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THE EFFECT OF VARIATIONS IN BLOOD SUPPLY ON THE SECRETION RATE AND COMPOSITION OF PAROTID SALIVA IN Na^+-DEPLETED SHEEP

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Sheep with permanent unilateral parotid fistulae may be maintained in good health indefinitely provided that sufficient $NaHCO_3$ is added to the dietary intake to compensate for the Na^+ lost each day in the 1–4 l. of hypertonic alkaline parotid saliva. It has been shown (Denton, 1956*a*) that withdrawal of this supplementary Na^+ intake results in Na^+ depletion and, as the extent of this increases, the ratio Na^+/K^+ in the parotid saliva falls progressively from a normal value of 18·0 (180:10 m-equiv/l.) to as low as 0·06 (10:180 m-equiv/l). Restoration of the dietary $NaHCO_3$ supplement, in an amount sufficient to correct the Na^+ depletion, causes the Na^+/K^+ ratio in the parotid saliva to return to normal.

Gross Na^+ depletion causes a fall in plasma Na^+ concentration (Denton, 1956*b*). That the absolute plasma Na^+ concentration is not, by itself, the direct cause of the changed Na^+/K^+ ratio in parotid saliva in these circumstances is indicated by the observation (Denton, unpublished) that if the water intake of sheep is severely restricted during Na^+ depletion, the plasma Na^+ concentration increases above control period values, but the Na^+/K^+ ratio of parotid saliva falls as in Na^+ depletion alone. It has also been shown that gross Na^+ depletion significantly reduces total body water and plasma volume (Denton, 1956*b*); and such changes, by their influence on circulatory dynamics, might reduce the amount of Na^+ presented to the parotid cells/unit time even of the water-depleted animal with raised plasma Na^+ concentration. This could well underly the change observed in the Na^+/K^+ ratio of parotid saliva.

In order to obtain further information regarding the causes of the change in Na^+/K^+ ratio of parotid saliva during Na^+ depletion, it became necessary to determine the effects on parotid secretion, and in particular on the Na^+/K^+

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ratio, of altering the blood supply to the gland in conscious sheep, both normal and Na^+-depleted. For this purpose, two sheep were provided with bilateral carotid loops. The carotid sinus nerve was cut on the side of the parotid fistula. This loop served for measurements of blood pressure, and for stimulation of parotid secretion and blood flow by intra-arterial infusion of acetylcholine. The loop on the opposite side was not denervated; and since a baroceptor reflex has been shown to exist in the sheep (see 'Results'), reflex increases in systemic blood pressure could be produced by occlusion of this loop. In such reflexes the sympathetic nerves, including those to the parotid gland, are involved. Their influence on parotid secretion in such circumstances could be ascertained by reason of the fact that in one of the sheep the sympathetic nerve supply to the parotid gland was divided, in the other it was not.

A considerable fall in blood supply to the head and presumably to the parotid gland could be produced by occlusion or near-occlusion of both loops. The effect of this procedure was more severe in one of the sheep, which had had the left occipital artery ligated, than in the other which had not. The results of the work to be reported indicate that while some alteration in the Na^+/K^+ ratio of saliva can be produced by alterations in blood supply to the gland during Na^+ depletion, indicating the existence of a 'local factor' in the low Na^+/K^+ ratio found in this state, the major cause of this striking phenomenon must be sought elsewhere.

METHODS

Two sheep (P.F. 7 and P.F. 16) were prepared surgically for these experiments as follows: after induction of anaesthesia both sheep were provided with a left parotid fistula, the duct and papilla being enclosed in a skin tube so that a teat about 2 cm long was formed for easier collection (Denton, 1956*b*). At subsequent operations bilateral carotid loops (van Leersum, 1911) were prepared and the left baroceptor nerves divided. In sheep P.F. 16 the left superior cervical ganglion and a portion of the left cervical sympathetic trunk in the root of the neck were removed, and the left occipital artery ligated. The removal of the ganglion was confirmed histologically, and it was demonstrated at operation that stimulation of the cranial end of the divided sympathetic trunk did not cause the characteristic gush of parotid saliva (Coats, Denton, Goding & Wright, 1956).

The procedure of observation of animals in metabolism cages has been described previously (Denton, 1956*a*). In the present experiments, the saliva-collecting system of the metabolism cage was removed, washed with glass-distilled water, dried and replaced each day rather than at the end of each balance period.

