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A MICROPUNCTURE INVESTIGATION OF ELECTROLYTE TRANSPORT IN THE PAROTID GLANDS OF SODIUM-REPLETE AND SODIUM-DEPLETED SHEEP

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SUMMARY

1. Parotid secretion has been studied by micropuncture in sodium-replete and sodium-deficient sheep.

2. The osmolality of unstimulated primary saliva was slightly higher than in plasma and fell following cholinergic nerve stimulation. In sodium-depleted animals the osmolality of final saliva was hypotonic and exhibited flow dependency, whereas in sodium-replete animals it was always isotonic.

3. In sodium-replete sheep, the primary fluid sodium concentration was about 120–130 mmol l^{-1} but in final saliva it was about 167 mmol l^{-1} and showed little or no flow-dependency. In sodium-depleted sheep, the primary sodium concentration averaged only 82·2 mmol l^{-1} and it was concluded that sodium-depleted primary fluid contained some other unidentified solute that allowed it to remain approximately isotonic; in final saliva the unstimulated sodium concentration was about 40 mmol l^{-1} and it rose with increasing flow rate to a maximum of 114·9 mmol l^{-1}.

4. The primary fluid potassium concentration in sodium-replete animals did not differ significantly from that seen in sodium-depleted animals and the values were uninfluenced by stimulation; the over-all mean value was 11·2 mmol l^{-1}. In final saliva, in sodium-replete sheep, the potassium concentrations averaged 7·8 mmol l^{-1} but in sodium-depleted sheep the concentrations were between 5 and 10 times greater than in primary fluid.

5. It was calculated from the equilibrium pH that the primary bicarbonate concentration would have been about 35 mmol l^{-1}. In final saliva, where bicarbonate was measured directly, the concentrations were much greater and increased with stimulation to about 115 mmol l^{-1}.

6. The primary fluid phosphate and chloride concentrations were the same in both sodium-replete and sodium-depleted animals and were unchanged by stimulation; the mean concentration of phosphate was 13·0 mmol l^{-1} and of chloride, 53·0 mmol l^{-1}. In final saliva the phosphate concentrations were little changed but the chloride concentrations fell to an average value of 20·0 mmol l^{-1}. In final saliva it was found that the summed sodium and potassium concentrations exceeded the summed chloride, bicarbonate and phosphate (in mequiv l^{-1}) concentrations, on average by 13·9 mequiv l^{-1}, regardless of sodium status or flow rate.

7. The results indicate that secretion by the sheep parotid can be accounted for in

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terms of the standard two-stage model. Phosphate seems to enter the saliva only in the primary fluid and potassium and bicarbonate appear to enter at both primary and secondary sites; sodium and chloride enter at the primary level and can be reabsorbed in the ducts. Salt depletion causes the primary fluid concentrations of sodium and chloride to fall and the content of an unidentified solute to rise markedly while, at the ductal level, it causes normally quiescent sodium and potassium transport processes to become activated.

INTRODUCTION

The two-stage secretory model for salivary secretion requires the formation by the glandular endpieces of an isotonic primary saliva having approximately plasma-like electrolyte content, which is subsequently modified in the glandular ducts by reabsorption and secretion of various electrolytes (Thaysen, 1960; Young & van Lennep, 1979). Although originally enunciated to explain the electrolyte excretion patterns of the human parotid, the model has been understood to apply to all hypotonic secreting glands. Direct evidence in support of the model first came from micropuncture and duct perfusion studies on the rat mandibular gland (Martinez, Holzgreve & Frick, 1966; Young & Schögel, 1966; Young, Frömter, Schögel & Hamann, 1967) but subsequent corroborative studies have also been carried out in the salivary glands of mice, rabbits, cats, and the marsupial possum, *Trichosurus vulpecula* (Young & van Lennep, 1979). However, with the exception of one study on the cat sublingual gland (Kaladelfos & Young, 1973), all work to date has been carried out on a fairly homogeneous group of hypotonic-secreting glands and it is not clear whether the same model applies to the isotonic secreting glands. The applicability of this model to the isotonic secreting sheep parotid has been studied indirectly by comparing the composition of saliva rapidly expressed by the myoepithelium surrounding the endpieces when stimulated via the cervical sympathetic trunk (Blair-West, Coghlan, Denton, Nelson, Wright & Yamauchi, 1969) but, while this method indicated that ductal modification of endpiece fluid occurs, it does not evoke the same confidence as procuring endpiece fluid by micropunture. Since Compton & Young (1976) had shown the feasibility of doing this in the sheep parotid, it was decided to compare primary and final fluids of the unstimulated and cholinergically stimulated gland (Coats, Denton, Goding & Wright, 1956).

The sheep parotid in common probably with the parotids of all the Bovidae (Bott, Denton, Goding & Sabine, 1964; Young & van Lennep, 1978, 1979; Young, 1979), is atypical in a number of remarkable respects. It is isotonic-secreting (true, also of the sublingual glands of the Fissipaedia), and has an extremely vigorous 'spontaneous' secretory rate (i.e. secretion in the absence of all known secretory stimuli) (Coats & Wright, 1957). The composition of sheep parotid saliva is also quite remarkable since it is exceptionally rich in phosphate and the relative concentrations of sodium and potassium in it can be completely reversed according to whether the animal is sodium-replete or sodium-depleted (Denton, 1956; Blair-West, Coghlan, Denton & Wright, 1967).

The present study was undertaken specifically to determine whether the two-stage model could account for the formation of sheep parotid saliva and to ascertain

whether the high phosphate content of the saliva arises as the result of secretion by the endpieces or the ducts. In addition, by studying salt-replete and salt-deprived sheep, it was hoped to learn whether the remarkable reversal of sodium and potassium concentrations seen during salt deprivation is the consequence of a change in the activity of the cells of the ducts or the endpieces. The results obtained suggest that the two-stage hypothesis does apply to the sheep parotid and that phosphate enters the saliva largely, if not exclusively, in the primary secretion. Furthermore, it appears that the changes in salivary sodium and potassium content brought about by salt deprivation are mainly due to changes in the transport activity of ductal cells.

A preliminary report of part of this work has already been published (Compton & Young, 1976).

METHODS

Thirty merino or crossbred sheep of either sex (twenty-three ewes and seven wethers) were studied, fourteen sodium-replete and sixteen sodium-depleted. The sodium-replete sheep were maintained on a diet of lucern chaff with adequate salt supplementation for at least 1 week and were loaded with 15 g sodium chloride by rumen tube on the eve of the experiment. Sheep were depleted of salt by the establishment of a unilateral parotid duct fistula through which the saliva was allowed to drain and escape (Denton, 1957). The severity of the developing sodium deficiency was monitored by performing daily determinations of the sodium and potassium concentration in the parotid saliva; an animal was considered as being suitably salt-depleted when, on the morning of the proposed experiment, the parotid salivary sodium concentration was less than 70 mmol l^{-1} and the potassium concentration exceeded 50 mmol l^{-1}.

