EFFECTS OF FLOW RATE, DURATION OF STIMULATION AND MINERALOCORTICOIDS ON THE ELECTROLYTE CONCENTRATIONS OF MANDIBULAR SALIVA FROM THE RED KANGAROO (*MACROPUS RUFUS*)

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SUMMARY

Mandibular saliva was collected at eight flow rates ranging between 0.052 ± 0.0059 (S.E. of mean) and 4.294 ± 0.0717 ml min^{-1} from anaesthetized red kangaroos receiving ipsilateral intracarotid infusions of acetylcholine. The concentrations of sodium (4.04 ± 0.759 to 75.9 ± 4.64 mmol l^{-1}) and chloride (51.2 ± 2.46 to 85.0 ± 6.90 mmol l^{-1}) and the osmolality (99.7 ± 4.88 to 178.9 ± 13.50 mosmol kg^{-1}) were positively correlated with salivary flow rate over most or all of the flow range, whereas the concentrations of potassium (50.3 ± 2.03 to 19.7 ± 3.16 mmol l^{-1}), calcium (5.43 ± 1.696 to 1.26 ± 0.055 mmol l^{-1}), magnesium (259.8 ± 49.3 to 19.0 ± 1.88 μmol l^{-1}), hydrogen ions (457.7 ± 107.3 to 69.3 ± 5.64 nmol l^{-1}) and phosphate (2.22 ± 0.171 to 0.27 ± 0.040 mmol l^{-1}) were negatively correlated with flow rate. Salivary bicarbonate concentration (15.6 ± 1.76 to 21.9 ± 1.83 mmol l^{-1}) showed little flow dependency except possibly at high levels of stimulation. Spontaneous secretion was not observed during anaesthesia.

During continuous stimulation of flow at two rates (0.5 and 2.0 ml min^{-1}) for periods of 90 min, rest transients were observed for sodium, potassium, calcium, chloride and phosphate in the initial sample and the sodium concentration rose by 17–56 % during the first 60 min of steady-state flow and stimulation.

Indications that the gland was capable of responding rapidly to changes in endogenous mineralocorticoid levels were confirmed by intracarotid infusion of aldosterone at $80\,\mu$g h^{-1}. With the mean salivary flow rate lying between 1.3 and 1.4 ml min^{-1}, the salivary Na$^+$/K$^+$ ratio began to fall at 45–60 min of aldosterone infusion and after 4 h of infusion had fallen to 0.62 ± 0.116. Administration of deoxycorticosterone acetate (DOCA) for 21 days at 0.25 or 0.3 mg kg^{-1} 12 h^{-1} caused a further lowering of the Na$^+$/K$^+$ ratio to 0.09 ± 0.013 at similar flow rates. Biopsy showed this increase to be associated with a moderate level of hypertrophy of the intralobular ducts of the gland. Two types of intralobular duct were identified on the basis of glycogen granulation after DOCA administration.

It was concluded that the mandibular gland of the red kangaroo, with its high secretory capacity, its responsiveness to low levels of mineralocorticoids and its saliva

being essentially a markedly hypotonic sodium/potassium chloride solution, is functionally better adapted to act as the primary source of saliva used as an external coolant than is the parotid gland.

INTRODUCTION

Early studies on the adrenalectomized opossum raised doubts as to the importance of salt-retaining adrenal-steroids in marsupials (Silvette & Britton, 1936, 1938; Hartman, Smith & Lewis, 1943). Subsequently, two species of Australian marsupial were shown to be less tolerant of adrenalectomy (Buttle, Kirk & Waring, 1952; Reid & McDonald, 1968). Thus although both the American opossum (Brownell, Beck & Besch, 1967; Johnston, Davis & Hartcroft, 1967) and the Australian marsupials (Weiss & McDonald, 1966a,b, 1967; Coghlan & Scoggins, 1967) produce aldosterone and/or corticosterone, their renal response to adrenalectomy appears to differ considerably. With the exception of the red kangaroo parotid gland (Beal, 1986), the response of non-renal marsupial tissues to mineralocorticoids has not been investigated. The parotid gland of chronically Na^+-replete kangaroos shows no enhancement in its ability to retain sodium during acute increases in arterial aldosterone levels for periods up to 4 h, whereas administration of mineralocorticoids at high levels over a period of days induces hypertrophy of the intralobular ducts with concurrent increases in sodium-transporting ability. Provided the parotid saliva is retained within the digestive tract, this slow response to mineralocorticoids should not be detrimental to sodium regulation in the kangaroo.

However, kangaroos use saliva spreading on their forelimbs as a means of thermoregulation during heat stress. Preliminary studies of both saliva flow (A. M. Beal, unpublished data) and salivary gland blood flow (Needham, 1982) indicate that the mandibular gland is a major contributor to the saliva secreted during thermoregulatory salivation. If the kangaroo mandibular gland is like the parotid gland in requiring a high level of mineralocorticoid over a long period before sodium retention is induced, sodium deficiency would occur due to export of saliva to the exterior before appropriate changes in salivary sodium occurred. The observation that the mandibular glands of grey kangaroos from a sodium-deficient environment have hypertrophy of the intralobular ducts (Blair-West et al. 1968) may be an indication that the mandibular gland, like the parotid gland, requires induction of sodium transport.

The only data on the composition of kangaroo mandibular saliva are the concentrations of the main electrolytes in a single sample obtained at a 'moderately fast flow rate' from a red kangaroo of unknown sodium status (Forbes & Tribe, 1969). The main anion was phosphate and the sum of the ions bicarbonate, phosphate and chloride was 2·5 times the sum of the cations sodium and potassium. This suggests that there must be at least one other major cation in kangaroo mandibular saliva or that there was a substantial error in the measurement of one or more of the ions.

This paper reports an investigation of the secretory capacity of the mandibular glands of Na^+-replete red kangaroo and the composition of mandibular saliva under a

range of experimental conditions. To assess the suitability of mandibular saliva for spreading as a coolant, the electrolyte concentrations were measured in saliva obtained over a range of flow rates up to maximum, during sustained secretion at stable flow rates, and during treatment with mineralocorticoids.

MATERIALS AND METHODS

Experimental procedures

Six adult red kangaroos were used, three males weighing 33–48 kg and three non-lactating females weighing 23·5–27 kg. Each animal had one common carotid artery exteriorized in a skin loop not less than 1 month before the first experiment. The kangaroos were maintained on a diet of lucerne chaff, supplement pellets and drinking water containing 25 mmol l^{-1} NaCl + 25 mmol l^{-1} NaHCO$_3$.

Two days before each experiment the animals were lightly anaesthetized with ketamine hydrochloride (Ketalar; Park Davis, Australia) given at rates of 8–18 mg kg^{-1}. This level was sufficient to tranquillize the animal so that it would lie unrestrained on its side in a normal resting position with its head raised and swallowing reflex unaffected for about 30 min. The skin overlying one superficial lateral tail vein was infiltrated with 1 % lignocaine hydrochloride in saline (David Bull Laboratories, Victoria), to produce local anaesthesia, and the vein was then cannulated with a vinyl cannula (0·97 mm i.d., 1·27 mm o.d.; Dural Plastics, NSW) using the technique of Seldinger (1953). To ensure that the kangaroos were sodium-replete, each animal was given 60 ml of 2 mol l^{-1} NaCl solution by slow intravenous injection *via* this cannula. The cannula was then filled with heparinized saline (1000 i.u. ml^{-1}) and covered with a bandage. Food was removed 15–16 h before the commencement of each experiment but the saline drinking solution was available until the experiment began.

On the day of experiment, anaesthesia was induced by intravenous injection of 5 % sodium pentobarbitone in saline through the tail vein cannula at rates of 25–36 mg kg^{-1}. Anaesthesia was maintained with sodium pentobarbitone throughout the experiment using the corneal reflex as a guide to the level of anaesthesia. The animals were positioned on one side (carotid loop side up) with an electrically heated pad under the thorax to maintain normal body temperature and with an air cushion under the hind quarters to prevent pressure damage to the hip and thigh region. The trachea was intubated with a cuffed endotracheal tube which was shortened so that the dead space of the respiratory tract was not increased. A solution of NaCl:KCl (150:4 mmol l^{-1}) or Hartmann's solution (lactated Ringer: Na$^+$, 131; K$^+$, 5; Ca^{2+}, 2; Cl$^-$, 111; lactate, 29 mmol l^{-1}) was infused intravenously at 1·0–2·0 ml min^{-1} for the duration of each experiment to minimize changes in body fluid composition resulting from transpiration and salivary loss. The carotid artery loop was cannulated with a polyethylene cannula (0·58 mm i.d., 0·96 mm o.d.; Dural Plastics, NSW) which was inserted 10 cm in the direction of the heart using the technique of Seldinger (1953). The duct of the mandibular gland ipsilateral to the carotid artery loop was catheterized with a nylon tube (0·75 mm i.d., 0·94 mm o.d.; Portex Ltd,

England) or with a polyethylene tube (0·8 mm i.d., 1·2 mm o.d. or 0·97 mm i.d., 1·27 mm o.d.; Dural Plastics, NSW). This catheter was inserted 3 cm into the duct through its orifice in the mouth. Saliva was collected into polystyrene and poly-propylene sample tubes which were closed except for a 20 wire gauge air-bleed. The distal end of the salivary catheter was positioned about 10 cm below the duct orifice and the dead space in the catheter was 0·1–0·17 ml. The parotid duct was also catheterized and the catheter was blocked with a spigot to prevent fluid loss *via* this gland. In the course of each experiment the spigot was removed to allow a sample of parotid saliva to be collected at a flow rate between 1 and 2 ml min^{-1}.