Saliva collections from the skin teat were made either into a stainless steel kidney-dish, or more often a stainless steel device with a very steep incline was inserted into the metabolism cage in which the animal was standing (Fig. 1). The saliva fell on this device and ran rapidly to a collecting cylinder. The sheep had been in metabolism cages and had been handled most days for a period of six months before these experiments were carried out. Thus they were quite undisturbed by the presence of an observer making a saliva collection and stood quietly during intracarotid infusions which lasted 2–12 hr (see below): there was rarely any increase of cardiac rate during the infusion.

In acute experiments, blood pressure was recorded on smoked paper on a kymograph from a cannula tied into the femoral artery. In survival experiments the blood pressure was recorded

from the carotid loop on the side on which the carotid sinus nerve had been cut. The measurement was made with the animal standing quietly in its cage with the head erect. The cuff, which was made from the type used to measure the blood pressure of babies, had a window covered with surgical glove rubber on the inner surface (Verney & Vogt, 1938). This cuff was snugly applied to the carotid loop with the aid of a large paper clip. The cuff was inflated above systolic pressure and then gradually released. The pressure was recorded as that at which a pulse was first unequivocally palpable cranial to the inflated cuff. The pressure was also taken by auscultation (Fig. 1) and

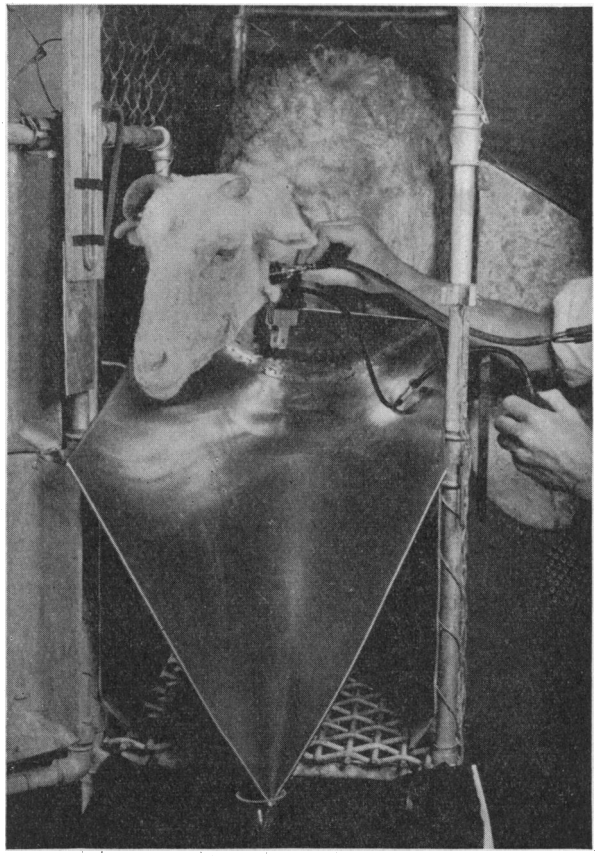

Fig. 1. Measurement of systemic blood pressure in denervated carotid loop by auscultation; the stainless steel device for collecting saliva is in place; the teat of the parotid fistula can be seen on the cheek.

the comparison showed that the pressures recorded by the above method were consistently 6–10 mm Hg less than systemic systolic blood pressure recorded by auscultation. The right carotid loop was occluded by application of an ordinary spring clothes peg after protecting the site of application by wrapping with several layers of dental rubber.

Infusions into the common carotid artery via the carotid loops were made through a 23-gauge needle connected to a polythene tube which was temporarily fixed to the loop with adhesive tape. This polythene tube led to a small manifold which was arranged so that more than one fluid could be infused into the artery at once if desired. A mercury pump was used for the infusion.

This consisted of a very fine capillary delivering a spray of mercury into a pressure bottle from a column. This displaced the fluid to be infused and the rate was adjusted by varying the height of the column. The infusions of ACh (2×10^{-5} in a solution of 5% glucose in glass-distilled water) were made by this technique. A geared electric motor with constant voltage supply was used to depress the plunger of a 50 ml. syringe for the infusion of NaCl solution. Full aseptic precautions were taken in the preparation of infusion fluids and the apparatus. There were no reactions to the infusion. For chemical methods, see Denton (1956*a*).

The animals were maintained on a daily ration of 0·6 kg of lucerne chaff containing approximately 20 m-equiv Na^+. They were given by rumen tube a supplementary intake of 600 m-equiv $NaHCO_3$/day. Na^+ depletion was produced by withholding the supplementary $NaHCO_3$ intake or reducing it to 100 m-equiv $NaHCO_3$/day. In these circumstances, the saliva composition was consistently in the range Na^+ 50–100 m-equiv/l.; K^+ 140–80 m-equiv/l. Frequent analyses showed that the urinary Na^+ excretion was less than 1 m-equiv/day. The animals could be maintained in this state of Na^+ depletion for 6–8 weeks, though the external Na^+ balance showed them to be depleted of as much as 400–700 m-equiv of Na^+ (Denton, 1956*b*).