Anaesthesia was induced with sodium thiopentone (400–500 mg) or sodium pentobarbitone (350–450 mg) injected I.V. The animals were then intubated with a Magill tube and thereafter maintained under anaesthesia with 2 % halothane in oxygen with positive pressure ventilation. Throughout each experiment, the blood pressure was monitored via a catheter placed in the transverse facial artery and, periodically, blood samples were taken for immediate measurement of serum electrolyte concentrations and blood gas tensions. Surgery was carried out on the non-fistulated parotid gland under aseptic conditions, in order to avoid effects due to infection, particularly at the gland surface. The parotid main duct was exposed close to the gland and the periductal fibres of the secretomotor (Moussu's) nerve were separated from the duct, sectioned and prepared for placement on an electrode; the ipsilateral cervical sympathetic nerve trunk was divided at midneck in order to avoid any uncontrolled gland stimulation. A polyethylene cannula was tied into the main duct and arranged so that saliva could be collected anaerobically into a syringe from a side arm. The saliva front was maintained at a height of 10 cm above the gland hilum in order to facilitate collection of micropuncture samples; it had been established in preliminary experiments that this small increase in outflow resistance changed neither the gland secretory rate nor the salivary composition. In the first seven experiments (Compton & Young, 1976) a small branch of the carotid artery caudal to the gland was cannulated to allow close-arterial infusion of acetylcholine chloride (10^{-4} mol l^{-1}) but in all subsequent experiments Moussu's nerve was stimulated electrically with 20 V impulses at 20 Hz, using a unipolar electrode.

The parotid gland was exposed and the overlying skin flap was turned back and supported on a copper retractor in such a way as to form a cavity into which paraffin oil could be poured so as to cover the surface of the gland completely. The gland was viewed under a dissecting microscope with the aid of a fibre-optic light source and was divested of its tough fibrous capsule and then protected by a layer of oil. Micropuncture samples were collected in oil-filled glass micropipettes as described previously (Martinez *et al.* 1966; Young & Schögel, 1966; Young & Martin, 1971). In the sheep parotid, the secretory endpieces consist of long tortuous tubules and, from the morphology, it is clear that only the endpieces themselves and an occasional intercalated duct would have been accessible to the micropuncture pipette which is only inserted to a depth of 20–30 μm below the gland surface (Young & van Lennep, 1978; van Lennep, Kennerson & Compton, 1977). When a short column of black-stained paraffin oil is injected so as to outline the lumen of an endpiece, it is flushed away in the stream of the continuously secreted primary

fluid; this contrasts with what is seen in other species where the oil column remains in place. With careful use of suction, it often proved possible to hold the column of oil stationary whilst aspirating samples of quite large volume ($\sim$ 100 nl).

Final saliva was collected fron the cannula placed in the main duct of the gland, both in the unstimulated state and during stimulation, and the salivary flow rate was determined from the sample volume and its collection time (Blair-West *et al.* 1969). At the conclusion of each experiment, the animal was killed and the gland removed and weighed so as to enable flow rates to be expressed per gram of secretory tissue. There was no significant difference in the mean gland weights for sodium-replete and sodium-depleted animals; the mean wet weight for all glands was 12·0 g (S.D. = 2·2).

The concentrations of sodium and potassium in final saliva were determined by flame photometry and chloride by a colorimetric procedure, using a Technicon[R] Autoanalyser; bicarbonate was estimated as total carbon dioxide also using a Technicon Autoanalyser, and osmolality by melting point determination. In micropuncture samples the concentrations of sodium and potassium were determined in the first seven experiments with a picomole flame photometer (Young & Shagrin, 1968) and in all subsequent experiments with an Aminco[R] helium glow photometer. In preliminary experiments it was demonstrated that there was negligible interference between sodium and potassium present in standard solutions in proportions comparable to those seen in saliva but, in any case, both machines were calibrated daily with solutions containing appropriate mixtures of sodium and potassium. Osmolality in micropuncture samples was determined from melting point using a Clifton[R] Biological Cryostat (Young & Shagrin, 1968) and Cl by coulombometric titration (Young, Martin, Asz & Weber, 1970). All micropuncture samples were analysed without dilution except in the case of phosphate determinations. Phosphate in all samples was estimated by a scaled down version of the colorimetric procedure of Kuttner & Cohen (1927). The pH of samples of whole blood and of final saliva were determined at 37 °C using a Radiometer[R] pH meter and micro-glass-electrode, type E5021*a*. The samples were collected anaerobically and pH was determined as soon as practicable after the experiment. For micropuncture samples, a pH-sensitive capillary glass electrode suitable for samples of volume 100–200 nl was used. Although micropuncture samples were collected anaerobically, their small volume made it seem possible that appreciable escape of carbon dioxide through the paraffin oil would occur. To obviate this, the pH of each sample was determined in a chamber filled with oil (at 37 °C) that had been gassed continuously with 5 % carbon dioxide in air for at least 2 h. By directly measuring the p_{CO_2} of comparable volumes of plasma, gassed in the same system, we estimate that the p_{CO_2} in our samples at the time of pH measurement was somewhat lower than 38 mm Hg, on average about 30 mm Hg. From a knowledge of the pH and the CO_2 tension, it was possible to estimate the bicarbonate content of the samples using the expanded form of the bicarbonate-carbon dioxide equilibrium equation, assuming a value of 6·13 for pK_1' and 9·7 for pK_2' (Edsall & Wyman, 1958); in the case of samples of final saliva this was not necessary since sample volumes were large enough to permit direct determination of bicarbonate (as total carbon dioxide).

For micropuncture samples, the data were divided, simply, into unstimulated and stimulated groups since we had no way of determining the individual secretory rate of each sample of primary saliva collected. For final saliva we were able to use the conventional plot of electrolyte concentration against flow rate. The data were stored on computer disk and sorted by computer into flow rate 'bins', each containing approximately equal numbers of samples; the bin size was set to be an integral multiple of 10 $\mu l\ g^{-1}\ min^{-1}$. For each bin, the mean concentration and flow rate, each $\pm$ S.E. of mean, were calculated. Throughout the paper, mean values are quoted with the standard error of the mean (S.E. of mean) and the number of observations (n) immediately following. Mean values were compared using Student's t test.

RESULTS

Effect of sodium deprivation on plasma composition

In Table 1 are set out the measured electrolyte concentrations, the osmolality and the pH of the plasma samples obtained from sodium-replete and sodium-depleted sheep. Salt depletion caused highly significant reductions in the plasma concentrations

of sodium (by 7·3 mmol l^{-1}) and bicarbonate (by 8·0 mmol l^{-1}) and increases in the concentrations of potassium (by 1·5 mmol l^{-1}) and chloride (by 11·0 mmol l^{-1}). There was also a significant reduction in phosphate concentration (by 0·24 mmol l^{-1}) and in pH (from 7·49 to 7·36).

Final saliva

The results of our analysis of final saliva are shown in Figs. 1–3 and in Table 2.