Effect of salivary flow rate (10 experiments; three males, two females)

Salivary secretion was stimulated by intracarotid infusion of acetylcholine chloride at rates ranging from 6·5 to 560 nmol min^{-1} using a variable-speed syringe pump. Each kangaroo underwent two experiments which were not less than 1 month apart. In the first experiment, salivary secretion was stimulated so as to produce a low flow rate (<0·125 ml min^{-1}) with secretion being increased subsequently to maximum through a series of predetermined flow rate ranges by increasing the rate of acetylcholine infusion. The flow rate ranges used were <0·125, 0·125–0·25, 0·25–0·5, 0·5–1·0, 1·0–2·0, 2·0–3·0, 3·0–4·0 and >4·0 ml min^{-1}. In the second experiment, maximal flow was stimulated initially and then a series of decreasing salivary flow rates falling within the same flow intervals was obtained by reducing the rate of acetylcholine infusion. During any period of stimulation, salivary collection was not commenced until the salivary flow was well established and reasonably constant. Two or more timed samples were taken during every flow interval (except the <0·125 ml min^{-1} interval) and the results obtained from their analysis were averaged to provide mean values for each flow rate interval of each experiment. Rest periods of 10–60 min were allowed between periods of gland stimulation: the shortest rest periods followed the period of lowest stimulation and the longest rest periods followed maximal stimulation. Blood samples (6–8 ml) were taken from the carotid artery before the first acetylcholine infusion and thereafter were taken at the end of each period of gland stimulation.

In four additional experiments, four of the kangaroos were pretreated over the 12 h before the experiment with spironolactone (Aldactone; Searle & Co.) at a rate of 2 mg kg^{-1}. The spironolactone was dissolved in ethyl oleate and was given intra-muscularly in two doses at 12 h and 1 h before commencement of the experiment. All other aspects of the protocol were identical with that given above.

In another four experiments, saliva secretion was stimulated by intracarotid infusion of methacholine chloride (Sigma Chemical Co., USA) at rates ranging from 30 to 470 nmol min^{-1} to obtain nominal flow rates of 1·0 and 4·0 ml min^{-1}. All other experimental procedures were as described above for the acetylcholine infusions.

Effect of duration of stimulation (six experiments; two males, one female)

Each animal underwent two experiments, not less than 1 month apart, in which two flow rates were investigated. Salivation was stimulated by intravenous infusion

of acetylcholine chloride (Sigma Chemical Co., USA) at rates varying between 18–112 and 45–295 nmol min^{-1} to produce salivary flow rates approximating 0·5 and 2·0 ml min^{-1}, respectively. Each flow rate was maintained for 90 min with a rest period of 120 min between the two periods of stimulation. The periods of low and high salivary flow were stimulated in opposite order in the two experiments on each animal. Serial salivary samples of 10 min duration were taken throughout the periods of stimulation and blood samples were taken from the carotid artery immediately before and after each period of stimulation. Collection of the first sample of saliva commenced as soon as the appropriate flow rate was established, which was always within 5 min of commencement of acetylcholine infusion and within 1–2 min of commencement of saliva flow.

Intracarotid aldosterone infusion (15 experiments; two males, three females)

Because the response to this treatment was rather variable, each animal underwent three infusion experiments. In seven experiments, at least one per animal, salivation was maintained without a break for the 5 h of saliva collection whereas in the other experiments salivation was not stimulated during the first hour of aldosterone administration. Stable salivary flow rates between 1·0 and 1·5 ml min^{-1} (depending on the size of the animal) were stimulated by intracarotid infusion of acetylcholine. Four 15-min serial samples of saliva were collected before *d*-aldosterone (Sigma Chemical Co., USA) was infused in saline (0·1 ml min^{-1}) at 80 μg h^{-1} for 4 h into the ipsilateral carotid artery. Serial 15-min samples of saliva were collected for the 3 or 4 h of salivary secretion. Blood samples (5 ml) were taken before commencement of sampling and thereafter at hourly intervals during the infusion.

Chronic deoxycorticosterone administration (five experiments; two males, three females)

Each kangaroo was given deoxycorticosterone acetate (DOCA) dissolved in ethyl oleate (5 or 6 mg ml^{-1}) intramuscularly for 21 days. Husbandry of the kangaroos before this treatment, cannulation of the tail vein and carotid artery and stimulation of salivation were as described above. The cannulae were left in the artery and vein for the 21 days of this treatment and were kept patent with heparin (5000 i.u. ml^{-1}) between samplings. On the first day of the treatment (day 0) the kangaroos were anaesthetized with sodium pentobarbitone, salivation was stimulated by intracarotid acetylcholine infusion to approximately 25 % of maximum sustainable flow and four serial 10- to 15-min samples of parotid saliva were collected. Immediately following this collection, the animals were given DOCA at the rate of 0·25 mg kg^{-1}. Thereafter the kangaroos were injected with DOCA at the rate of 0·25 mg kg^{-1} 12 h^{-1} (two animals) or 0·3 mg kg^{-1} 12 h^{-1} (two animals) at approximately 08.00 and 20.00 h each day for 21 days. Under the conditions outlined for day 0, four serial 10- to 15-min samples of saliva were collected at the day 0 flow rate for each animal between 09.30 and 11.00 h on days 1, 3, 6, 10, 14 and 21 of DOCA administration. Blood samples (5 ml) were taken before and after salivary collection. Several procedures were used to offset the sodium gaining and potassium losing actions of the DOCA

injections. Throughout the period of DOCA administration the kangaroos were fed lucerne chaff only (the supplement pellets had added salt), were given drinking water containing $50-100 \, \text{mmol} \, l^{-1}$ potassium derived equally from KCl and K_2CO_3 and, during each saliva collection, they were given an infusion containing potassium at concentrations varying between 5 and $125 \, \text{mmol} \, l^{-1}$. The concentration of potassium in the drinking solution and in the infusate depended on the elapsed time through the DOCA treatment and on the changes in plasma sodium and potassium observed in each animal. The kangaroos had access to food and drinking solution except during saliva collection.

Samples of tissue were taken by excision biopsy from the contralateral mandibular glands of two of the animals (one male and one female) before, and at 21 days of, DOCA administration. The tissue was fixed in buffered formalin, embedded in methacrylate or paraffin wax and after sectioning, stained by haematoxylin/eosin (H & E), periodic acid Schiff (PAS) and Alcian Blue combined with the previous stains. Some sections were pretreated with diastase before PAS staining. The area of the sections occupied by acinar cells, striated/intralobular ducts and excretory ducts was estimated by tracing the image of the section projected onto a graphics tablet. The height of the cells lining the striated ducts was measured using an eyepiece micrometer.

At the end of each period of anaesthesia, the kangaroos were kept on the heated pad and air cushion until $15-30 \, \text{min}$ after the endotracheal tube had been removed. The endotracheal tube was removed when the swallowing reflex had returned and the animals were monitored over the following $15-30 \, \text{min}$ to ensure that respiration was unimpaired. Removal of cannulae and catheters was done while the kangaroos were anaesthetized. At the end of each experiment, the anaesthetized kangaroos were given a single intramuscular injection of procaine penicillin and dihydrostreptomycin (Streptopen injection; Glaxo Australia Pty Ltd; Victoria) at a rate of $1 \, \text{ml} \, 12 \, \text{kg}^{-1}$ as a safeguard against infection. Since kangaroos are essentially night-feeding animals, experiments during the daylight hours do not interfere with their food intake and thus the experimental animals gained weight during the period of these experiments.

Analytical procedures

Blood plasma and saliva were analysed for sodium, potassium, calcium, magnesium, chloride, phosphate, hydrogen ion and osmolality, and additionally saliva was analysed for bicarbonate. Blood samples were taken into plastic syringes heparinized with one drop of heparin ($5000 \, \text{i.u.} \, \text{ml}^{-1}$) and centrifuged at $2200 \, g$ for $10 \, \text{min}$ to obtain plasma for analysis. Microhaematocrit determinations were made in triplicate on blood spun at $12\,000 \, g$ for $10 \, \text{min}$ in a microhaematocrit centrifuge (Hawksley). Salivary pH was measured at $36 \, °C$ under anaerobic conditions using thermostatted Radiometer microelectrodes. Saliva and plasma were analysed in duplicate for sodium, potassium, calcium and magnesium by atomic absorption spectroscopy using appropriate ionization suppressants. Duplicate estimations of the chloride concentration in plasma and saliva were made using a Radiometer chloride titrator (model CMT 10). Total inorganic phosphate concentrations in saliva and

plasma were determined in duplicate using the method of Baginski, Foa & Zak (1967). The osmolality of plasma and saliva was estimated by freezing point depression using a Knauer osmometer. The bicarbonate concentration of saliva was determined by the titration procedure of Gyory & Edwards (1967) modified for 0·2-ml aliquots of sample.