RESULTS

As a preliminary to subsequent experiments, the existence of a 'carotid sinus reflex' mechanism in the sheep was established. Dissection of ten heads showed that on each side two nerves arose from the region of the junction of the common carotid and occipital arteries—there is no internal carotid artery in the sheep. One nerve, *A*, arose from the superficial surface of the occipital

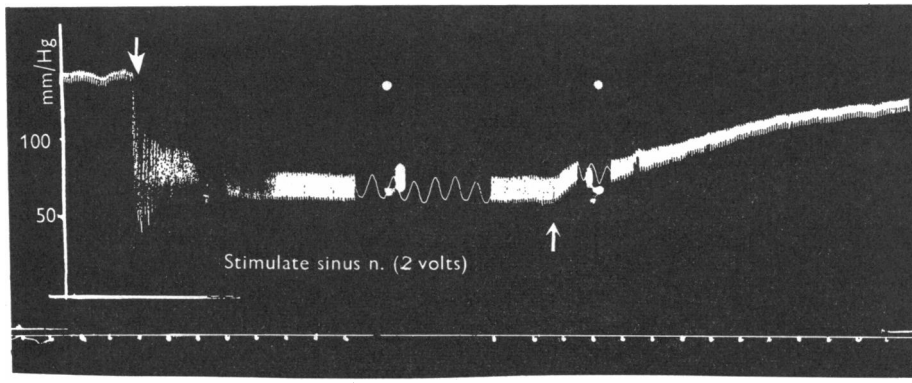

Fig. 2. The effect on systemic arterial blood pressure in an anaesthetized sheep of 5 min faradic stimulation of the central end of the baroceptor nerve *A* (sinus nerve, see text); time marker 30 sec.

artery and joined the IX nerve as it passed beneath the superior border of the posterior belly of the digastric muscle. Faradic stimulation of the central end in acute experiments caused a rapid fall in blood pressure from 140 to 60–70 mm Hg and cardiac slowing (Fig. 2). There was no accommodation after 5 min stimulation (O'Connor, 1955).

On ceasing stimulation blood pressure returned gradually to normal. After bilateral vagal section at the level of the hyoid, stimulation caused a

much slower fall in arterial pressure to 90–100 mm Hg. Stimulation of the central end of the IX nerve distal to its junction with this nerve (A) had no effect on blood pressure.

The second nerve, *B*, arose from the deep surface of the carotid artery about 3–5 mm caudal to the origin of the occipital artery, ascended parallel to nerve *A* and entered the skull between the IX nerve and the superior cervical ganglion. Stimulation of the central end of this nerve had no effect on heart rate or blood pressure; it was, however, cut in the 'survival' animals at the same time as nerve A.

TABLE 1. The effect of Na^+ depletion and the carotid sinus reflex on blood pressure (measured by palpation)

Sheep and day (December 1955)	Na^+ balance	Blood pressure (mm Hg) (no. of observations)	Blood pressure with innervated carotid loop occluded (mm Hg) (no. of observations)
P.F. 7			
5	Normal	90 (2)	122±18 (2)
9	Normal	98± 4 (4)	120± 5 (3)
12	Normal	80± 5 (6)	94± 7 (8)
16	4 days' depletion	69± 5 (6)	119±15 (8)
19	7 days' depletion	76± 4 (3)	—
23	2 days positive	93± 3 (10)	104±26 (8)
P.F. 16			
5	Normal	91± 6 (8)	110±12 (6)
9	4 days' depletion	82± 6 (4)	87± 5 (3)
16	11 days' depletion	77± 3 (10)	97±10 (6)
18	13 days' depletion	79± 2 (7)	91± 7 (6)
20	2 days positive	83± 4 (10)	93± 5 (7)
22	Normal	85± 6 (7)	102± 6 (4)
	Observations made with sheep on its side		
P.F. 7			
21*	Normal	130±15 (7)	150±17 (8)
7†	Na^+-depleted	132±10 (4)	175±17 (15)
12	Normal	104± 5 (5)	122±10 (6)
P.F. 16			
5	Normal	105± 3 (2)	115± 3 (4)

* 1954.

† January 1956.