Osmolality (Fig. 1 and Table 2). In sodium-replete animals it was found that salivary osmolality was close to that of plasma at all flow rates; in unstimulated saliva the

TABLE 1. Composition of sheep plasma in sodium-replete and sodium-depleted animals. Values are means ± S.E. of mean; n = number of samples

	Sodium-replete ($n = 9$)	Sodium-depleted ($n = 12$)	P
mmol l^{-1}			
[Na]	147·9 ± 0·8	140·6 ± 1·0	< 0·001
[K]	3·8 ± 0·2	5·3 ± 0·3	< 0·001
[Cl]	96·2 ± 1·4	107·2 ± 2·1	< 0·001
[HCO_3]	23·3 ± 1·2	15·3 ± 1·0	< 0·001
[PO_4] total	1·34 ± 0·08	1·01 ± 0·10	< 0·025
mosmol $kg_{H_2O}^{-1}$			
Osmolality*	284·0 – 300·0	259·0 – 293·0	—
pH	7·49 ± 0·06	7·36 ± 0·03	< 0·05

* Osmolality was only measured for plasma from three sheep in each series. In earlier studies on these and other sodium-replete sheep, the mean plasma osmolality was 297·5 ± 1·6 mosmol $kg_{H_2O}^{-1}$ ($n = 15$); from a series of mildly sodium-depleted sheep, it was 289·1 ± 2·6 mosmol $kg_{H_2O}^{-1}$ ($n = 15$); these means differ significantly ($P < 0·01$).

mean osmolality was 281·0 ± 4·8 mosmol $kg_{H_2O}^{-1}$ ($n = 16$) and it rose significantly ($P < 0·05$) by 14 mosm $kg_{H_2O}^{-1}$ following stimulation. Examination of the responses of individual animals revealed that, in some cases, the saliva was close to isotonic at all flow rates whereas, in others, there was a definite flow dependency; in those cases where flow dependency was absent, the salivary sodium concentrations were always high, often around 170 mmol l^{-1}, whereas in the cases exhibiting flow dependency, the sodium concentrations were lower, usually less than 145 mmol l^{-1}. Of our fourteen sodium-replete sheep, four showed unequivocal flow-dependent osmolality patterns. In sodium-depleted animals, the flow-dependency of salivary osmolality was much more marked (Fig. 1) so that osmolality rose from 223·3 ± 6·8 mosmol $kg_{H_2O}^{-1}$ ($n = 13$) in unstimulated saliva to 276·0 ± 5·1 mosmol $kg_{H_2O}^{-1}$ ($n = 5$) in maximally stimulated saliva ($P < 0·01$).

pH. The pH of final saliva was remarkably constant and exhibited little or no flow-dependency. Furthermore there was no significant difference in the salivary pH of the sodium-replete and sodium-depleted groups. The mean pH for all samples studied was 8·06 ± 0·02 ($n = 125$).

Sodium and Potassium (Figs. 2 and 3, Table 2). In sodium-replete animals, the final salivary sodium concentration was always high and the potassium concentration low. In four of the fourteen animals studied, a slight increase in sodium concentration

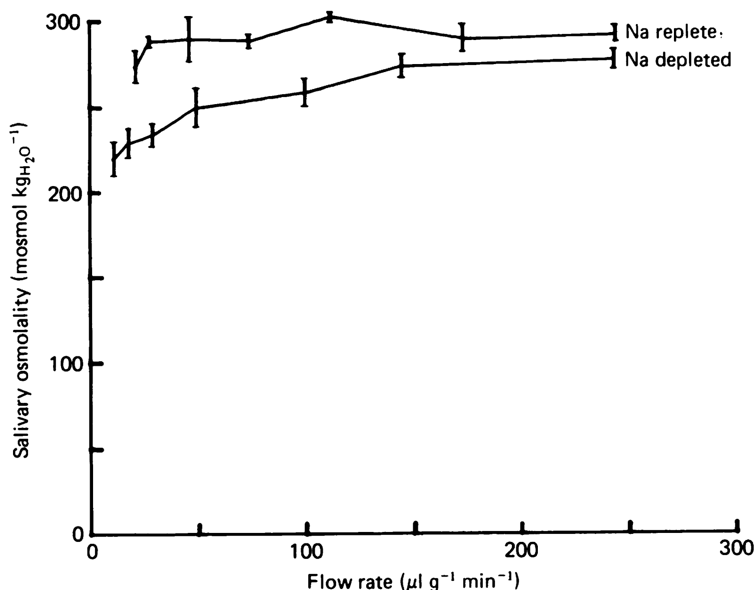

Fig. 1. The relation between the osmolality of the final saliva of the sheep parotid and salivary flow rate in sodium (Na)-replete and sodium (Na)-depleted animals. Samples collected at the lowest flow rate were from unstimulated glands and all other samples were collected during parasympathetic nerve stimulation or during close-arterial infusion of acetylcholine. Each point is the mean of five to eight samples ± S.E. of mean.

TABLE 2. Composition of sheep parotid saliva collected from the main ducts of unstimulated glands ('unstimulated saliva') and from glands undergoing parasympathetic nerve stimulation ('stimulated saliva') in sodium-replete and sodium-depleted sheep. Only samples for which all electrolyte measurements sodium, potassium, chloride, bicarbonate, phosphate (Na, K, Cl, HCO_3, PO_4) had been performed have been included. The ionic charge on the phosphate ions has been calculated in mequiv l^{-1} from the pH of the sample and the molar concentration of inorganic phosphate, assuming a pK_2' value of 6·8. The summed cation concentration, less the summed anion concentration (in mequiv l^{-1}) has been expressed as the 'residual anion' concentration. Values are means ± S.E. of mean; n = number of samples studied

	Sodium-replete			Sodium-depleted		
	Unstimulated ($n=25$)	Stimulated ($n=37$)	P	Unstimulated ($n=25$)	Stimulated ($n=38$)	P
Na (mmol l^{-1})	162·2 ± 2·3	166·7 ± 1·4	n.s.	40·1 ± 6·1	92·4 ± 5·2	< 0·001
K (mmol l^{-1})	7·4 ± 1·0	8·0 ± 0·5	n.s.	94·8 ± 5·2	62·9 ± 3·5	< 0·001
Cl (mmol l^{-1})	25·5 ± 1·2	18·3 ± 0·9	< 0·001	16·9 ± 1·0	20·1 ± 1·1	< 0·05
HCO_3 (mmol l^{-1})	97·8 ± 2·1	113·4 ± 1·7	< 0·001	67·6 ± 3·3	97·4 ± 2·8	< 0·001
PO_4 (mmol l^{-1})	17·0 ± 1·6	14·5 ± 1·3	n.s.	20·2 ± 1·7	11·7 ± 0·7	< 0·001
(mequiv l^{-1})	33·0 ± 3·1	28·1 ± 2·5	n.s.	39·0 ± 3·2	22·7 ± 1·4	< 0·001
Residual anions (mequiv l^{-1})	13·2 ± 1·8	14·9 ± 1·3	n.s.	11·4 ± 3·1	15·1 ± 0·9	n.s.
Osmolality* (mosmol $kg_{H_2O}^{-1}$)	281·0 ± 4·8	294·8 ± 2·5	< 0·05	223·3 ± 6·8	255·6 ± 5·6	< 0·01
Flow rate (µl g^{-1} min^{-1})	23·8 ± 1·0	157·0 ± 17·4	—	18·7 ± 1·4	111·6 ± 14·8	

* The numbers of samples studied for osmolality were only 16, 24, 13 and 23, respectively.

with increasing secretory rate was noticed but in the remaining ten animals no flow dependency was demonstrable. The concentrations averaged between 162 and 168 mmol l^{-1} at all flow rates although, in one sheep (showing flow dependency) they ranged between 138 and 150 mmol l^{-1} and, at the opposite extreme, in another (not showing flow dependency) they ranged between 170 and 180 mmol l^{-1}. In no case was a reversed flow dependency, with concentration falling with increasing secretory

