THE EFFECT OF Na+ DEPLETION ON THE Na+:K+ RATIO OF THE PAROTID SALIVA OF THE SHEEP

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It is well known that the continued removal of gastric juice from animals or man does not radically alter the composition of the juice (Dragstedt & Ellis, 1930; Katsch & Mellinghoff, 1933). This was found despite the loss of up to half the body chlorides (Lim & Ni, 1925–6).

McCance (1938) reviewed the literature on the effect of salt deficiency on the electrolyte composition of body secretions. In his own experiments he produced depletion by a deficient diet and repeated sweats. The depletion was great enough to reduce the extracellular chlorides of his subjects by 40–50%. He found that in mixed saliva, gastric secretion and sweat there was a fall in Na^+ concentration and a rise in K^+ concentration. However, the changes in the first two were small, i.e. roughly reciprocal changes in Na^+ and K^+ of about 5–10 m-equiv/l. In the case of sweat glands, the Na^+ and Cl^- loss in each episode decreased with increasing depletion. Whereas initially 150–180 m-equiv Na^+ were lost each time, finally (7th–8th day) only 60–80 m-equiv were lost in about the same volume. There was a small gradual rise (5–10 m-equiv) in the K^+ loss.

Gross Na^+ depletion has been produced in dogs by pancreatic fistulae (Gamble & McIver, 1928; Hartmann & Elman, 1929; Johnston & Ball, 1930). The total base concentration of the pancreatic secretion decreased as the total base of the plasma decreased. The observations of Johnston & Ball (1930), though not conclusive on this point, indicate that there was no rise of K^+ in the pancreatic juice as the animal became Na^+ depleted.

This paper is concerned with reporting a rather striking relation between the electrolyte balance of the sheep and the composition of parotid gland secretion.

The normal sheep secretes 4–8 l. of alkaline parotid saliva each day. This buffers the products of bacterial fermentation in the rumen. The transplantation of one parotid papilla and duct on to the cheek permits the subtraction of

2–3 l. of this fluid each day. Given adequate replacement of Na^+, a sheep with such a fistula remains in good condition indefinitely. If Na^+ is withheld a large negative Na^+ balance is rapidly produced. In the course of electrolyte balances made to study the response of the kidney to the Na^+ depletion so produced, it was observed that, by the time the animal was depleted of 500–800 m-equiv of Na^+ ion, the parotid saliva had changed from a predominantly Na^+ secretion to a predominantly K^+ secretion.

METHODS

Cross-bred Merino sheep were used.

Each sheep was put in a stainless steel metabolism cage. The posterior section had a floor of 2·5 cm stainless steel mesh. Below this a very fine stainless steel mesh sloped at 45° and led to the faeces collection bin. Urine passed through the fine mesh and ran down a stainless steel collecting well to a vessel below. Because of the nature of sheep faeces, separation of faeces and urine was virtually complete. The anterior portion of the cage also had a mesh floor, and saliva from the fistula dripped through this, and ran rapidly from a sloping catchment system into a container. There was a rubber dividing section, and the design was effective in preventing any significant contamination of either compartment. There was a food bin in front and a water bin at the side. A sheep can be kept indefinitely in such a cage.

In the course of a balance experiment the sheep was on a constant diet. It was put on the diet at least 10 days before the experiment began. At the same time each day the saliva and urine were collected. The animal was given its supplementary electrolyte intake by way of a rubber tube passed into the rumen. It was weighed, blood was collected, and then it was fed. The electro-lyte composition of the diet was determined, and the constancy of the batch being fed was checked several times. When it was necessary, precautions were taken to ensure that the animal received the same dry weight each day, allowing for the variable water content of some plant foods. The sheep generally ate its 24 hr ration within 4 hr. Water was freely available and the intake was measured. The faeces were collected and analysed at appropriate intervals during the balance period.