Digital compression of the carotid artery at the origin of the occipital artery caused a similar fall in blood pressure to stimulation of nerve *A*; simultaneous compression of the artery at points 2–3 cm. above and below this region raised the arterial pressure by 10–15 mm Hg. The normal blood pressure, measured in the left loop by compression with a cuff and palpation or auscultation, was 80–100 mm Hg, when the animal stood quietly in its cage with the head erect (Table 1). If, however, the sheep were taken out of its cage and gently secured on its side, the blood pressure rose to 100–130 mm Hg and in sheep P.F. 7, as in other sheep tested, this procedure inhibited the continuous secretion of parotid saliva characteristic of the ruminant, as also did continued stimulation of the cervical sympathetic in anaesthetized sheep (Coats *et al.* 1956). Occlusion of the right (innervated) carotid loop for 3–10 min

raised the blood pressure by 10–50 mm Hg. If this were done with the animal lying on its side, blood pressures as high as 170–190 mm Hg were recorded. During occlusion, the cardiac rate usually decreased by 5–20 beats/min.

It was concluded that a baroceptor reflex exists in the sheep and that nerve *A* described above corresponds to the carotid sinus nerve described by de Castro (1926–28) in the bull.

The effect of reduction of blood flow on parotid secretion

In these experiments the blood flow to the head and hence to the parotid gland was altered by restricting the flow in the carotid arteries. Either the left (denervated) or right (innervated) arteries, or both, were occluded. Alternatively, the right artery was occluded, and a manometer cuff applied to the left artery. This cuff could be inflated so that graded constriction was obtained and when desired the pressure could be raised to 80–100 mm Hg, at which level only a small thrill was palpable cranial to the cuff. The changes in cranial blood pressure produced by such procedures were first determined directly as follows. In P.F. 16, whilst in normal Na^+ balance, a manometer cuff was applied to the left carotid loop as for measurement of blood pressure by auscultation. As far cranial to this cuff as possible, a 20-gauge hypodermic needle was inserted in the artery. This needle was connected to a mercury manometer by a polythene tube, through which a slow flow of heparinized saline was maintained by a side arm leading to a motor-driven syringe. The initial reading was 90 mm Hg. On occlusion of the right carotid loop, the pressure rose to 100–104 mm Hg. The manometer cuff on the left loop was now inflated. When the cuff pressure reached 70–80 mm Hg, the blood pressure in the loop had fallen to 50 mm Hg. When the cuff pressure exceeded 100 mm Hg, the blood pressure in the loop had fallen to 35–40 mm Hg and the sheep commenced to stagger and shake. On releasing the right loop, the blood pressure in the left loop rose within 30 sec to 106 mm Hg, although the cuff on the left loop was still inflated to 80 mm Hg pressure. If the right loop were left patent, and only the left loop occluded, there was little effect on blood pressure measured in the left loop as above until the cuff pressure exceeded 80 mm Hg. Increase of the cuff pressure until it produced occlusion (150 mm Hg) caused the blood pressure in the left loop to fall to 50–60 mm Hg. This did not disturb the animal.

Simultaneous occlusion of both arteries had little effect on P.F. 7 apart from a noticeable tendency to yawn and grind the teeth. When the procedure was carried out while the animal was in Na^+ depletion, this behaviour was somewhat more noticeable, in addition to which the animal sometimes became restless and appeared apprehensive. In P.F. 16, in which the left occipital artery had been ligated, 1–3 min occlusion of both arteries caused the sheep to stagger and fall down. It recovered within 30 sec of releasing one loop. If,

however, one loop were completely occluded and the cuff on the other inflated until a pulse was just barely palpable cranial to the cuff for 3–5 min before occlusion, the animal withstood this with no more disturbance than P.F. 7. However, when depleted of Na^+, P.F. 16 could not tolerate occlusion of both arteries however produced.

Normal Na^+ balance. Complete occlusion of both carotid arteries had little effect on either rate of secretion or electrolyte composition. Thus, in an experiment on P.F. 7, the control secretion rate was 2·3 ml./min. After 20 min occlusion of right loop, with cuff on left loop at 90 mm Hg, secretion rate was 1·90 ml./min. During the next 10 min, with both loops occluded, the rate was 2·3 ml./min; during release of the left loop only for 10 min it was 2·3 ml./min and during 30 min with both loops released, 2·3 ml./min. No changes in composition occurred. Similar results were obtained with P.F. 16 (Table 2).