Statistical procedures

The data for salivary and plasma electrolyte concentrations were analysed initially by one-way analysis of variance across the eight predetermined flow intervals. If a significant variance ratio was obtained, differences between any two flow intervals were found using Tukey's w procedure as described by Steel & Torrie (1960). The data for the effect of duration of stimulation were analysed across the nine time periods of each of the two flow rates using the above techniques. The data for steroid administration were analysed using the paired t-test procedure.

RESULTS

Effect of salivary flow rate

Mean values for the haematocrit, plasma osmolality and the plasma concentrations of sodium, potassium, calcium, magnesium, chloride and phosphate from carotid arterial blood were estimated for each of the eight flow rate intervals (Figs 1–4). No significant differences were found between the flow rate intervals in any of the above parameters. Salivary flow rates exceeding 4·0 ml min^{-1} were obtained in six of the 10 experiments (four out of five animals). Hence the values for these flow rates are given as open circles in Figs 1–4 to draw attention to the lower number of replicates.

Salivary cations

The concentration of sodium in mandibular saliva was positively correlated with flow rate over the entire flow range, increasing from $4·04 \pm 0·759$ to $75·9 \pm 4·64$ mmol l^{-1} (Fig. 1). Considerable variation in concentration was observed between animals and between experiments on the same animal. At maximum flow, salivary sodium concentrations of 102 and 54 mmol l^{-1} were obtained in two experiments on the same animal. Within experiments on the same animal there appeared to be a direct relationship between plasma and saliva sodium concentration but across the whole range of data the relationship was not significant.

The concentrations of potassium, hydrogen ion, calcium and magnesium in the saliva were all negatively correlated with flow over the entire flow range (Figs 1, 2). Salivary potassium exceeded the plasma potassium concentration substantially at all flows, declining from $50·5 \pm 2·03$ to $19·7 \pm 3·16$ mmol l^{-1}. As for sodium, there was considerable variation in potassium concentrations between animals and between experiments on the same animal. Statistical comparison of the two experiments on each animal showed that the differences in Na$^+$/K$^+$ ratio between the experiments were significant ($P < 0·05$) at all except the lowest flow rate. When the Na$^+$/K$^+$

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ratios of these two groups of experiments were compared to those of animals treated with spironolactone the low ratio group had significantly lower ratios at five of the eight flow rates ($P<0.05$), whereas the high ratio group and the spironolactone
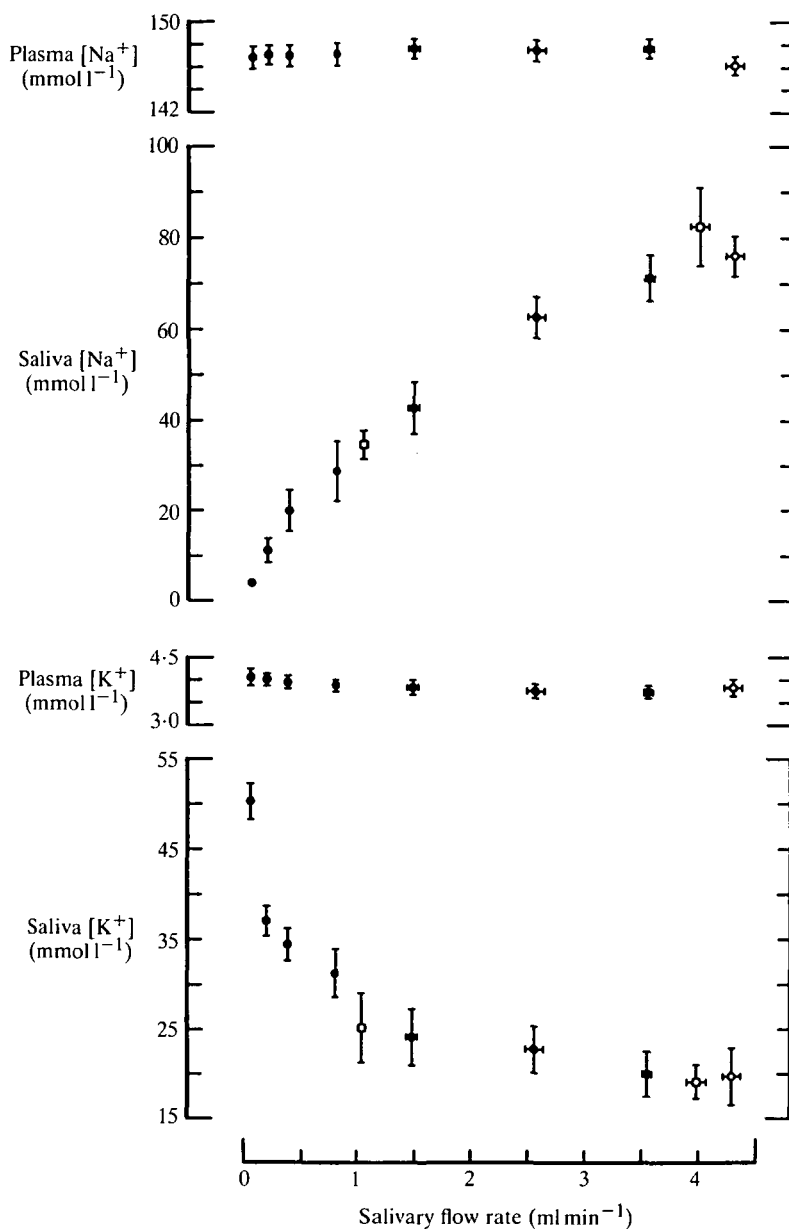

Fig. 1. Sodium and potassium concentrations of saliva, obtained by acetylcholine stimulation from the mandibular gland of red kangaroos, regressed on salivary flow rate. Sodium and potassium concentrations of arterial plasma are given for each flow rate (closed circles; $N = 10$; means ± S.E. of mean). Only four animals contributed to the highest flow rate (open circles; $N = 6$). Salivary sodium and potassium concentrations are given for two flow rates during methacholine stimulation (open squares; $N = 4$).
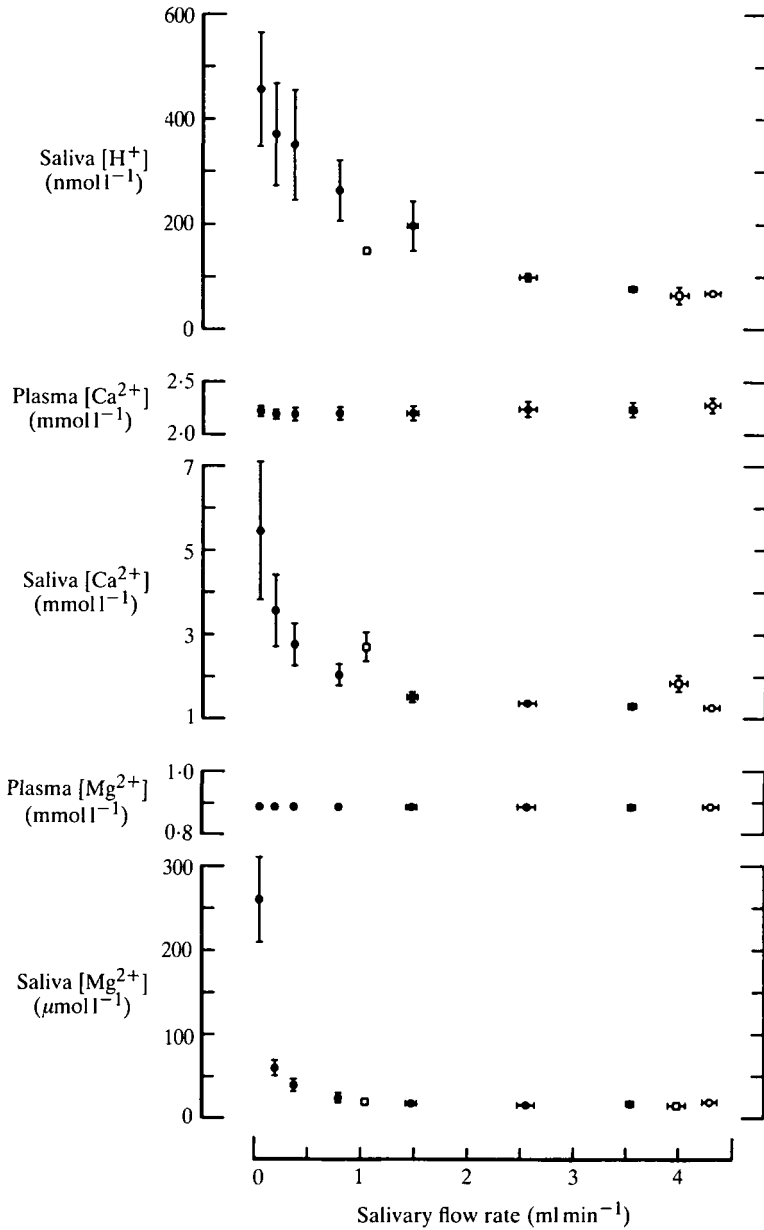
Fig. 2. Hydrogen ion, calcium and magnesium concentrations of saliva, obtained by acetylcholine stimulation from the mandibular gland of red kangaroos, regressed on salivary flow rate. Calcium and magnesium concentrations of arterial plasma are given for each flow rate (closed circles; $N = 10$; means $\pm$ s.e. of mean). Only four animals contributed to the highest flow rate (open circles; $N = 6$). Salivary hydrogen ion, calcium and magnesium concentrations are given for two flow rates during metacholine stimulation (open squares; $N = 4$).