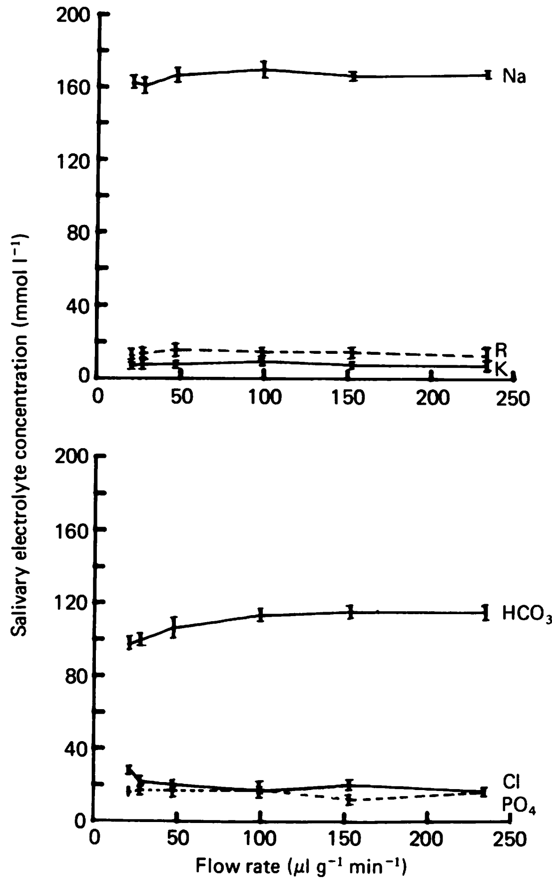

Fig. 2. The relation between the electrolyte composition of the final saliva of the sheep parotid and salivary flow rate in sodium(Na)-replete animals. Gland stimulation was achieved as described in the legend to Fig. 1; each point is the mean of seven to fifteen samples $\pm$ S.E. of mean. The symbol 'R' indicates residual anions, i.e. the summed sodium and potassium concentration less the summed chloride (Cl), bicarbonate (HCO_3) and phosphate (PO_4) concentration, expressed in mequiv l^{-1}.

rate (as reported by Beal, 1979), ever observed. Salivary potassium concentrations averaged $7{\cdot}8 \pm 0{\cdot}5$ mmol l^{-1} ($n = 62$), and showed no flow dependency, but, in the four cases that exhibited a sodium flow-dependency, a small reciprocal potassium flow-dependency was also to be observed. In the most marked of these cases, the potassium concentration was 16 mmol l^{-1} in the spontaneous secretion and fell to around 9 mmol l^{-1} at high flow rates.

In sodium-depleted animals, a marked flow-dependency, both for sodium and potassium, was seen in all cases. In unstimulated saliva, the sodium concentration averaged $40 \cdot 1 \pm 6 \cdot 1$ mmol l^{-1} ($n = 25$) and, with increasing secretory rate, the concentration rose to a mean of $114 \cdot 9 \pm 4 \cdot 8$ mmol l^{-1} ($n = 8$) at a flow rate of 200 μl g^{-1} min^{-1}. In contrast, potassium concentrations were high at low secretory rates, and fell as secretory rate increased so that the summed sodium and potassium concentrations tended to remain constant; in unstimulated saliva the potassium

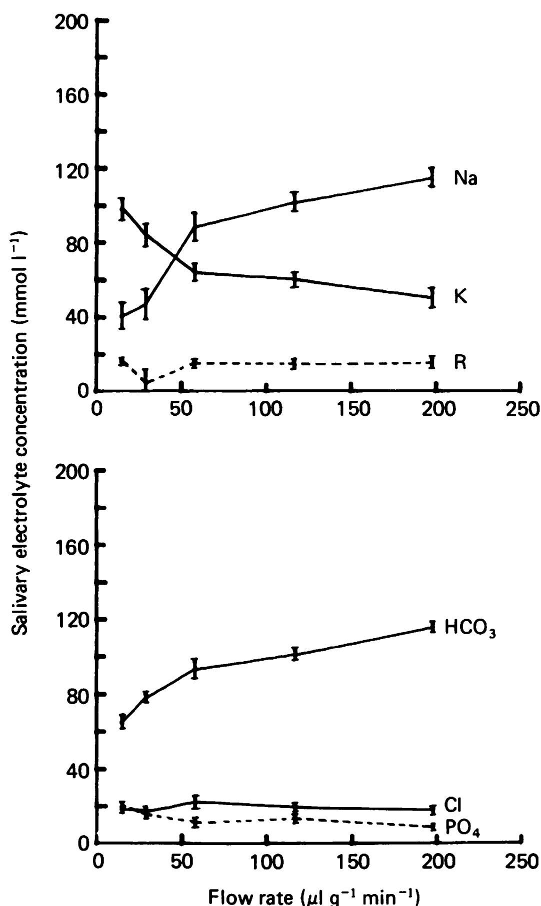

Fig. 3. The relation between the electrolyte composition of the final saliva of the sheep parotid gland and salivary flow rate in sodium(Na)-depleted animals. Gland stimulation was achieved as described in the legend to Fig. 1; each point is the mean of eight to twenty-two samples $\pm$ S.E. of mean. The symbol 'R' is defined in the legend to Fig. 2.

concentration averaged $94 \cdot 8 \pm 5 \cdot 2$ mmol l^{-1} ($n = 25$), and at maximum flow rates it had fallen to $50 \cdot 1 \pm 4 \cdot 9$ mmol l^{-1} ($n = 8$).

Bicarbonate (*Figs. 2 and 3, Table 2*). In general it was found that sodium deprivation had little effect on the concentrations and excretory patterns of salivary anions. On average, the bicarbonate concentrations in saliva from sodium-depleted animals were lower than in saliva from sodium-replete animals, as would be expected in view of the osmolality difference, but the over-all excretory pattern was similar. In un-

stimulated saliva the concentration averaged $97 \cdot 8 \pm 2 \cdot 1$ mmol l^{-1} ($n = 25$) in Na-replete samples, and $67 \cdot 6 \pm 2 \cdot 1$ mmol l^{-1} ($n = 25$) in sodium-depleted samples and, at flow rates in excess of 200 μl g^{-1} min^{-1}, the corresponding mean concentrations were $114 \cdot 7 \pm 3 \cdot 5$ mmol l^{-1} ($n = 7$) and $115 \cdot 6 \pm 2 \cdot 0$ mmol l^{-1} ($n = 8$).

Phosphate (*Figs. 2 and 3, Table 2*). Phosphate concentrations showed no flow dependency in sodium-replete saliva and the average of all samples studied was $15 \cdot 5 \pm 1 \cdot 0$ mmol l^{-1} ($n = 58$); after correction for sample pH, this represents a charge concentration of about 30 mequiv l^{-1} (assuming a pK_2' value for phosphate of 6·8: Edsall & Wyman, 1958). In sodium-depleted animals, the phosphate concentration showed a definite flow-dependency, averaging $20 \cdot 2 \pm 1 \cdot 7$ mmol l^{-1} ($n = 25$) in samples of unstimulated secretion, and falling to lower values than seen in sodium-replete saliva at high secretory rates; at flow rates of 200 μl g^{-1} min^{-1}, the concentration averaged $8 \cdot 4 \pm 0 \cdot 9$ mmol l^{-1} ($n = 8$). These concentrations represent a fall in charge concentration from 38 to 17 mequiv l^{-1}.