TABLE 2. The effect on secretion and on Na^+/K^+ ratio of parotid saliva of bilateral occlusion of carotid loops in sheep P.F. 16 in normal Na^+ balance

Time (min)	Secretion rate (ml./min)	Ratio: Na^+/K^+
0– 30	1·92	18·7
30– 63	1·06	24·8
64– 95	1·52	26·1
96–128	1·25	24·6
129–148	1·39	22·1
152	Right (innervated) loop occluded, left (denervated) loop at 100 mm Hg: during last 5 min of next period left loop at 130–140 mm Hg	
152–172	1·00	22·4
172	Both loops released	
172–190	1·36	22·1
191–211	1·15	21·4
212–272	2·17	25·3
273–333	2·28	26·4
334–374	2·25	25·2
375–405	2·27	28·8
406–418	2·58	26·1

Sodium depletion. In both sheep, 4–13 days' Na^+ depletion caused the arterial pressure to fall by 10–20 mm Hg (Table 1). In another experiment on sheep no. PF. 16, in which the blood pressure was recorded by auscultation, it was found that after 2 days' Na^+ depletion, which usually results in a negative Na^+ balance of 300–500 m-equiv (Denton, 1956*b*), the blood pressure fell from 90/67 mm Hg (mean of 30 readings) to 79/59 mm Hg (mean of 13 readings). In both sheep occlusion of the right (innervated) carotid loop continued to cause a reflex rise in blood pressure during Na^+ depletion (Table 1). The pressure usually rose above the value normal for the same sheep before Na^+ depletion (Fig. 3).

Occlusion for 20–30 min of both loops in P.F. 7, or occlusion of the right loop

and near-occlusion of the left loop in P.F. 16, caused a definite reduction of secretion rate and Na^+/K^+ ratio of saliva. On simultaneous release of the arteries, the secretion rate and Na^+/K^+ ratio of the saliva was increased considerably above the control level for a period of 10–30 min (Fig. 4). The reduction of secretion rate occurred immediately upon occlusion; and if during occlusion one artery were temporarily released, there was a gush of 5–10 drops of secretion from the fistula within 10 sec. The post-occlusion period

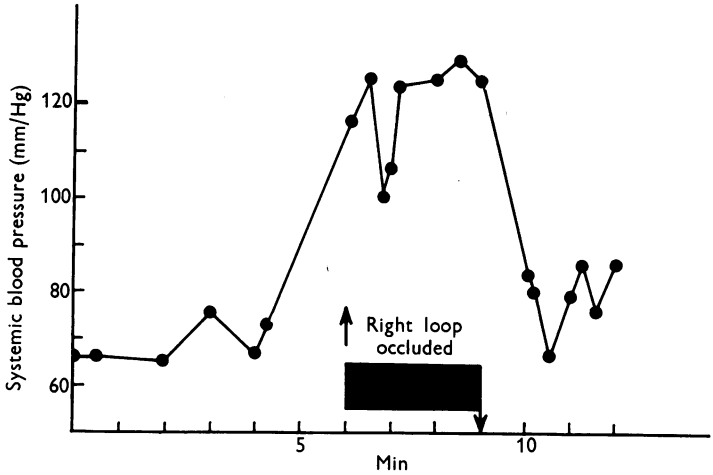

Fig. 3. The effect on systemic blood pressure measured in the left (denervated) carotid loop of occlusion of the right (innervated) loop. Sheep P.F. 7, Na^+-depleted.

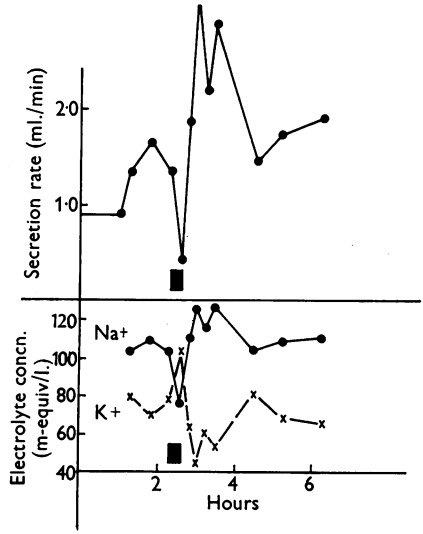

Fig. 4. The effect on parotid secretion rate and saliva composition of occlusion (at signal) of the contralateral (innervated) carotid loop and compression of the ipsilateral (denervated) loop by 80 mm Hg pressure: sheep P.F. 16, Na^+-depleted.

of increased secretion occurred if either artery alone were released. Occlusion of the left artery alone in either animal caused a slight increase in secretion rate.

Thus the blood supply to the parotid gland was reduced in a conscious animal. This reduced the salivary Na^+/K^+ ratio of a Na^+-depleted sheep but had little effect when Na^+ balance was normal.