Operative. Operations to make parotid fistulae and carotid loops were carried out under 'Pentothal' curare, cyclopropane and oxygen anaesthesia. The method of making a parotid fistula will be described in detail elsewhere. No experiments were made until a month after operation. Histological study of parotid glands from animals which were killed after having had a fistula for 2–6 months did not show any difference between the two sides. The sheep (P.F. 1—a 4-year-old cross-bred ewe), on which the bulk of the study reported in this paper was made, has had a fistula for 13 months. It has secreted over 100,000 m-equiv of Na^+. Its present weight (34 kg—December 1954) is 2 kg heavier than before the fistula was made.

Chemical methods. Na^+ and K^+ in urine and saliva were determined with a Beckman flame spectrophotometer (Wynn, Morris, Simon, McDonald & Denton, 1950). Chloride was determined in urine and saliva by the method of Volhard. 25 ml. aliquots of saliva were titrated and there was no difficulty in determining the end-point. The solutions were cooled before titration. 5 ml. ali-quots of urine were gently heated with activated charcoal and transferred quantitatively through Whatman No. 41 filter-paper to a second flask before addition of 0·1 N-$AgNO_3$, A.R. concentrated nitric acid, cooling and titration. Using this technique, the end-point was quite definite. Phos-phate in saliva was determined by the method of Fiske & SubbaRow (1925). All glassware used in chemical determinations was washed twice in distilled water. All determinations were made in duplicate and were repeated if agreement was not ±2%. The methods were frequently standard-ized to check that they would recover equivalent amounts of cation and anion from a standard solution, and that this recovery could also be made from the biological solutions.

Determination of the percentage loss of saliva during collection. During a 24 hr collection of saliva, volume loss might occur as a result of evaporation and as a result of 'wetting' of the surface area of the collecting system. At the end of each balance the whole collecting well and mesh were washed with distilled water and the washings analysed. The electrolyte recovered was added to the total of electrolyte secreted, and as this total was divided by the volume of saliva actually collected, it follows that the concentrations of all electrolytes as expressed are too high by a factor equal to the percentage water loss. In order to determine the magnitude of this factor the following experiments were done.

A large glass container was suspended 2 ft. above the mesh floor of the cage. A burette tap and dropper were used as the outlet, and the system was arranged to give a requisite number of drops of sheep's saliva per minute and to drain completely. The drops fell through the mesh, drained along the sloping collecting system, and fell down the well into a filter funnel and collecting receptacle below.

The results of three experiments are as follows:

(1) Volume of saliva = 2 l.; time of dripping = 30 hr; temperature in cage = 23° C; volume lost = 62 ml. = 3·1 %. On this day the cage temperature was higher than is usual in our experimental room (15–20° C).

(2) In this instance, the drops of saliva were allowed to run for 7 cm across a piece of sheep-skin and fall from the dependent point of this. The cage was wet with saliva before starting as was usual in any 24 hr balance period under consideration. Volume of saliva = 0·50 l.; time of dripping = 23 hr; temperature = 19° C; volume lost = 35 ml. = 7 %.

(3) Conditions as in (2) except that the time was increased to 48 hr. Volume of saliva = 2·0 l.; loss = 90 ml. = 4·5 %.

In the course of twenty acute experiments in which right and left parotid ducts were cannulated and the saliva analysed (Coats, Denton & Wright, 1955, unpublished), the average Na^+ and K^+ composition found was $Na^+ = 169$ m-equiv/l., $K^+ = 7$ m-equiv/l. McDougall (1948) gives an average composition of $Na^+ = 177$ m-equiv/l., $K^+ = 8$ m-equiv/l., for sheep's saliva collected from cannulated ducts. The Na^+ and K^+ sum of these figures is about 3–8 % lower than the composition as collected in our experiments under control balance conditions uncomplicated by additional electrolyte intake (Table 2, Section (*a*)). The evaporative water loss does not, however, bear upon the main point of this paper which is the $Na^+:K^+$ ratio of the saliva, and no correction for it has been made.