Chloride and residual anions (*Figs. 2 and 3, Table 2*). No consistent pattern of flow dependency was observed for chloride in either group of sheep; in sodium replete sheep the concentrations fell and in sodium-depleted sheep they rose, both changes being very small. The over-all mean chloride concentration was $20 \cdot 0 \pm 0 \cdot 5$ mmol l^{-1} ($n = 125$). After conversion of molar phosphate concentrations to the equivalent charge concentrations, it was possible to calculate the 'residual anion concentration' in mequiv l^{-1} as the difference between the summed sodium and potassium concentrations and the summed chloride, bicarbonate and phosphate concentrations. The results were similar in both groups of sheep: there was a nett residual anion concentration of $13 \cdot 9 \pm 0 \cdot 9$ mequiv l^{-1} ($n = 125$) which exhibited no evidence of flow dependency.

Primary saliva (*Table 3*)

The results of the analysis of samples of primary saliva obtained by micropuncture are shown in Table 3.

Osmolality. The respective osmolalities of unstimulated and stimulated samples collected from sodium-replete glands did not differ significantly from sodium-depleted ones ($P > 0 \cdot 4$). For all sheep studied, the mean osmolality in unstimulated samples was $309 \cdot 2 \pm 4 \cdot 8$ mosmol $kg_{H_2O}^{-1}$ ($n = 45$) and following stimulation it fell significantly ($P < 0 \cdot 01$) to a mean value of $290 \cdot 6 \pm 4 \cdot 8$ mosmol $kg_{H_2O}^{-1}$ ($n = 36$).

Sodium. In sodium-replete animals, the sodium concentration in unstimulated samples was $120 \cdot 2 \pm 2 \cdot 7$ mmol l^{-1} ($n = 74$), a value significantly ($P < 0 \cdot 001$) lower than in plasma, the mean difference being about 28 mmol l^{-1}. Following stimulation, the concentration rose significantly ($P < 0 \cdot 01$) to reach a mean value of 132·6 mmol l^{-1}, although still remaining significantly lower than in plasma ($P < 0 \cdot 05$). In sodium-depleted animals, the mean unstimulated primary sodium concentration was much lower and did not change significantly following stimulation; the over-all mean was $82 \cdot 2 \pm 2 \cdot 5$ mmol l^{-1} ($n = 151$).

Potassium. In contrast to sodium, the concentrations of potassium were significantly higher than in plasma in all animals studied. Despite marked differences in the potassium concentrations found in the final saliva of replete and depleted animals (Table 2, Figs. 2 and 3), the concentrations of this ion in the primary saliva were not

TABLE 3. Composition of primary saliva collected by micropuncture from sheep parotid glands in the unstimulated state and during stimulation, either by intra-arterial injection of acetylcholine or by electrical stimulation of the parasympathetic nerve supply to the gland. Values are means ± S.E. of mean; n = number of samples studied

	Sodium-replete			Sodium-depleted		
	Unstimulated	Stimulated	P	Unstimulated	Stimulated	P
Na (mmol l^{-1})	120·2 ± 2·7 (n = 74)	132·6 ± 2·8 (n = 64)	< 0·01	81·8 ± 3·1 (n = 71)	82·5 ± 3·8 (n = 80)	n.s.
K (mmol l^{-1})	11·5 ± 1·0 (n = 74)	9·0 ± 0·8 (n = 63)	n.s.	12·6 ± 1·5 (n = 71)	11·4 ± 1·5 (n = 79)	n.s.
Cl (mmol l^{-1})	53·3 ± 4·4 (n = 11)	49·8 ± 1·9 (n = 63)	n.s.	58·5 ± 4·2 (n = 32)	53·5 ± 3·4 (n = 39)	n.s.
PO_4 (mmol l^{-1})	14·3 ± 1·2 (n = 12)	13·2 ± 1·2 (n = 22)	n.s.	12·7 ± 1·5 (n = 15)	12·2 ± 1·8 (n = 20)	n.s.
(mequiv l^{-1})*	26·4 ± 2·1 (n = 12)	24·5 ± 2·4 (n = 22)	n.s.	22·4 ± 3·1 (n = 15)	23·1 ± 3·6 (n = 20)	n.s.
Osmolality (mosmol $kg_{H_2O}^{-1}$)	311.5 ± 4·7 (n = 19)	296·0 ± 7·0 (n = 13)	< 0·05	307·6 ± 7·2 (n = 26)	287·4 ± 6·0 (n = 23)	< 0·05

* The charge concentration attributable to $H_2PO_4^-$ and HPO_4^{2-} was calculated from the pH and the molar phosphate concentration assuming a value of 6·8 for the pK_2' (Edsall & Wyman, 1958).

significantly different in the two groups ($P > 0{\cdot}2$), nor did stimulation cause any significant change ($P > 0{\cdot}15$). The mean primary fluid potassium concentration for all samples studied was $11{\cdot}2 \pm 0{\cdot}64$ mmol l^{-1} ($n = 287$), a value significantly greater than the mean potassium concentration in the final saliva of sodium-replete animals ($P < 0{\cdot}01$) and significantly less than the concentration in the final saliva of sodium-depleted sheep ($P < 0{\cdot}001$).

pH. As was the case with final saliva, the pH of the primary saliva was remarkably constant and showed no significant difference between Na-replete and sodium-depleted animals. Similarly, stimulation had no significant effect on the measured values. The mean pH of all primary fluid samples studied was $7{\cdot}69 \pm 0{\cdot}20$ ($n = 40$) which was significantly lower ($P < 0{\cdot}001$) than the mean pH recorded in final saliva even when allowance was made for the slight difference in p_{CO_2} at which the two sets of measurements were made.

Chloride and phosphate. As was the case for potassium, the concentrations of chloride and phosphate in primary saliva did not differ significantly between sodium-replete and sodium-depleted animals. Similarly, in neither group did stimulation cause any significant alteration in concentration. The mean primary fluid chloride concentration for all samples studied was $53{\cdot}0 \pm 1{\cdot}6$ mmol l^{-1} ($n = 145$) and the mean phosphate concentration was $13{\cdot}0 \pm 0{\cdot}8$ mmol l^{-1} ($n = 69$); knowing the pH of primary saliva, we calculated the mean charge concentration for phosphate to be about 24 mequiv l^{-1} ($n = 69$). The concentration of chloride in primary saliva was significantly ($P < 0{\cdot}001$) greater than in final saliva but, in the case of phosphate, no significant difference was demonstrable (except in unstimulated samples from sodium-depleted sheep).

Bicarbonate and residual anions. Bicarbonate concentrations could not be determined directly in samples of primary saliva because of the small sample volumes. However, from a knowledge of the mean pH of the samples (7·69) and the probable p_{CO_2} at which the pH was measured (30 mm Hg), it can be calculated from the carbon dioxide–bicarbonate equilibrium equation that the bicarbonate concentration at equilibrium would have been about 35 mmol l^{-1}.

DISCUSSION

The present results allow us to conclude that the two-stage secretory model can account for the electrolyte excretion patterns of the sheep parotid in much the same way as it does for other salivary glands. The studies throw new light on the secretory mechanisms of the sheep parotid, particularly its handling of bicarbonate and phosphate, and the analysis of the primary fluid poses some interesting and unexpected problems that demand careful consideration.