treatment had similar ratios at all flow rates. In all experiments, the Na^+/K^+ ratio of parotid saliva collected mid-experiment was in the Na^+-replete range of 18–20. Substantial variation in hydrogen ion concentration was also observed, particularly at low flow rates. At the lowest flow rate, salivary hydrogen ion concentrations of 1115 and 67 nmol l^{-1} (pH 5·94 and 7·16) were obtained in two experiments on the same animal. Salivary calcium concentrations ranged between 5·43 ± 1·696 and 1·26 ± 0·055 mmol l^{-1}, always exceeding the plasma calcium level at flow rates below 0·125 ml min^{-1}. Salivary magnesium concentrations were considerably lower than the corresponding plasma magnesium concentrations, falling from 260 ± 49·3 to 19 ± 1·8 μmol l^{-1}.

Salivary anions

The concentration of phosphate was negatively correlated with flow over the entire flow range (Fig. 3), the maximum concentration being less than 50 % of the mean plasma concentration. Both chloride and bicarbonate concentrations were positively correlated with flow over most but not all of the flow range (Fig. 3). The chloride concentration fell with increasing flow up to approximately 0·2 ml min^{-1} and then increased with flow progressively to a maximum of 85·0 ± 6·90 mmol l^{-1}. Salivary chloride showed more variation in concentration than any other ion measured. Below 1–2 ml min^{-1}, salivary bicarbonate concentration averaged 15·2 ± 1·11 mmol l^{-1} and no consistent changes in bicarbonate concentration were observed. Above this flow rate, bicarbonate concentration increased to a maximum of 21·9 ± 1·83 mmol l^{-1}.

Salivary osmolality

Osmolality was positively correlated with flow at flow rates above 0·2 ml min^{-1} (Fig. 4). In parallel with sodium, potassium and chloride, there was considerable variability in the osmolality of saliva from different animals and from the same animal in different experiments. At maximum flow, salivary osmolalities of 243 and 139 mosmol kg^{-1} were obtained in separate experiments on the same animal. In another experiment, salivary osmolality at maximum flow was less than that at the lowest flow (88 %). Overall, the maximum osmolality of mandibular saliva was 62·6 ± 3·73 % of the plasma osmolality.

Methacholine stimulation

With the exception of salivary calcium, saliva produced using methacholine as the stimulant (open squares, Figs 1–4) had similar ion concentrations to that produced using acetylcholine stimulation. The calcium concentration of methacholine-stimulated saliva was higher than that obtained by acetylcholine infusion ($P < 0.02$). There were no significant differences in plasma calcium concentrations between the two treatments.

Effect of duration of stimulation

At the lower flow rate (0·5 ml min^{-1}), consistent trends were found for sodium, calcium and phosphate only. Salivary sodium rose progressively to give an average
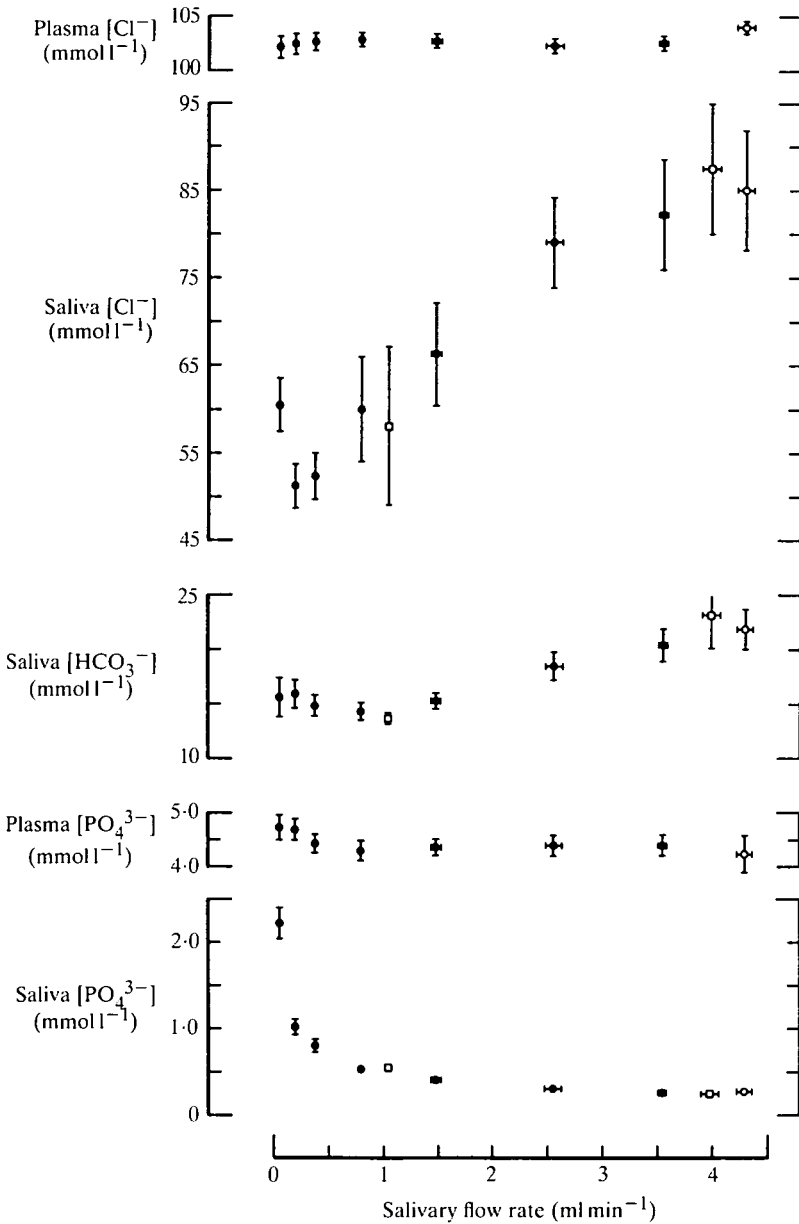
Fig. 3. Chloride, bicarbonate and phosphate concentrations of saliva, obtained by acetylcholine stimulation from the mandibular gland of red kangaroos, regressed on salivary flow rate. Chloride and phosphate concentrations of arterial plasma are given for each flow rate (closed circles; $N = 10$; means ± s.e. of mean). Only four animals contributed to the highest flow rate (open circles; $N = 6$). Salivary chloride, bicarbonate and phosphate concentrations are given for two flow rates during metacholine stimulation (open squares; $N = 4$).
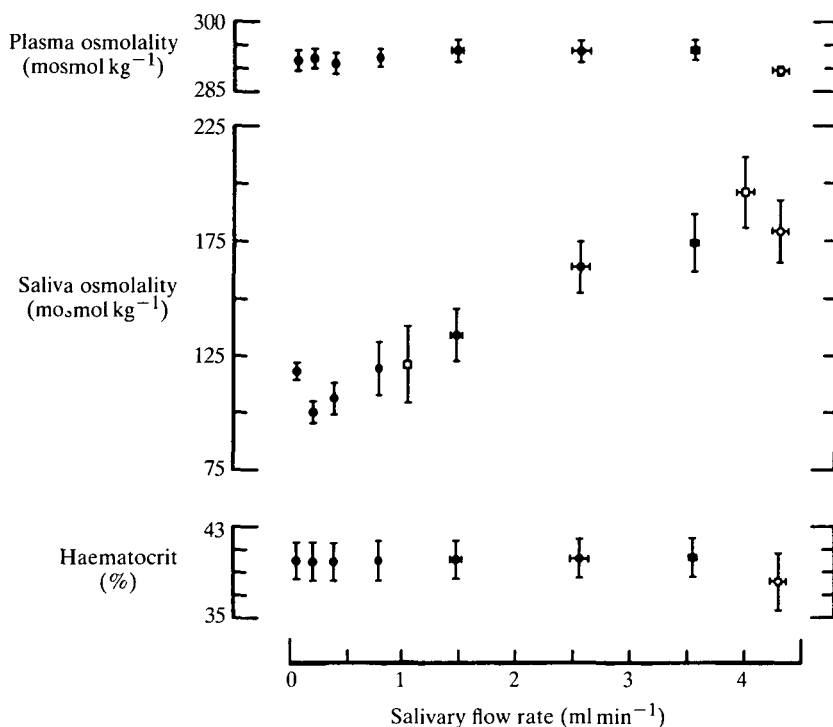
Fig. 4. Osmolality of saliva, obtained by acetylcholine stimulation from the mandibular gland of red kangaroos, regressed on salivary flow rate. Plasma osmolality and the haematocrit of arterial blood are given for each flow rate (closed circles; $N = 10$; means ± S.E. of mean). Only four animals contributed to the highest flow rate (open circles; $N = 6$). Salivary osmolality is given for two flow rates during methacholine stimulation (open squares; $N = 4$).

increase of 6 mmol l^{-1} over the first 70 min of collection. Both calcium and phosphate fell over the initial 10–20 min so that the first salivary sample had significantly higher concentrations of calcium and phosphate than some or all of the subsequent samples (Fig. 5; Table 1).

