to meet changing conditions in the rumen (Bailey & Balch, 1961*b*, *c*; Bailey, 1961). The results reported in this communication show, however, that the influence of the saliva in changing the composition of the rumen fluid varied with the different ions studied, the influence of saliva being greater with Na than with K, chloride or phosphate.

The mixed saliva of cows, like the parotid saliva of sheep (Kay, 1960), remains approximately isotonic with blood (Bailey, 1959, unpublished) notwithstanding the considerable differences in the content of the various ions at different times and with different diets. It follows that, in addition to acting in a buffering capacity and to providing minerals necessary for the nutrition of the micro-organisms, the saliva must play an important part in ensuring that the tonicity of the rumen contents does not differ greatly from the tonicity of the blood. This effect would help to limit the movement of water across the rumen epithelium and thereby contribute to the maintenance of those levels of free fluid in the ventral reticulo-rumen that have been shown to be important for optimum mechanical functioning (Schalk & Amadon, 1928; Balch, 1958). Further, the dry-matter content of the digesta in the various regions of the reticulo-rumen influences the rate of breakdown of cellulose in those regions (Balch & Johnson, 1950). It remains to be shown to what extent the rate of secretion and composition of saliva can be changed in order to counter alterations in the contents of free liquid or of dry matter in the reticulo-rumen. The results of these experiments suggest that in general the direct response to changes of this kind would be fairly small.

SUMMARY

1. The concentrations of sodium, potassium, chloride and inorganic phosphate ions 1, 4, 8 and 14 h after the beginning of a meal were determined in mixed saliva and rumen fluid of four cows receiving five different diets.

2. The concentration of Na in rumen fluid was proportional to, but usually lower than, the concentration in saliva. The most important source of Na in the rumen fluid was the saliva.

3. The concentrations of chloride in rumen fluid were invariably greater than those in saliva and the amounts of chloride supplied by the diets were probably equal to or greater than those supplied by the saliva. In all but one instance the chloride in the diet increased the concentrations of this ion in rumen fluid 1 h after a meal; the concentration decreased thereafter and approached the saliva levels 14 h after the meal.

4. The concentration of K in rumen fluid was proportional to, but invariably higher than, the concentration in saliva. The amounts of K supplied by the diets constituted a greater fraction of the rumen K than did the dietary Na of the Na in the rumen. In all but one instance the K in the diet increased the concentration of this ion in rumen fluid 1 h after a meal; the concentration decreased thereafter to low values 14 h after the meal.

5. The concentration of inorganic phosphate in rumen fluid was higher with some rations than with others, but the differences could not be attributed to variations in the phosphate concentrations in saliva or the amounts consumed in the food.

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