<div align="center">RESULTS</div>

<div align="center">*The volume of saliva and electrolyte composition*</div>

In order to establish a base-line for salivary composition with varying food intake but adequate electrolyte maintenance, a number of collections of saliva was made with the sheep on a different diet each time (Table 1). This comparison indicates that:

(i) In confirmation of the observations on horses (Colin, 1886; Scheunert & Trautmann, 1921) and oxen (Ellenberger & Hofmeister, 1887; Block, 1939), the activity of the parotid gland was related to the type of food eaten—i.e. to its physical properties and, as in the case of oxen, to the amount of rumination it provoked. There was very much less rumination when the animal was feeding on fresh lucerne alone.

(ii) The rate of saliva secretion was not a simple function of electrolyte balance. In all the above experiments the Na^+ intake was adequate to counter the fistula loss. In all instances the urinary Na^+ excretion was 100 m-equiv/day or greater.

(iii) The composition of the saliva was virtually the same at the slow rate as at the fast rate. This is important in relation to what is to follow.

TABLE 1. The composition of sheep saliva in relation to the diet (Sheep P.F. 1)

Diet	Period of collection (days)	Mean volume collected daily	pH	Na$^+$	K$^+$	Cl$^-$	HPO$_4^{2-}$	HCO$_3^-$
0·8 kg oaten chaff (water content 10%) 0·3 kg fresh green lucerne finely chopped (water content 65%) 595 m-equiv NaHCO$_3$ Free access to water	12	3·17	8·5	178	16	17	18	140
0·5 kg oaten chaff 1·0 kg fresh lucerne 595 m-equiv NaHCO$_3$ Free access to water	6	2·68	—	180	12	11	13	—
1·0 kg fresh lucerne 595 m-equiv NaHCO$_3$ Free access to water	4	1·47	—	180	14	12	—	—

The effect of withholding supplementary Na$^+$ *intake*

The supplementary intake was withdrawn gradually (Fig. 1). The features are:

(i) There was a gradual decrease in the saliva volume. Whereas the average volume/day in the control period was 2·68 l., the average over the 11 days of Na$^+$ depletion was 1·71 l.

(ii) Commensurate with the negative Na$^+$ balance (1100 m-equiv were lost from the fistula in the first 3 days) the Na$^+$ concentration of the saliva decreased from 180 to 60–80 m-equiv/l. There was an almost equivalent increase in the K$^+$ concentration. It rose from 10 to 110–120 m-equiv/l. Thus the saliva retained its hypertonicity, and apart from a small increase of HPO$_4^{2-}$ with an equivalent decrease of HCO$_3^-$, the anion pattern also was unchanged.

(iii) Within 48 hr of Na$^+$ withdrawal, the Na$^+$ excretion by the kidney was reduced to virtually nil. At the same time the renal K$^+$ excretion was greatly reduced so that on 8 of the 11 days, the urine K$^+$ concentration was less than plasma concentration—a quite uncommon finding. Thus, in the face of gross Na$^+$ depletion, the animal had ceased excreting Na$^+$ in the urine, greatly reduced it in the saliva, and 'transferred' the large urinary K$^+$ excretion characteristic of the herbivora to the parotid secretion.

Whilst it is the purpose of this paper to deal solely with the parotid gland, as distinct from analysis of adaptation in terms of total balance, it can be pointed out that in the later stages of depletion the animal was virtually in Na$^+$ equilibrium. The external balance showed that the fistula Na$^+$ loss was countered by the net Na$^+$ gain from food. This has been borne out by the frequent observation that a sheep with a fistula can survive for months if it be

given an adequate amount of the usual diet and access to rock salt or a dose of 50 g of $NaHCO_3$ every 2–3 weeks. The parotid gland secretion then has a permanently high K^+ concentration.