The effect of raised blood pressure on parotid secretion during Na^+ *depletion*

The sheep (P.F. 7) was Na^+-depleted. When three control collections had established that the secretion rate of the left parotid gland was constant, the right carotid loop was completely occluded for 10–15 min. Saliva collection was continued during and after occlusion. The cardiac rate was observed throughout. It was found that occlusion of the right common carotid artery caused a definite increase of secretion rate and that this sometimes persisted

TABLE 3. The effect of carotid occlusion on the Na^+/K^+ ratio of parotid saliva in a Na^+-depleted sheep P.F. 7

Time (min)	Secretion rate (ml./min)	Ratio: Na^+/K^+	Cardiac rate (beats/min)
0–10	1·25	0·77	48
17	Right carotid loop (innervated) clamped		
24–34	2·20	0·98	50, 48, 48
36	Carotid loop unclamped		48
38–48	2·55	1·76	60, 54, 50
55	Left carotid loop (denervated) clamped		
56–66	1·85	1·75	48
72	Carotid loop unclamped		
73–83	1·30	1·52	48

for an hour or more after the cessation of occlusion. The persistence of the change was not greatly influenced by occlusion of the left carotid loop immediately following release of the right loop. This increase of secretion rate was associated with a clear-cut rise of salivary Na^+/K^+ ratio. Table 3 is typical of five such experiments. Occlusion of the right loop usually had little effect on secretion in P.F. 16, which had had the superior cervical ganglion removed on the side of the fistula.

The question arises from the experiments on short duration occlusion whether the increase of secretion rate was caused by the rise of systemic blood pressure (Fig. 3), or whether it was a consequence of increased blood flow through the gland. This latter possibility might be inferred from the fact that after occlusion of the right loop virtually all blood to the head would be carried by the left artery, apart from the right occipito-vertebral anastomosis, and the parotid gland vasculature might partake in a general vaso-dilatation consequent on the opening up of many anastomotic channels. Consequently, an experiment was made on the effect of right loop occlusion whilst an infusion of ACh was running into the left carotid loop. This experiment would be pertinent

to a third possible consideration, namely that the rise of systemic blood pressure, which was evoked by the right carotid sinus reflex, resulted from vasoconstriction which also involved the vasculature of the left parotid gland, and hence the secretory response to rise of blood pressure was not as great as would otherwise have been the case. In this instance, such an effect would be eliminated by the infusion of ACh into the left parotid gland vasculature.

It was found in three experiments that occlusion of the right loop during an ACh infusion into the left loop had virtually no effect on the salivary secretion rate or on the Na^+/K^+ ratio of the saliva (Fig. 5). Since the ACh infusion was running into the left loop it was not possible to measure directly that a

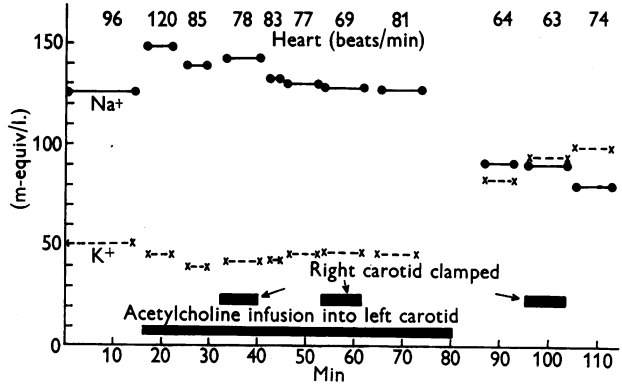

Fig. 5. The effect of intracarotid ACh infusion (lower signal) and carotid loop occlusion (upper signal) on saliva composition of sheep P.F. 7 in moderate Na^+ depletion; ●, Na; ×, K.

blood pressure rise occurred. However, in each instance when the right loop was occluded there was the usual decrease of cardiac rate, as can be seen from the figures of the average cardiac rate for each collection period at the top of Fig. 5. As such change of cardiac rate usually occurred when the blood pressure was measured and found to rise, it seems a likely assumption that the figures indicate that the blood pressure rose upon right loop occlusion in this instance.