At the higher flow rate (2·0 ml min^{-1}), all ions except magnesium showed statistically significant changes in concentration during the 90-min stimulation (Fig. 5; Table 1). Again, the sodium concentration increased by approximately 7–8 mmol l^{-1} over the first 70 min of collection. The concentrations of sodium, potassium, calcium, chloride and phosphate in the initial sample were significantly higher than those in some or all of the samples collected during the following 80 min. The hydrogen ion and bicarbonate concentrations in the saliva were reasonably stable during the first 50 min of collection but thereafter hydrogen ion concentrations fell and bicarbonate concentrations rose. At the higher flow rate, differences in

Fig. 5. Flow rate and electrolyte concentrations of mandibular saliva obtained at constant flow rates approximating to 0·5 and 2·0 ml min^{-1} by continuous acetylcholine stimulation for 90 min ($N = 6$; means ± S.E. of mean).

Fig. 5

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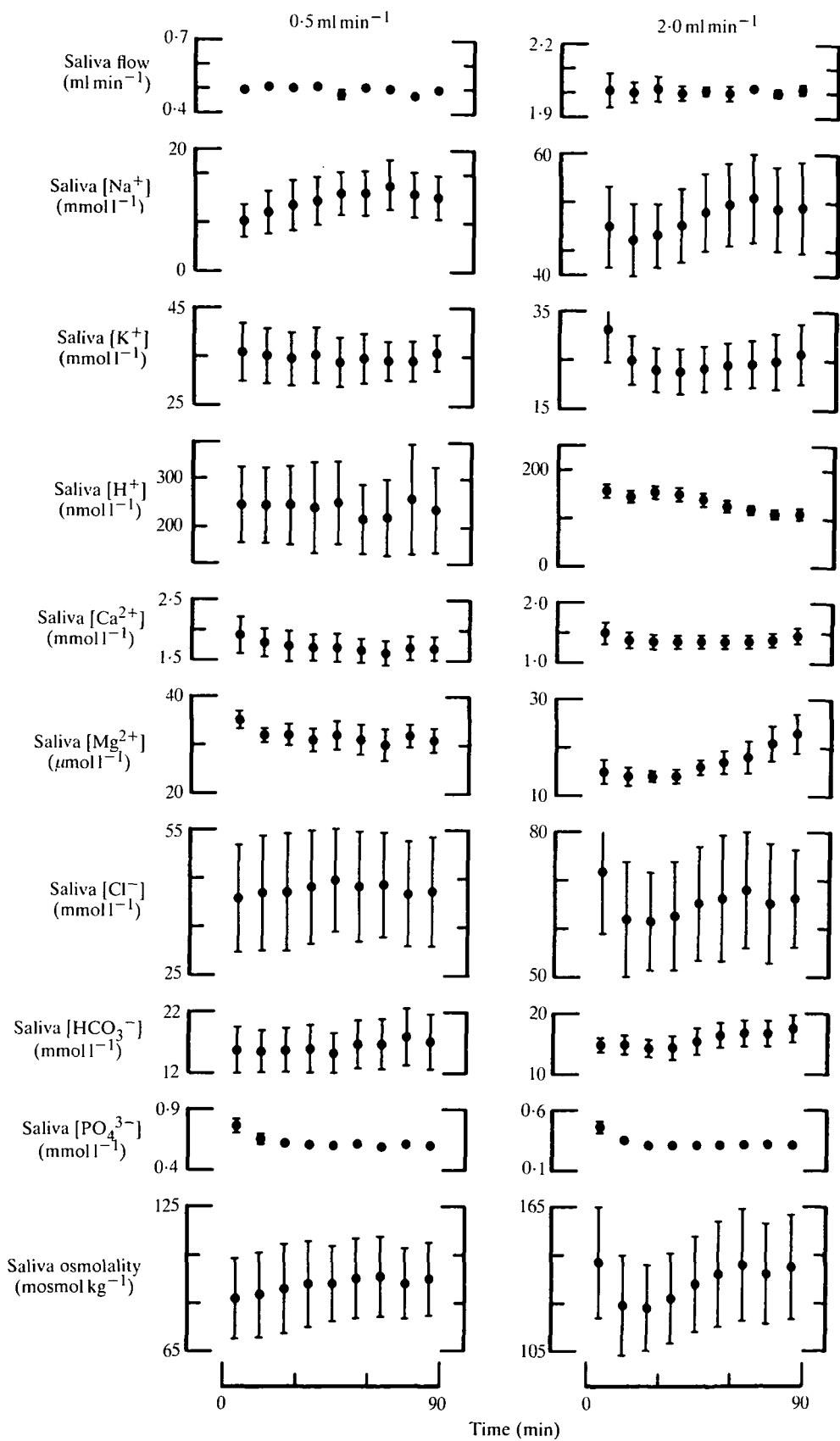
salivary hydrogen ion concentration between animals were much less than at the lower flow rate. Reflecting the above responses, the osmolality of the initial salivary sample was always elevated. Salivary osmolality reached minimum levels after 10–20 min of stimulation and then rose again.

Large differences in osmolality of mandibular saliva were observed between animals and, to a degree, were characteristic of particular individuals. These differences in osmolality reflected the variation in sodium, potassium and chloride in the saliva of different animals.

Table 1. *Summary of the final w procedure analyses comparing the concentrations of electrolytes in saliva collected during sustained secretion at 0·5 and 2·0 ml min^{-1} for periods of 90 min*

Salivary parameter		Comparison Period of stimulation							
		2	3	4	5	6	7	8	9
					0·5 ml min^{-1}				
[Sodium]	1	NS	NS	*	**	**	***	**	**
	2	—	NS	NS	NS	NS	**	NS	NS
[Calcium]	1	NS	NS	NS	NS	*	*	NS	NS
[Phosphate]	1	**	***	***	***	***	***	***	***
					2·0 ml min^{-1}				
[Sodium]	1	*	NS	NS	NS	NS	NS	NS	NS
	2	—	NS	NS	NS	NS	*	NS	NS
	3	—	—	NS	NS	NS	*	NS	NS
[Potassium]	1	**	***	***	***	***	***	**	**
[Calcium]	1	NS	*	*	*	*	*	NS	NS
[H$^+$]	1	NS	NS	NS	*	***	***	***	***
	2	—	NS	NS	NS	*	***	***	***
	3	—	—	NS	NS	***	***	***	***
	4	—	—	—	NS	**	***	***	***
	5	—	—	—	—	NS	*	**	**
[Chloride]	1	**	**	*	NS	NS	NS	NS	NS
[Bicarbonate]	1	NS	NS	NS	NS	*	**	**	**
	2	—	NS	NS	NS	*	**	**	**
	3	—	—	NS	NS	**	**	**	**
	4	—	—	—	NS	*	**	**	**
[Phosphate]	1	***	***	***	***	***	***	***	***
Osmolality	1	*	**	NS	NS	NS	NS	NS	NS
	2	—	NS	NS	NS	NS	*	NS	*
	3	—	—	NS	NS	NS	*	NS	*

Provided the initial variance ratios were significant, all possible comparisons were tested and omission of a comparison from this table means no significant differences were found.

Differences are indicated as levels of significance (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = not significant).

P is the probability of erroneous rejection of the null hypothesis (i.e. type 1 error).