(iv) The sensitivity of this response to change of electrolyte balance was shown on the seventh morning of the withdrawal period (Fig. 1, day 13) when 150 m-equiv of $NaHCO_3$ were given. There was a clear-cut change in the composition of the saliva collected over the next 24 hr, whereas there was no change in urine composition (Fig. 1). The saliva Na^+ rose 14 m-equiv/l., and the K^+ fell 17 m-equiv/l.
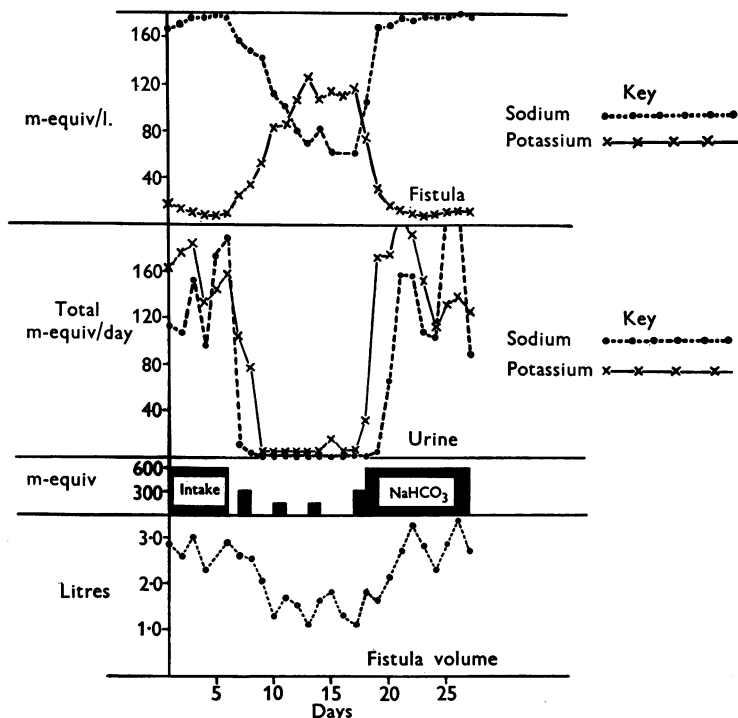

Fig. 1. The effect of withholding the daily intake of 595 m-equiv of $NaHCO_3$ on the volume and Na^+ and K^+ concentrations of the parotid saliva. The Na^+ concentration fell, and the K^+ concentration rose. The change in daily excretion of Na^+ and K^+ by the kidney is also shown. Sheep P.F. 1.

The effect of Na^+ depletion on the parotid saliva composition

The observations recorded above have been extensively repeated in the course of total balance studies analysing the renal response of this animal to excess Na^+ subtraction. This is clearly shown in Table 2. Each section of this table is made up from a number of periods under identical conditions. The sections (c), (d), (e) and (f) differ from the first two sections (a) and (b) in that,

TABLE 2. The relation of parotid saliva composition to Na⁺ balance (Sheep P.F. 1)

Total no. of days	Total volume (l.)	Saliva Average vol/day (l.)	Saliva Total (m-equiv)			Mean composition (m-equiv/l.)				Daily intake: food (kg); electrolyte (m-equiv)	Average vol/day (l.)	Urine Average excretion/day (m-equiv)		
			Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	$Na^+ + K^+$			Na^+	K^+	Cl^-
(a) Control period—without K⁺ supplement														
11	33·34	2·78	5928	365	412	178	11	13	189	1·0 lucerne 0·5 chaff 595 NaHCO₃	0·82	146	153	118
(b) Na⁺ depletion—from 3 days after Na⁺ withdrawal—without K⁺ supplement														
6	8·52	1·42	635	1003	96	75	118	11	193	1·0 lucerne 0·5 chaff	1·65	0·5	5	100
(c) Control period—with K⁺ supplement														
77	201·09	2·61	37733	4278	2622	188	21	13	209	0·5 lucerne chaff 0·5 chaff 595 NaHCO₃ 200 KHCO₃	1·25	173	396	152
(d) First 3 days of negative Na⁺ balance—with K⁺ supplement														
15	32·07	2·14	4379	1740	389	137	54	12	191	0·5 lucerne chaff 0·5 chaff 200 KHCO₃	0·72	5	182	162
(e) Na⁺ depletion—from 3 days after Na⁺ withdrawal—with K⁺ supplement														
29	46·48	1·60	3220	5976	408	69	129	9	198	0·5 lucerne chaff 0·5 chaff 200 KHCO₃	1·22	1·3	189	156
(f) First 3 days of Na⁺ replacement after negative balance—with K⁺ supplement														
12	24·83	2·07	3983	951	290	160	38	12	198	0·5 lucerne chaff 0·5 chaff 595 NaHCO₃ 200 KHCO₃	1·25	95	397	112