It appeared desirable to determine the effect on salivary Na^+/K^+ ratio of occlusion of the right artery for a much longer period. Consequently, after a period in normal electrolyte balance, the $NaHCO_3$ supplement was withdrawn from P.F. 7 (Fig. 6) and the salivary Na^+/K^+ ratio was observed to fall progressively as the sheep became more depleted of Na^+. Between the 3rd and 4th day of negative balance the right loop was well covered with fine sheet rubber, and kept occluded for 24 hr. The occluding device was moved frequently during this period. Fig. 6 shows that this procedure made little or no difference to the slope of the curve of salivary Na^+ and K^+ concentrations. No measurements were made to determine whether the blood pressure remained elevated, but the diversion of flow would have been sustained. On the 7th day

of negative balance, 120 m-equiv $NaHCO_3$ were given by rumen tube, and the usual unequivocal parotid response to a change of external Na^+ balance occurred.

Occlusion of the right (innervated) carotid loop caused a transient rise of secretion rate and salivary Na^+/K^+ ratio during Na^+ depletion. The experiments did not define conclusively whether this was due to the rise of systemic blood pressure or increase of blood flow.

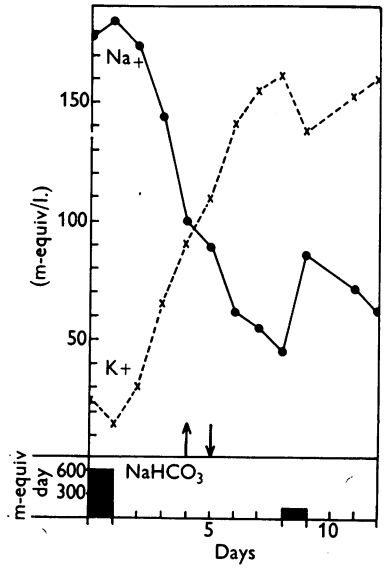

Fig. 6. The effect on salivary Na^+ and K^+ concentrations of 24 hr continuous occlusion (between arrows) of the right (innervated) carotid loop; sheep P.F. 7, progressive Na^+ depletion; ●, Na; × K: black columns show $NaHCO_3$ (m-equiv/day).

Intracarotid acetylcholine infusion

Normal sheep. In control experiments before Na^+ depletion was produced, ACh (2×10^{-5} in 5% glucose solution) was infused at 0·6 ml./min into the left (denervated) carotid loop. Secretion immediately increased from a basal rate of 1·0–1·5 to 6–8 ml./min. During the next 30–90 min, the rate gradually decreased to 3–5 ml./min, at which it remained for the duration of the infusion. This large and protected increase in secretion caused little or no change in the salivary Na^+/K^+ ratio. Occlusion of the innervated carotid loop on the opposite side (right) for 10–15 min during such infusions did not alter the Na^+/K^+ ratio.

Na^+-depleted sheep. Eleven experiments were made in which ACh was infused for periods of 1–11 hr. On the morning of the experiment the usual small supplement of 100 m-equiv $NaHCO_3$ was withheld. After 2–3 hr control collection of saliva, ACh infusion was begun and collection continued. A substantial increase in secretion occurred, together with evidence of

vasodilatation—reddening of the skin of the face and upper neck. The cardiac rate varied between 50 and 80/min and the animals were not disturbed.

Table 4, which summarizes the results of the eleven experiments, shows that in all instances ACh infusion increased the Na^+/K^+ ratio, and in most experiments the ratio was higher in the last collection during infusion than in the first. The changes produced correspond to a rise of 25–50 m-equiv in salivary Na^+ concentration, and a reciprocal fall in salivary K^+ concentration. However, it was clear from the infusions of a long duration that the Na^+/K^+ ratio did not change continuously but remained constant during the last 1–3 hr. The change produced in the ratio appeared to be related to the degree of Na^+ depletion, being greatest when Na^+ depletion was greatest.

TABLE 4. The effect of intracarotid acetylcholine infusion on salivary Na^+/K^+ ratio of Na^+-depleted sheep

Sheep	Duration of acetylcholine infusion (min)	Control Na^+/K^+ ratio (secretion rate, ml./min)	First collection after infusion started: Na^+/K^+ ratio (secretion rate, ml./min)	Last collection during infusion: Na^+/K^+ ratio (secretion rate, ml./min)
P.F. 7	12	0·76 (1·20)	1·77 (4·10)	2·30 (4·0)
	56	2·50 (1·45)	3·21 (4·20)	2·73 (2·0)
	195	0·66 (0·97)	1·05 (2·42)	1·98 (1·82)
	225	0·56 (0·95)	0·97 (3·48)	1·27 (1·82)
	235	0·27 (1·27)	1·30 (11·14)	1·16 (3·57)
	257	0·43 (1·04)	1·78 (6·0)	1·71 (3·26)
P.F. 16	682	0·13 (0·65)	0·64 (3·94)	1·34 (1·57)
	453	2·58 (1·42)	2·63 (3·19)	3·67 (2·0)
	372	1·18 (0·47)	1·87 (3·76)	1·95 (1·91)
	130	0·81 (1·25)	1·69 (4·23)	1·31 (2·87)
	220	0·26 (0·60)	2·69 (5·94)	1·82 (4·67)