Aldosterone infusion

Plasma sodium concentration rose during all aldosterone infusion experiments (t_{14} test $= 8·039$; $P < 0·001$), the mean increase being $1·85 \pm 0·230$ mmol l^{-1} over 4 h (Fig. 6). Plasma potassium fell in all but one experiment ($t_{14} = 5·475$; $P < 0·001$), the mean fall in potassium concentration being $0·38 \pm 0·064$ mmol l^{-1}. Plasma osmolality rose in most experiments giving a mean increase of $3·63 \pm 1·060$ mosmol kg^{-1} ($t_{14} = 3·426$; $P < 0·01$).
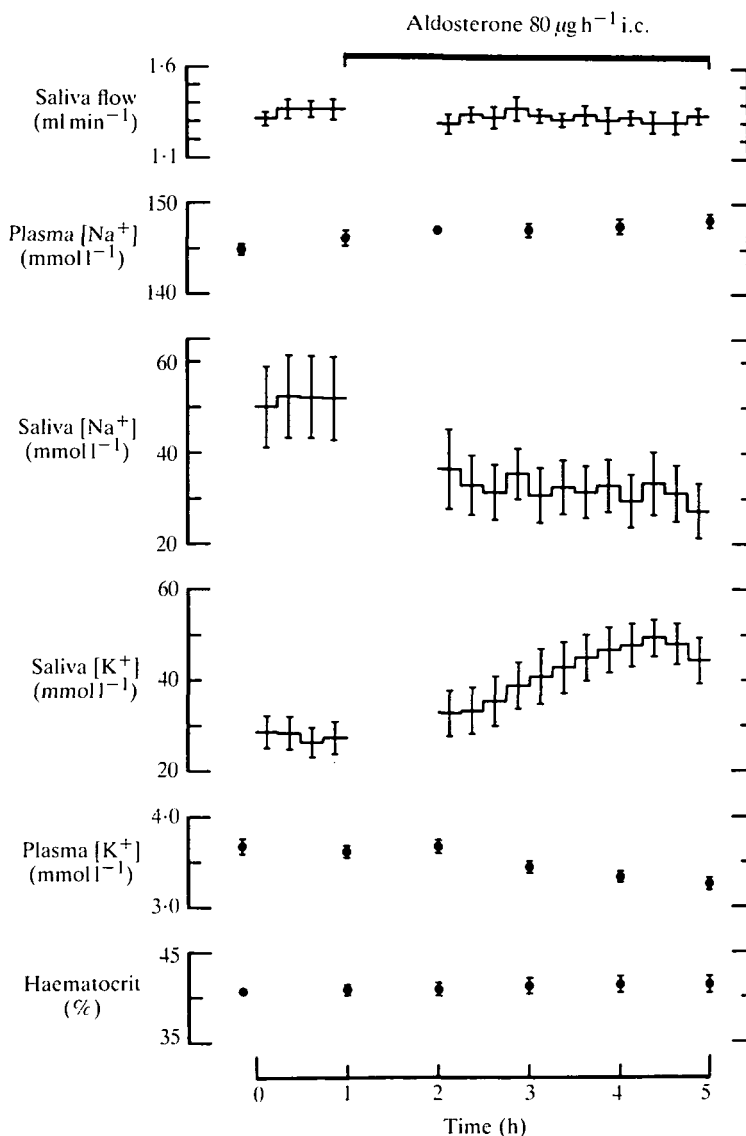
Fig. 6. Flow rate, sodium concentration and potassium concentration for mandibular saliva before and during a 4-h ipsilateral intracarotid (i.c.) infusion of aldosterone at $80\,\mu$g h^{-1}. Plasma sodium and potassium concentrations and the haematocrit are given at hourly intervals ($N = 15$; means $\pm$ S.E. of mean).
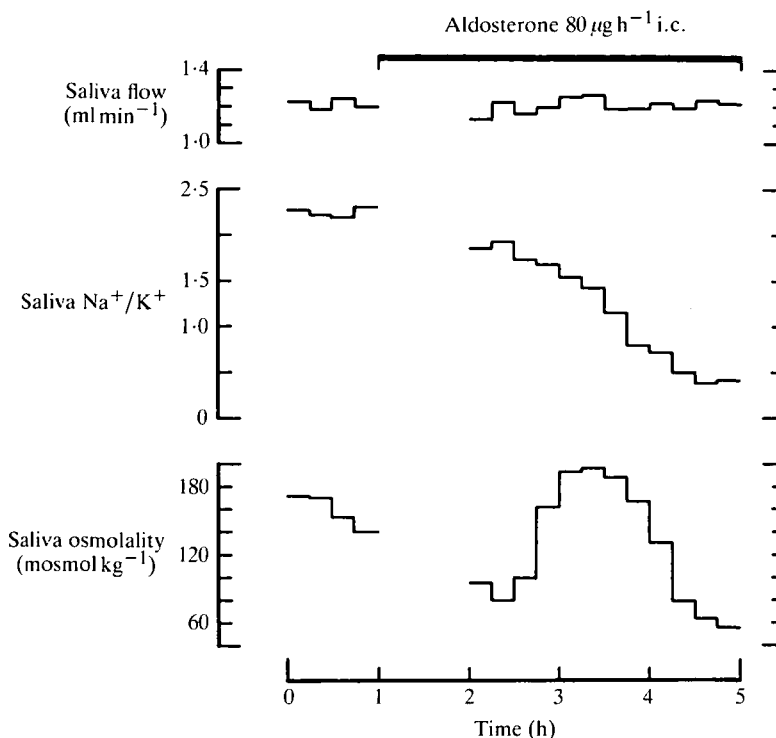
A. M. BEAL

Fig. 7. One of the several examples of a 4-h ipsilateral intracarotid (i.c.) infusion of aldosterone at $80 \mu g h^{-1}$ in which the mandibular salivary flow rate was constant, the salivary Na^+/K^+ ratio fell progressively but salivary osmolality was highly variable.

A fall in salivary sodium concentration was associated with all but two aldosterone infusions and salivary potassium concentration rose in all experiments (Fig. 6). As a consequence, the Na^+/K^+ ratio of the saliva fell from $2 \cdot 5 \pm 0 \cdot 68$ to $0 \cdot 62 \pm 0 \cdot 116$ after 4 h of aldosterone infusion ($t_{14} = 3 \cdot 153$; $P < 0 \cdot 01$). In the seven experiments in which samples were collected during the first hour of aldosterone administration, the Na^+/K^+ ratio had fallen significantly by 45–60 min of infusion ($t_{14} = 2 \cdot 97$; $P < 0 \cdot 05$). Salivary osmolality showed a tendency to fall in the majority of experiments but overall changes in osmolality were not significant. In 10 of the experiments, the Na^+/K^+ ratios of parotid saliva taken at the beginning of salivary collection and shortly after cessation of mandibular collection following the 4 h aldosterone infusion were $18 \cdot 81 \pm 0 \cdot 630$ and $18 \cdot 0 \pm 0 \cdot 237$ at flow rates of $1 \cdot 20 \pm 0 \cdot 04$ and $1 \cdot 17 \pm 0 \cdot 053$ ml min^{-1}, respectively.

An unexpected response by the mandibular gland occurred during some aldosterone infusions and was highlighted by the results from one particular kangaroo. In two of three experiments and in the absence of any obvious changes in experimental conditions, the salivary Na^+/K^+ ratio fell progressively as anticipated after commencement of aldosterone infusion, but, during the infusions, osmolality and both sodium and potassium concentrations rose simultaneously to values exceeding the initial control values before falling again at the end of the experiment (Fig. 7).

During these infusions, plasma sodium increased from 148·3 to 150·2 mmol l^{-1} and potassium fell from 3·30 to 3·09 mmol l^{-1} in a progressive manner.

Chronic DOCA administration

Plasma sodium concentration rose over the first 3 days of DOCA injection ($t_4 = 3·758$; $P < 0·05$) and did not increase during the remainder of the DOCA treatment (Fig. 8). Changes in plasma potassium concentration during the 21 days of steroid treatment were not statistically significant whereas the fall in haematocrit during the first 10 days was significant ($t_4 = 21·14$; $P < 0·001$).

Salivary sodium concentration fell progressively during the period of DOCA administration whereas salivary potassium rose over the initial 3 days and then plateaued or remained elevated at values lower than the 3-day peak value (Fig. 8). As a consequence the Na$^+$/K$^+$ ratio fell from 1·00 ± 0·098 to 0·09 ± 0·013 ($t_4 = 8·658$; $P < 0·001$) by 21 days of steroid treatment. The concentrations of both ions were significantly different from the untreated state by day 1 of treatment ($t_4 = 13·2$ and 7·607; $P < 0·001$ and 0·01, respectively).

Histological examination of mandibular gland tissue from two of the kangaroos showed some moderate changes in the development of the intralobular ducts over the period of DOCA injection (Fig. 9). The mean height of the intralobular duct cells increased by 19·4 and 24·7 % ($P < 0·001$) and the lobular volume occupied by intralobular ducts increased by 12·0 and 13·2 % (Table 2). After the DOCA treatment, the cells in about half of the intralobular duct cross-sections were heavily granulated (Fig. 9B). The granules were not stained by H & E or by Alcian Blue (pH 2·5) procedures but were stained by PAS and this was prevented by diastase treatment prior to staining. Consequently, the granules were identified as glycogen. The remainder of the intralobular duct sections showed virtually no granulation (Fig. 9B) as was the case for all duct sections in tissue taken before DOCA administration (Fig. 9A).

DISCUSSION

In red kangaroos, the mandibular gland has approximately half the mass of the parotid gland (Forbes & Tribe, 1969), the weight of the two mandibular glands being 0·54 ± 0·03 g kg^{-1} body weight (Porter, 1981). Using the above approximation the maximum flow for all experiments (4·01 ± 0·123 ml min^{-1}) was equivalent to 450 ± 33·9 μl min^{-1} g^{-1} wet weight of gland. Thus the maximum secretory rate of the mandibular gland was double that of the kangaroo parotid gland under the same experimental conditions (Beal, 1984) and is high relative to the secretory rates reported for mandibular glands in other species (Schneyer & Schneyer, 1967). As with the parotid gland, the kangaroo mandibular shows no evidence of spontaneous secretion during sodium pentobarbitone anaesthesia.