Osmolality

In both sodium-replete and sodium-depleted animals the osmolality of primary fluid was observed to fall significantly by about 15–20 mosmol $kg_{H_2O}^{-1}$ following stimulation of secretion (Table 3). Although plasma osmolality was not determined in all of the sheep studied in the present series, it seems clear that the osmolality of the unstimulated samples was slightly hypertonic, since the plasma osmolality in

sodium-replete sheep in our laboratory averages only 297·5 mosmol $kg_{H_2O}^{-1}$ and it falls during sodium-depletion (Table 3). Most previous micropuncture studies have demonstrated a plasma-like osmolality for primary saliva and have failed to reveal a significant difference between the osmolalities of unstimulated and stimulated samples (Young & van Lennep, 1979). In two studies, in the rabbit parotid (Mangos, McSherry & Arvanatakis, 1973) and in the immature rat mandibular gland (Holzgreve, Martinez & Vogel, 1966), a slight degree of hypertonicity was reported although unfortunately, in neither was an attempt made to distinguish between unstimulated and stimulated samples. Since the generally accepted theory for formation of the primary fluid involves active secretion of solute with water following by osmosis, it is to be expected that the primary fluid will be isotonic or slightly hypertonic and, if the secretory process involves the so-called secretion canaliculi, that it will not change much with changing secretory rates (Young & van Lennep, 1978; 1979). However, following stimulation of solute secretion one would expect the osmolality of the primary fluid to increase whereas we have observed a decrease, implying that the hydraulic resistance of the endpiece epithelium has decreased concomitantly with an increase in solute transport.

Our studies show clearly that the osmolality of the final saliva of the sheep parotid can exhibit flow dependency and that the gland can only be considered as isotonic-secreting in salt-replete animals. In most of our sodium-replete sheep, the salivary osmolality was approximately plasma-like at all flow rates but in a few there was a definite tendency for the saliva collected at resting flow rates to be hypotonic. Since the saliva in these cases showed lower than average sodium concentrations, and higher than average potassium concentrations, it seems likely that the development of hypotonicity does not take place unless there is some degree, however slight, of sodium-depletion, or a raised plasma aldosterone due to some other cause such as operative stress (Blair-West, Boyd, Coghlan, Denton, Goding, Wintour & Wright, 1964). Another recent study (Beal, 1979) has reached a similar conclusion.

Sodium and potassium

The sodium content of the primary saliva of sodium-replete sheep, although significantly lower than in plasma, was similar to that seen in micropuncture studies of other glands (Young & van Lennep, 1979) and would not have occasioned much further comment here. However, the concentrations seen in sodium-depleted sheep are exceptionally low ($\sim$ 82 mmol l^{-1}), so low, in fact, that the summed concentration of all measured cations and anions falls short of the measured osmotic activity of the samples by more than 100 mosmol $kg_{H_2O}^{-1}$. There is no ready explanation why the measured sodium concentration might be so low; either it is due to an experimental artifact or the primary saliva must contain appreciable quantities of one or more osmotically active solutes additional to those measured. Three potential artifacts might give rise to such a result.

(i) The collected samples of primary fluid from the endpieces may have been contaminated by aspiration of sodium-poor fluid from the gland duct system. This seems unlikely, since such contaminating fluid would be hypotonic and rich in potassium and chloride (see Table 2), and the mixture would be hypotonic with relatively high concentrations of potassium and chloride; in fact, however, the fluid was isotonic

and, despite the remarkable difference in sodium concentration, the concentrations of potassium, chloride and phosphate were the same as in sodium-replete samples.

(ii) The collected samples may have contained large quantities of protein which, by occupying volume, would result in falsely low estimates of sodium concentration. This could not be exportable protein, not only because sheep parotid saliva is especially poor in protein, but also because there was a disproportionate alteration in the concentration of the several ions present. In any case there is no histological evidence to suggest that the capacity of the gland to synthesize and secrete exportable protein increases during sodium depletion (Blair-West *et al.* 1969). Another possibility is that the samples became contaminated with aspirated microvilli which are extremely profuse on the luminal surface of the endpiece epithelium of the sheep parotid (Blair-West *et al.* 1969; van Lennep *et al.* 1977). This, too, seems unlikely, since we would have to postulate that the contamination occurred in sodium-depleted but not sodium-replete animals, not to mention that, again, we would expect to see the effect manifested not only as a disturbance in the sodium concentration, but also as a disturbance in the concentrations of potassium, chloride and phosphate. In any case we examined some of our samples under dark-ground illumination and electron microscopically and never saw more than an occasional microvillus.

(iii) The samples may have contained some substance that interfered with the microdetermination of sodium. This, too, seems unlikely, since the low sodium concentrations were also observed in those experiments in which we measured sodium with the aid of a picomole flame photometer (Young & Shagrin, 1968) rather than a helium-glow photometer. Furthermore, we have never observed such interference during use of either machine when measuring samples of final saliva, primary saliva from other glands, plasma and various synthetic solutions made up to resemble primary saliva in composition.

If we may accept that the low sodium concentrations are not artifactual, it still remains for us to determine what substance made up that part of the osmotically active solute (~ 100 mosmol $kg_{H_2O}^{-1}$) not accounted for by sodium, potassium, chloride, phosphate and bicarbonate. Whatever it is, it is removed in the ducts since there is no sign of its presence in final saliva, where the total electrolyte content is sufficient to account for the measured osmolality. It seems unlikely to be protein, calcium, or any other of the commonly encountered divalent ions, since it is difficult to imagine that such compounds would be secreted in high concentrations in the primary fluid, only to be completely reabsorbed in the ducts. It seems more likely that primary saliva contains an organic compound of low molecular weight, perhaps a metabolic substrate that is reabsorbed and utilized by the duct cells. Such a compound would have to be non-ionized, or at least poorly ionized, since the summed sodium and potassium concentrations in primary fluid correspond quite closely to the summed chloride, phosphate and estimated bicarbonate concentrations.

The potassium concentration of primary saliva, which averaged 11·2 mmol l^{-1}, did not differ significantly between sodium-replete and sodium-depleted sheep and was affected only slightly or not at all by stimulation. The values are of similar size to those reported for the mandibular glands of the rabbit (Mangos *et al.* 1973; Young & van Lennep, 1979) and the cat (Kaladelfos & Young, 1974). On the basis of the available micropuncture data, Young & van Lennep (1979) have concluded that

potassium is concentrated in the cells of the endpiece by a basally located (sodium–potassium) ATPase and that the potassium ion enters the saliva from the cytoplasm across the apical membrane by a passive process. Such a mechanism seems equally likely to operate in the sheep parotid.

The sodium and potassium excretory curves, seen both in sodium-replete and sodium-depleted sheep, are similar to those described previously by many other authors (Coats & Wright, 1957; Kay, 1960; Blair-West *et al.* 1967; Young & van Lennep, 1979; Young, 1979). In the sodium-depleted animals, the excretion curves are very similar to those described for most other mammalian glands; according to Young's (1979) classification, both curves would be referred to as type A and the only feature that warrants comment is the rather high potassium concentrations observed, particularly at maximum flow rates. In sodium-replete animals, the sodium and potassium patterns are less typical since they commonly show no flow dependency (Fig. 3) or, in those cases where some flow-dependency is apparent, flow curves conforming to type F (for sodium) and type A (for potassium) of Young's (1979) classification.

The interpretation of the potassium excretory curves is relatively straightforward. In the sodium-depleted animals, where the final salivary potassium concentration always exceeded the primary fluid levels, it is clear that ductal potassium secretion occurred, just as has been postulated for all other salivary glands so far studied (Young & van Lennep, 1979; Young, 1979): the ductal secretory mechanism appears to have a limited transport rate, so that although the potassium concentration achieved in final saliva is high in resting samples, it falls asymptotically towards the primary level as flow rate is increased. In sodium-replete animals, where the final salivary potassium concentration was always lower than in the primary fluid, it appears that there is no ductal secretory mechanism involved and that potassium is actually reabsorbed during passage of the saliva to the exterior.