throughout the whole period of the balance, the animal was given 200 m-equiv of K^+ each day as $KHCO_3$. This was done to avoid the situation of very low K^+ excretion (Fig. 1) in a group of renal studies being conducted at the same time. It had no effect on the characteristic salivary change during Na^+ depletion. Sections (b) and (e) show saliva composition as from the third day after withdrawal of the Na^+ supplement until the end of the withdrawal period. In all instances, by the end of the first 3 days of Na^+ withdrawal, the animal had a Na^+ deficiency of over 500 m-equiv.

Table 2 shows:

(i) An unequivocal difference between Na^+ : K^+ ratio of saliva during control (sections (a) and (c)) and during Na^+ depletion (sections (b) and (e)).

The periods of transition in the balance (sections (d) and (f)) show an intermediate composition. This commensurate relation between Na^+ balance and saliva composition was clearly shown in the daily analyses from which this table has been compounded, in the same way as is shown in Fig. 1. The urinary Na^+ excretion reflected the state of Na^+ balance. In all control periods it was over 100 m-equiv/day, whereas during depletion it averaged 1 m-equiv/day. During the transition periods (sections (d) and (f)) it showed an intermediate composition as did the saliva.

(ii) The volume of saliva secreted during the depletion period was approximately 1 l. day less than during the control period. However, rate of secretion and composition were not directly related. The daily volume under depletion conditions on an oaten chaff diet (section (e)) was greater than the daily volume under control conditions when the animal had only fresh green lucerne to eat (Table 1). This fact that changed composition was not directly caused by decreased secretion rate has been confirmed frequently in short-term experiments. Over the course of several hours the parotid saliva secretion rate in a sheep may vary 20-fold according as the animal is eating, ruminating, standing quietly or is intentionally frightened. The composition of the saliva of the animal in normal Na^+ balance remains constant over these large changes of secretion rate.

(iii) The provision of 200 m-equiv/day of additional K^+ intake during the control period resulted in the average K^+ concentration of the saliva being 10 m-equiv/l. higher. The average Na^+ concentration was also 9 m-equiv/l. higher. Thus the osmotic pressure of the saliva was increased.

The effect of a very large Na^+ intake on the composition of parotid secretion

The control conditions were a daily diet of 0·4 kg lucerne chaff; 0·4 kg oaten chaff; 595 m-equiv $NaHCO_3$; free access to water.

Two types of experiment were made:

(a) The daily Na^+ intake was doubled.

(b) The animal was not fed for 4 days, during which time the saliva volume

decreased to 0·35 l./day by the last day. Thus the Na^+ intake, which was continued, was in large excess of fistula loss and there was a great increase of urine Na^+ excretion. In both instances there was a rise in the $Na^+:K^+$ ratio of the saliva, mainly as a result of decreased K^+ concentration. The Na^+ concentration in the first experiment remained unaltered. In the second, the average volume/day during the last 3 days of starvation was 0·5 l., and the average composition was $Na^+ = 229$ m-equiv/l.; $K^+ = 9$ m-equiv/l.; $Na^+:K^+$ ratio = 25. The control $Na^+:K^+$ ratio was 15.

Another feature of the experiment in which the Na^+ intake was doubled was that the daily volume of saliva decreased. Whereas in the control period an average of 2·8 l. was secreted each day, in the 4-day period of doubled $NaHCO_3$ intake the average was 2·0 l./day. This is interesting in view of the finding of Krasusky, Krynskaya and Kotlyarevskaya (1940) that intra-ruminal administration of organic and mineral acids stimulates parotid secretion in sheep with chronic fistulae.