Unlike kangaroo parotid saliva, which has a specialized composition typical of foregut-fermenting herbivores, the saliva of the kangaroo mandibular gland is
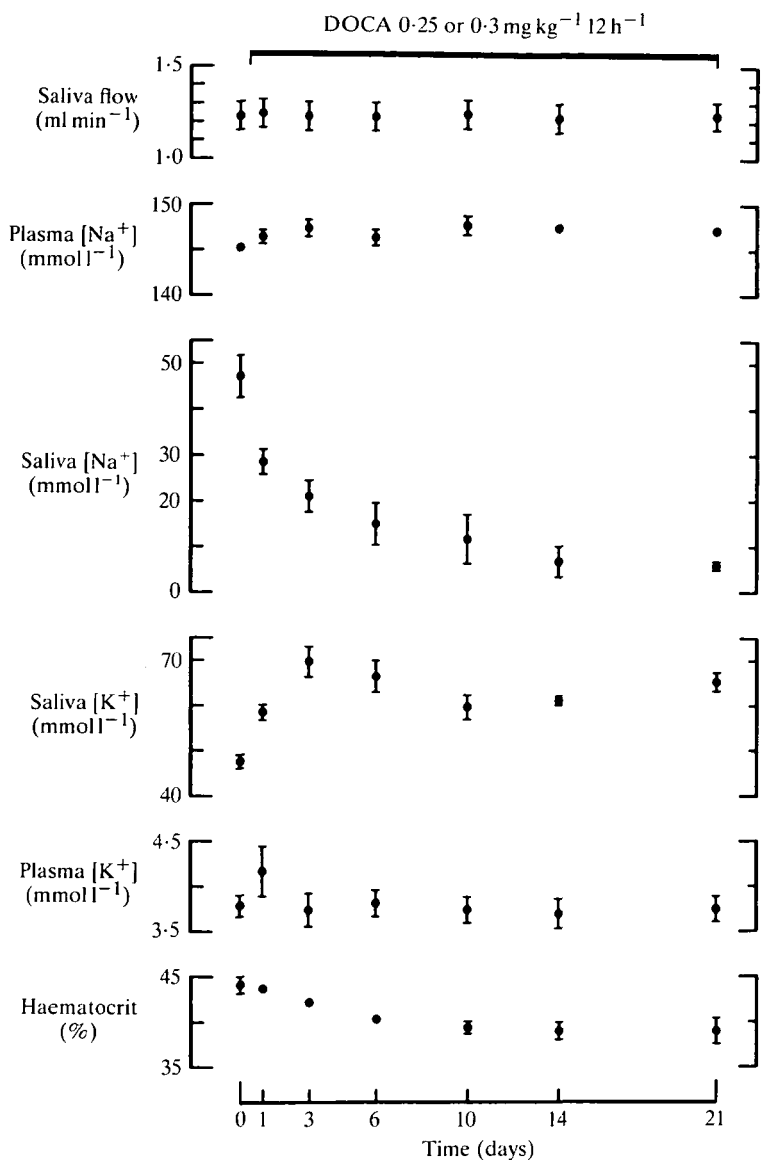
A. M. BEAL

Fig. 8. Flow rate, sodium and potassium concentrations of mandibular saliva, sodium and potassium concentrations of arterial plasma and haematocrit on days 0, 1, 3, 6, 10, 14 and 21 of intramuscular deoxycorticosterone acetate (DOCA) injection at 0·25 or 0·3 mg kg^{-1} 12 h^{-1} ($N = 5$; means ± s.e. of mean).

essentially a sodium/potassium chloride solution. The sodium and potassium concentrations had positive and negative curvilinear relationships, respectively, with flow rate with the sodium concentration rarely exceeding 100 mmol l^{-1} and potassium concentrations tending to level off at about 20 mmol l^{-1}. These features are common to many mandibular salivas from eutherian species (Young & Schneyer, 1981).
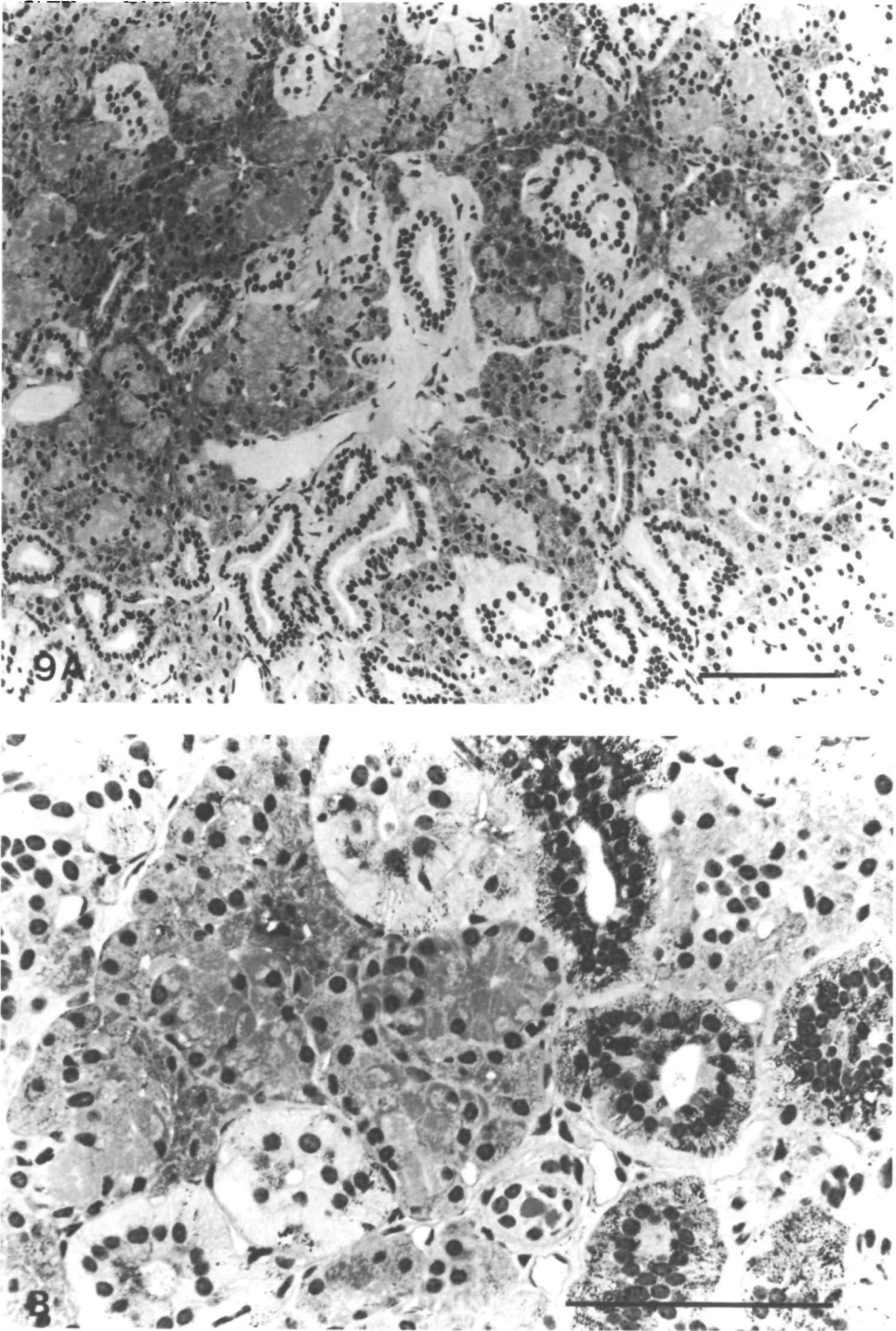
Fig. 9. Mandibular gland from a female red kangaroo showing the effect of deoxy-corticosterone acetate (DOCA) administration on intralobular duct histology. Fixed in buffered formalin, methacrylate sections stained with periodic acid Schiff and haema-toxylin. (A) The pretreatment, Na^+-replete state. Scale bar, $100\,\mu m$. (B) The effect of 21 days injection of DOCA at $3\,mg\,kg^{-1}\,12\,h^{-1}$. Scale bar, $100\,\mu m$.

The calcium concentration of kangaroo mandibular saliva was some 20–100 times that of kangaroo parotid saliva (Beal, 1984) and the calcium excretion curve resembles that typical of potassium. Similar negative flow rate dependency has been reported for human labial saliva (Wiesmann, Boat & Di Sant'Agnese, 1972), and for cat and rat mandibular salivas (Nielsen & Petersen, 1970; Martinez, Quissel, Wood & Giles, 1975; Compton, Martinez, Martinez & Young, 1981). Although ionized calcium was not measured, it is reasonable to predict that, at the lower flow rates, salivary ionized calcium concentrations would exceed those of plasma since salivary pH, phosphate concentrations and protein concentrations (55–550 mg l^{-1}; A. M. Beal, in preparation) are all relatively low. The observation that during methacholine stimulation the salivary calcium levels were higher than during acetylcholine stimulation may indicate that sympathetically stimulated saliva has a lower calcium concentration. Methacholine is more exclusively muscarinic than acetycholine which could have some nicotinic action on cervical sympathetic ganglia. The pattern of magnesium excretion was similar to that reported for a number of eutherian glands (Young & Schneyer, 1981) and closely approximated the values found in kangaroo parotid saliva at equivalent flow rates. At all flow rates the total magnesium concentrations were well below the expected levels of ionized magnesium in arterial plasma.