The sodium excretory curves are more difficult to interpret in relation to the primary fluid sodium concentrations. The form of the curve in sodium-depleted animals (Fig. 3) would suggest that ductal sodium reabsorption at a limited rate was taking place from a fluid with a high sodium content (cf. Young, 1979) but, in fact, the primary fluid sodium concentration was only 82·2 mmol l^{-1}, the plasma-like osmolality of the primary fluid being made up by an unidentified solute. Since this unidentified solute is absent from final saliva, it follows that it must have been removed in the ducts; this would have freed water, some of which would tend to be reabsorbed osmotically and, if sodium were not also being absorbed in the ducts, the luminal sodium concentration would tend to rise closer to plasma-like values. Hence, since sodium concentrations remained low, particularly at low flow rates, it seems reasonable to interpret the sodium flow curve as arising from the simultaneous reabsorption in the ducts of some sodium along with the unidentified solute. In those sodium-replete sheep in which no sodium flow-dependency was observed, the sodium concentrations found in the final saliva were considerably higher than were found in the primary fluid although the osmolality was not greater. As in the case of sodium-depleted sheep, it must be inferred that the unidentified solute responsible for raising the osmolality of primary fluid to isotonic levels has been reabsorbed along with some water so that all unreabsorbed solutes become secondarily concentrated.

Since, in contrast to sodium-depleted animals, the ducts do not appear to reabsorb sodium, the concentration of this ion rises and the saliva remains isotonic.

The above arguments draw us to the conclusion that the ducts of the sheep parotid are like those of most other mammalian salivary glands, being capable of reabsorbing sodium and secreting potassium. However, the ducts of the sheep parotid appear to be unique in possessing the capacity to cease all net sodium and potassium transport activity when the animal is sodium-replete. In those few sodium-replete sheep in which some minor ductal sodium–potassium transport activity was present, it seems reasonable to postulate that aldosterone secretion resulting from operative stress (Blair-West *et al.* 1964) was responsible.

Beal (1979), in a recent study on the sodium-replete sheep parotid, has reported sodium concentrations in final saliva similar to those described in our paper, but at unstimulated flow rates he actually observed an increase in sodium concentration (to around 190 mmol l^{-1}) associated with a sharp increase in phosphate concentration and in acidity. We have not observed such sodium patterns in the present experiments, although they have been encountered by others from time to time (cf. Fig. 1 (1) in the paper of Thaysen & Tarding, 1974). Evidently several distinct processes operate more or less independently. At the primary level, one factor, which we suspect is phosphate loading, operates to cause an increase in primary phosphate content with an associated increase in sodium concentration, and a second factor, consequent on sodium depletion, acts to trigger secretion of an unidentified solute, with an associated decrease in sodium concentration. At the same time, sodium depletion acts at the ductal level via increased plasma aldosterone concentrations and sensitization of the gland to aldosterone action (Blair-West, Coghlan, Denton, Goding & Wright, 1963) to stimulate reabsorption of sodium and secretion of potassium, processes which cease when sodium repletion occurs. The ductal changes induced by sodium-depletion are of sufficient magnitude to obscure any changes induced in the primary fluid.

Anions

Bicarbonate. For technical reasons it has not proven possible to measure the pH of primary fluid *in situ* and, indeed, the present study is the first in which pH has been measured on aspirated samples equilibrated *in vitro* with carbon dioxide at a known tension. Without knowing whether the carbon dioxide–bicarbonate system is at equilibrium *in vivo* and what the intraluminal carbon dioxide tension is, we cannot assume that the measured pH values are the same *in vivo* but we can use them to calculate the primary fluid bicarbonate concentration: this averaged 35 mmol l^{-1}. Previously it has been postulated by some authors (see Young & van Lennep, 1979, for review of this literature) that a high primary fluid bicarbonate concentration is the major source of this ion in saliva; the present studies allow this possibility to be excluded, at least for the sheep parotid. In view of the fact that the final salivary pH and bicarbonate content are appreciably greater than in the primary fluid, it is apparent that the ducts must be responsible for secreting bicarbonate, either directly or indirectly (by absorbing hydrogen ions). Furthermore, the bicarbonate excretory curve corresponds to type C of Young's (1979) classification, i.e. concentrations exceed the plasma level and rise progressively with increasing flow rate, which can

only be explained by invoking a stimulation of ductal bicarbonate transport concomitant with the increased production of primary fluid; such a phenomenon has been demonstrated in experiments on the rat and rabbit mandibular glands (Young & van Lennep, 1979; Case, Conigrave, Novak & Young, 1980.

Phosphate. The present studies are the first in which phosphate has been measured in primary fluid samples. The values are remarkably constant and appear not to be influenced by the sodium status of the animal. The close correspondence of the phosphate concentration in final and primary saliva makes it appear highly likely that there is no ductal secretion of phosphate and that virtually all phosphate appearing in final saliva gained access to it in the primary secretion. The flow-dependency observed in the phosphate excretory curve of the sodium-depleted sheep (Fig. 3) is simply explained as a secondary concentration of salivary phosphate due to water reabsorption from the hypotonic saliva.

Chloride. In most previously published micropuncture studies, chloride has been found in the primary fluid in concentrations close to those seen in interstitial fluid (Young & van Lennep, 1979) and it has been concluded that the ion distributes itself passively between lumen and interstitium. If this conclusion holds for the sheep parotid, where the primary fluid chloride concentration averaged 53 mmol l^{-1}, then it follows that the prevailing transepithelial potential difference would have to be about 20 mV, lumen negative. This would seem surprising since the morphology of salivary endpieces, and the few available electrophysiological data, make it seem likely that the epithelium is electrically leaky (Young & van Lennep, 1978, 1979). However, without a knowledge of what substances account for the unidentified solutes in primary saliva, it is pointless to speculate further on this question.

Residual anions. Finally, a word of comment is necessary on the so-called residual anion content of saliva. As mentioned above, divalent cations and anions are present in sheep parotid saliva only in very low concentrations (Blair-West *et al.* 1967) so that the summed sodium and potassium concentrations should be nearly equal to the summed chloride, bicarbonate and phosphate (in mequiv l^{-1}) concentrations. In fact, however, there was a consistent cation excess in final saliva of about 14 mequiv l^{-1}, both in sodium-replete and sodium-depleted sheep. This excess is too great to be attributable to an error in the value of the pK'_a for phosphate used for the conversion of molar phosphate concentrations to equivalent charge concentrations, so that one must conclude that another anion is indeed present in saliva in appreciable concentrations. Because of the uncertainty surrounding our estimate of the pH of primary saliva, we cannot be equally confident that such an anion is present in the precursor secretion although it seems quite likely, at least in sodium-replete sheep. The unidentified anion cannot be a protein, since sheep parotid saliva contains very little ($\sim 200\ \mu g\ ml^{-1}$) so that an organic anion of small molecular weight appears to be the only likely candidate. One possibility would be lactate, which is present in sweat, for example, in similar concentrations (Sato, 1977). However, in a few pilot studies on samples of final saliva we only detected small amounts of fatty acids, not exceeding 4 mg l^{-1} (short-chain from lactic to hippuric acid and cyclic compounds) and 12 mg l^{-1} (longer chain compounds). Even if present in higher concentrations in primary saliva, they seem unlikely to have made a significant contribution to the

unidentified osmotically active solute of sodium-depleted samples (see above) since at the pH obtaining, there would be commensurate need for a cation other than sodium, potassium, calcium or hydrogen in high concentrations.