TABLE 3. The effect of Na^+ depletion on the phosphate concentration of the saliva
(Sheep P.F. 1)

Condition of balance	No. of days	Saliva volume (l./day)	Total HPO_4^{2-} (m-equiv)	HPO_4^{2-} m-equiv/ day	Average m-equiv HPO_4^{2-}/l
Control period	12	2·78	409	34	12
Na^+ depletion period	11	1·71	390	35	21
Control period (increased HPO_4^{2-} intake)	21	2·36	2044	97	41
Na^+ depletion period (increased HPO_4^{2-} intake)	10	1·59	918	92	58

The effect of Na^+ *depletion on the phosphate composition of the saliva*

The phosphate concentration of the parotid secretion rose during Na^+ depletion. This rise was commensurate with the decrease of secretion volume so that the total phosphate secreted was unchanged. An increased phosphate intake caused an increased secretion of phosphate by the parotid (the animal was given 100 m-mole K_2HPO_4 per day throughout a balance study). When Na^+ intake was withheld, the same relation held with regard to concentration change and total secretion per day (Table 3).

DISCUSSION

The data recorded in this paper were derived from intensive study of one animal which has been used as its own experimental control. However, it is desired to emphasize that this phenomenon of reversal of cation proportions has been reproduced numerous times, without exception, in each of twenty sheep during the course of investigation of the cause of this parotid behaviour. Also in the studies of Na^+ depletion by the use of oesophageal fistulae, the

same change was seen in the mixed saliva as depletion occurred. It was observed in eight sheep, without exception, and the relation to Na^+ balance was shown in instances where isotonic saline was given intravenously during the course of the experiments (Denton, Wynn, McDonald & Simon, 1951; Denton, McDonald Munro & Williams, 1952). The figures presented in this paper do not represent the limit of change in saliva composition possible. In experiments, yet to be reported, on the effect of psychic stimulation in the context of altered electrolyte balance, specimens with a composition of $Na^+ = 5$–10 m-equiv/l., $K^+ = 150$–170 m-equiv/l., $Na^+:K^+$ ratio $= 0.03$ have been collected. Thus the change to a secretion of K^+ may be almost complete.

The experiments with large Na^+ intake suggest that the relationship between $Na^+:K^+$ ratio of parotid saliva and the external Na^+ balance is continued to some extent on the side of a positive Na^+ balance.

A reciprocal relationship between Na^+ and K^+ concentration in milk secreted by cows has been described (Barry & Rowland, 1953), but relation to external electrolyte balance has not been investigated. Gross Na^+ depletion in man has resulted from complete post-gastrectomy duodenal fistulae (Denton *et al.* 1951). The fluid lost from such a fistula is predominantly pancreatic secretion. As the patient became depleted of Na^+ the plasma Na^+ and fistula fluid Na^+ concentration decreased equivalently, but there was no rise of K^+ concentration of the secretion.

Consideration of this phenomenon of parotid secretion in the light of knowledge of the effect of adrenal hormones on the ratio of $Na^+:K^+$ in secretions will be the subject of discussion in a later paper.

SUMMARY

1. A sheep with a parotid fistula secreted 3 l. of alkaline saliva each day. The Na^+ concentration was approximately 180 m-equiv/l. and the K^+ concentration 10 m-equiv/l. ($Na^+:K^+$ ratio $= 18$). With a normal diet, and adequate replacement of Na^+, the sheep remained in good condition indefinitely.

2. If Na^+ replacement were withheld, the animal became grossly depleted of Na^+. The saliva volume decreased by approximately 1 l./day. The composition of the parotid secretion changed. Na^+ concentration fell to 60 m-equiv/l., and K^+ concentration rose to 120 m-equiv/l. ($Na^+:K^+$ ratio $= 0.5$).

3. There was a commensurate relation between the amount of Na^+ depletion and the $Na^+:K^+$ ratio of the parotid saliva.

4. During Na^+ depletion the phosphate concentration of the saliva rose. The total amount of phosphate secreted per day was unchanged.

5. During very large intake of Na^+, the $Na^+:K^+$ ratio of the parotid saliva rose.

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