There was little evidence that the ducts of the kangaroo mandibular gland secrete bicarbonate in response to cholinergic stimulation as occurs in some other glands (Young & van Lennep, 1979; Case, Conigrave, Novak & Young, 1980) since the bicarbonate concentrations were always lower than the expected plasma bicarbonate concentrations and showed little flow dependency except at the higher rates of flow and stimulation. Since β-adrenergic stimulation of this gland causes bicarbonate to increase to values well in excess of plasma concentrations (A. M. Beal, unpublished data) the higher values at high flow rates may result from nicotinic actions of high levels of acetylcholine. However, in this context it should be noted that methacholine caused increases in bicarbonate that equalled or were greater than those caused by acetylcholine (Fig. 3). Phosphate concentrations showed the typical negative curvilinear relationship with flow and, in contrast to the ruminant mandibular gland (Kay, 1960), were always well below the plasma concentrations at all flow rates. As a consequence of the low and relatively stable bicarbonate concentrations and the very low concentrations of phosphate, the main salivary anion was chloride and the shape

Table 2. *Intralobular duct cell height (mean ± S.E.M.) and intralobular duct volume as a percentage of lobular volume before and after intramuscular administration of deoxycorticosterone acetate (DOCA) at 0.25 mg kg^{-1} 12 h^{-1} for 21 days*

Kangaroo Identification	Sex	Intralobular duct cell height (μm)		Intralobular duct volume (%)	
		Before DOCA	21 days DOCA	Before DOCA	21 days DOCA
N.F.	F	18.5 ± 0.56	22.1 ± 0.53	24·7	27·7
D.F.	M	22.7 ± 0.61	28.3 ± 0.63	25·6	29·0

of the excretion curve for chloride mirrored the shape of the excretion curve for the sum of the major cations.

The previously published data for mandibular salivary composition was for a sample obtained at a flow of $0.13\ ml\ min^{-1}$ or $20.9\ ml\ min^{-1}\ g^{-1}$ tissue (Forbes & Tribe, 1969). Since this flow rate closely approximates the second slowest flow rate in the present study ($21.5 \pm 1.34\ ml\ min^{-1}\ g^{-1}$ tissue) comparison can readily be made. There is reasonable agreement in the concentrations of sodium and bicarbonate but substantial differences in the concentrations of potassium, chloride and phosphate, the potassium and chloride values in the current study being two and three times those of the previous study, respectively, and the phosphate levels being about 3 % of the value previously published. The most obvious difference in protocol between the two studies is the method of stimulation of salivation (electrical and pharmacological, respectively), but given that both methods are basically cholinergic it seems unlikely that they are the cause of such gross differences in the anion concentration of the saliva. Nor can these differences in concentration be explained by collection of saliva at different intervals after onset of stimulated flow.

At the commencement of stimulation, the mandibular gland showed positive rest transients for sodium, potassium, calcium, chloride and phosphate (Fig. 5) similar to those observed in various combinations for some glands of eutherian species (Burgen, 1956; Coats & Wright, 1957; Dreisbach, 1959; Petersen & Poulsen, 1968; Nielsen & Petersen, 1970). The size of the rest transients was dependent on the level of stimulation, as was reported for potassium in dog parotid saliva (Burgen, 1956) and calcium in cat mandibular saliva (Nielsen & Petersen, 1970). Although the phosphate transients appeared similar in magnitude at both levels of stimulation, after allowing for the volume collected in each sample, the transient at the higher level of stimulation was approximately three times that at the lower level of stimulation. Except for the potassium rest transient, the causes of the transients in the above ion concentrations have not been established. However, for a large ion like phosphate there are likely to be two mechanisms. Phosphate is believed to be secreted into the primary fluid and, being a relatively impermeant anion, concentrates at low flow rates by fluid withdrawal in the duct system (Compton, Nelson, Wright & Young, 1980). Consequently, high levels of phosphate are likely to occur in saliva trapped in the duct system of a resting gland and this phosphate would be flushed out at the onset of stimulated flow. Sheep mandibular saliva has phosphate concentrations of $5\ mequiv\ kg^{-1}$ or less at flow rates above $30\ \mu l\ min^{-1}\ g^{-1}$ tissue, whereas the stagnant saliva in a resting gland had a concentration of $159\ mequiv\ kg^{-1}$ (Kay, 1960). If this mechanism alone caused the phosphate rest transient, the increment in phosphate excretion during the transient would be similar at all levels of stimulation but, as this was not the case, a second mechanism, related to the level of stimulation, must also be involved.

During prolonged stimulation, there was a general trend for salivary sodium concentrations to rise by some $6-7\ mmol\ l^{-1}$ during the first 60 min of constant flow at either level of stimulation. In absolute terms this increase is small but relative to the prevailing concentrations at both low and high flow rates the increase was large

(56 and 17%, respectively). Since the increase in salivary sodium cannot be explained by concurrent increases in plasma sodium concentration, the rise in salivary sodium could result either from a progressive increase in primary fluid concentration or, more likely, from a small fall in the rate of ductal reabsorption during prolonged acetylcholine stimulation.

In spite of the animals having chronically high sodium intakes and thus being physically sodium replete, significant variations in mandibular salivary Na^+/K^+ ratio occurred between experiments on the same animal. This can be explained by differences in circulating mineralocorticoid levels between experiments since the gland responds to acute infusions of aldosterone. In contrast, the parotid gland of the red kangaroo under similar experimental conditions remains refractory to the acute aldosterone administration at the same high rate (Beal, 1986) and, except during long-term DOCA administration, all parotid samples collected during this study had Na^+/K^+ ratios in the Na^+-replete range. Clearly, the mandibular gland of the red kangaroo, unlike the parotid gland, is continuously responsive to low but fluctuating levels of endogenous mineralocorticoid during the Na^+-replete state. Furthermore, the onset of the response of the kangaroo mandibular gland was rapid relative to that of the sheep parotid gland, taking 45–60 min and about 70 min, respectively (Blair-West et al. 1963). As has been shown for the parotid gland of the Na^+-replete kangaroo (Beal, 1986), the mandibular gland responds to chronically high mineralocorticoid concentrations in the blood during DOCA administration with reduction of the salivary Na^+/K^+ ratio to values lower than that occurring after acute aldosterone infusion. In Na^+-deficient grey kangaroos, the percentage of mandibular gland volume occupied by the intralobular (striated) ducts was four times that of Na^+-replete animals (Blair-West et al. 1968). Some increase in both intralobular duct cell height and percentage lobular volume occurred concurrently with the lowered Na^+/K^+ ratio during DOCA administration. However, these histological changes were small relative to those reported for the grey kangaroo in the wild, possibly because the experimental period of high steroid influence was too short and/or the effect of the mineralocorticoid is potentiated by sodium deficiency. These histological data agree with those of Blair-West et al. (1968) in that the degree of mandibular duct hypertrophy was small relative to that found in the parotid gland following DOCA administration (Beal, 1986) or sodium depletion. Presumably, less hypertrophy of the mandibular ducts occurs during sodium depletion or DOCA administration because the ducts of this gland, in contrast to the parotid ducts, have already responded to the low levels of endogenous steroid in the Na^+-replete kangaroo. In rabbits fed low-sodium diets, the intralobular ducts of the mandibular gland develop a substantial degree of glycogen granulation (Compton et al. 1975; Young & van Lennep, 1978). A similar phenomenon occurred in some intralobular ducts of the kangaroo mandibular gland, where the increase in glycogen granulation was clearly a response to the high level of adrenal steroid since the animals were not sodium deficient. However, the kangaroo mandibular gland appears to have two populations of intralobular ducts, those which become granulated and those which remain ungranulated in the presence of high steroid levels. Two types of secretory

end piece, both serous, have been distinguished histologically in kangaroo mandibular glands (Forbes & Tribe, 1969) and thus each of the two types of intralobular duct may be associated with one species of end piece.

The large excursions in salivary osmolality seen during some aldosterone infusion experiments were due to parallel increases in sodium and potassium concentrations. A change in activity of a single salivary function, e.g. rate of primary fluid secretion or ductal sodium reabsorption, seems inadequate to explain this result since (1) the flow rate remained essentially constant throughout, (2) the Na^+/K^+ ratio of the saliva continued to fall irrespective of the actual ion concentrations, (3) the time course of the changes was too long to correlate with any oscillation in the level of anaesthesia or with the rate of gland stimulation, (4) the effect was not synchronized between experiments and (5) it cannot be explained by concomitant changes in plasma electrolytes.

Clearly the mandibular gland of the red kangaroo, with its high secretory capacity, its rapid responsiveness to low levels of mineralocorticoids and its saliva consisting essentially of a markedly hypotonic sodium/potassium chloride solution, is functionally better adapted to act as the primary source of saliva for external cooling than is the parotid gland.

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