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REFERENCES

BEAL, A. M. (1979). Parotid salivary flow and composition during close-arterial infusion of acetylcholine and atropine in conscious sodium-replete sheep. *Q. Jl. exp. Physiol.* **64**, 89–107.

BLAIR-WEST, J., BOYD, G. W., COGHLAN, J. P., DENTON, D. A., GODING, J. R., WINTOUR, M. & WRIGHT, R. D. (1964). The functional capacity of abdominal and transplanted left adrenal glands of sheep. *J. Physiol.* **175**, 78–80*P*.

BLAIR-WEST, J. R., COGHLAN, J. P., DENTON, D. A., GODING, J. R. & WRIGHT, R. D. (1963). The effect of aldosterone, cortisol and corticosterone upon the sodium and potassium content of sheep's parotid saliva. *J. clin. Invest.* **42**, 484–496.

BLAIR-WEST, J. R., COGHLAN, J. P., DENTON, D. A., NELSON, J., WRIGHT, R. D. & YAMAUCHI, A. (1969). Ionic, histological and vascular factors in the reaction of the sheep's parotid to high and low mineralocorticoid status. *J. Physiol.* **205**, 563–579.

BLAIR-WEST, J. R., COGHLAN, J. P., DENTON, D. A. & WRIGHT, R. D. (1967). Effect of endocrines on salivary glands. In *Handbook of Physiology*, section 6 (Alimentary Canal), vol. 2 (Secretion), pp. 633–664, ed. CODE, C. F. Washington: American Physiological Society.

BOTT, E., DENTON, D. A., GODING, J. R. & SABINE, J. R. (1964). Sodium deficiency and corticosteroid secretion in cattle. *Nature, Lond.*, **202**, 461–463.

CASE, R. M., CONIGRAVE, A. D., NOVAK, I. & YOUNG, J. A. (1980). Electrolyte and protein secretion by the perfused rabbit mandibular gland stimulated with acetylcholine or catecholamines *J. Physiol.* **300**, 467–487.

COATS, D. A., DENTON, D. A., GODING, J. R. & WRIGHT, R. D. (1956). Secretion by the parotid gland of the sheep. *J. Physiol.* **131**, 13–31.

COATS, D. A. & WRIGHT, R. D. (1957). Secretion by the parotid gland of the sheep: the relationship between salivary flow and composition. *J. Physiol.* **135**, 611–622.

COMPTON, J. S. & YOUNG, J. A. (1976). The concentration of Na^+ and K^+ in the primary and final saliva of sodium depleted lambs. *Proc. Aust. physiol. & pharmacol. Soc.* **7**, 143*P*.

DENTON, D. A. (1956). The effect of Na^+ depletion on the Na^+:K^+ ratio of the parotid saliva of the sheep. *J. Physiol.* **131**, 516–525.

DENTON, D. A. (1957). The study of sheep with permanent unilateral parotid fistulae. *Q. Jl. exp. Physiol.* **42**, 72–95.

EDSALL, J. T. & WYMAN, J. (1958). *Biophysical Chemistry*, vol. 1. New York: Academic.

HOLZGREVE, H., MARTINEZ, J. R. & VOGEL, A. (1966). Micropuncture and histologic study of submaxillary glands of young rats. *Pflügers Arch.* **290**, 134–143.

KALADELFOS, G. & YOUNG, J. A. (1973). Micropuncture and cannulation study of water and electrolyte excretion in the isotonic-secreting cat sublingual gland. *Pflügers Arch.* **341**, 143–154.

KALADELFOS, G. & YOUNG, J. A. (1974). Water and electrolyte excretion in the cat submaxillary gland studied using micropuncture and duct cannulation techniques. *Aust. J. exp. Biol. med. Sci.* **52**, 67–79.

KAY, R. N. B. (1960). The rate of flow and composition of various salivary secretions in sheep and calves. *J. Physiol.* **150**, 515–537.

KUTTNER, T. & COHEN, H. R. (1927). Micro colorimetric studies. I. A molybdic acid, stannous chloride reagent. The micro estimation of phosphate and calcium in pus, plasma, and spinal fluid. *J. biol. Chem.* **75**, 517–531.

LENNEP, E. W. VAN, KENNERSON, A. R. & COMPTON, J. S. (1977). The ultra-structure of the sheep parotid gland. *Cell & Tissue Res.* **179**, 377–392.

Mangos, J. A., McSherry, N. R. & Arvanitakis, S. N. (1973). Autonomic regulation of secretion and transductal fluxes of ions in the rat parotid. *Am. J. Physiol.* **225**, 683–688.

Martinez, J. R., Holzgreve, H. & Frick, A. (1966). Micropuncture study of submaxillary glands of adult rats. *Pflügers Arch.* **290**, 124–133.

Sato, K. (1977). The physiology, pharmacology and biochemistry of the eccrine sweat gland. *Rev. Physiol. Biochem. Pharmacol.* **79**, 51–132.

Thaysen, J. H. (1960). Handling of alkali metals by exocrine glands other than the kidney. In *The Alkali Metal Ions in Biology*, pp. 424–463, ed. Ussing, H. H., Kruhöffer, P., Thaysen, J. H. & Thorn, N. A. Berlin, Göttingen, Heidelberg: Springer.

Thaysen, J. H. & Tarding, R. (1974). Ductal reabsorption of Na in 1:1 exchange for K in sheep parotid gland. In *Secretory Mechanisms of Exocrine Glands*, pp. 464–472, ed. Thorn, N. A. & Petersen, O. H. Copenhagen: Munksgaard.

Young, J. A. (1979). Salivary Secretion of Inorganic Electrolytes. In *International Reviews of Physiology. Gastrointestinal Physiology III*, vol. 19, pp. 1–58, ed. Crane, R. K. Baltimore: University Park Press.

Young, J. A., Frömter, E., Schögel, E. & Hamann, K. F. (1967). A microperfusion investigation of sodium resorption and potassium secretion by the main excretory duct of the rat submaxillary gland. *Pflügers Arch.* **295**, 157–172.

Young, J. A. & Martin, C. J. (1971). The effect of a sympatho- and a para-sympathomimetic drug on the electrolyte concentrations of primary and final saliva of the rat submaxillary gland. *Pflügers Arch.* **327**, 285–302.

Young, J. A., Martin, C. J., Asz, M. & Weber, F. D. (1970). A Microperfusion investigation of bicarbonate secretion by the rat submaxillary gland. The action of a parasympathomimetic drug on electrolyte transport. *Pflügers Arch.* **319**, 185–199.

Young, J. A. & Schögel, E. (1966). Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. *Pflügers Arch.* **291**, 85–98.

Young, J. A. & Shagrin, J. (1968). Nanolitre micromethods in biological research: determination of sodium, potassium and melting point. *Proc. Aust. Assoc. clin. Biochem.* **1**, 333–336.

Young, J. A. & van Lennep, E. W. (1978). *The Morphology of Salivary Glands.* London, New York: Academic.

Young, J. A. & van Lennep, E. W. (1979). Transport in Salivary and Salt Glands: Salivary Glands. In *Membrane Transport in Biology*, vol. 4B, Transport Organs, pp. 563–674, ed. Giebisch, G. Berlin, Heidelberg, New York: Springer.