In these experiments, it could be reasoned that the infusion, by causing a large increase in secretion and hence further depleting the animal of Na^+, to some extent negated its own effect on the salivary Na^+/K^+ ratio. Accordingly in one experiment, on a sheep grossly depleted of Na^+, after 6 hr ACh infusion 4 M-NaCl was infused in addition for 70 min at 0·31 ml./min (Table 5). Thus 22 ml. containing 88 m-equiv Na^+ were given, corresponding almost exactly to the Na^+ lost in the 1062 ml. saliva secreted during the previous 6 hr. A small rise in salivary Na^+ concentration occurred but this never reached 100 m-equiv. The salivary Na^+/K^+ ratio was virtually unchanged during the last 2 hr of the ACh infusion.

Secretion rate and saliva composition in Na^+ *depletion*

Analysis of saliva collected from Na^+-depleted sheep during the course of days which included reduction of secretion by experiments already described, and periods of rumination and psychic stimulation when salivation was increased, indicated the existence of a relation between secretion rate and composition (Fig. 7). The concentration of Na^+ rose, and that of K^+ fell, in

TABLE 5. The effect of ipsilateral intracarotid infusion of acetylcholine and NaCl solutions on the salivary Na^+/K^+ ratio of Na^+-depleted sheep P.F. 16

Time (min)	Secretion (ml./min)	Ratio: Na^+/K^+
0– 37	0·65	0·13
39	Started ACh infusion* 1 ml./min	
39– 99	3·94	0·64
99–154	3·18	0·91
154–214	2·95	0·95
214–274	2·95	0·93
274–334	0·50	0·99
334–394	2·17	1·25
400	Started 4 M-NaCl infusion, 0·31 ml./min; ACh infusion continued	
400–430	2·20	1·30
430–470	2·08	1·47
470	NaCl stopped (total infused 22 ml. = 88 m-equiv Na^+); ACh infusion continued	
470–500	1·77	1·53
500–527	1·70	1·57
537–562	0·56†	1·43
562–596	1·09†	1·15
596–626	1·83	1·35
626–656	1·73	1·48
656–686	1·80	1·42
686–716	1·57	1·34
717	ACh infusion stopped	
717–744	0·66	1·30
762–792	0·33	0·46

* 2×10^{-5} in 5% glucose.

† Needle temporarily out of artery.

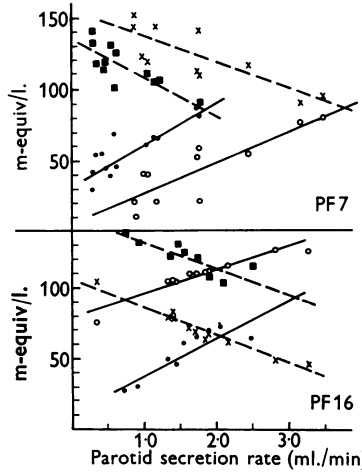

Fig. 7. The relation between salivary secretion rate and Na^+ and K^+ concentrations of Na^+-depleted sheep. P.F. 7; 9 days' depletion, Na^+ ○, K^+ ×; 16 days' depletion (small Na^+ supplement given between 9th and 16th days), Na^+ ●, K^+ ■. P.F. 16; 9 days' depletion, Na^+ ●, K^+ ■; 4 days' depletion, Na^+ ○, K^+ ×.

apparently linear fashion ($P < 0{\cdot}01$ in all instances) with increasing rate of secretion over the range observed (approx. 0·5–3·0 ml./min). As can be seen in Fig. 7, the greater the degree of Na^+ depletion, the further along the secretion-rate axis the Na^+ and K^+ concentration lines intersect and, as will be indicated in a subsequent publication, this arbitrarily chosen point probably represents a measure of the circulating humoral influence evoked by Na^+ depletion. However, as indicated by the data of Table 4, greater stimulation of secretion by intracarotid ACh infusion, even where rates of more than 5 ml./min were observed, did not cause the Na^+/K^+ ratio to rise to a value predicted by extrapolation of the lines shown in Fig. 7. In other words, these lines must really be curvilinear, tending to become parallel with the abscissa at higher secretion rates. Fig. 8 shows this effect in P.F. 7.

Thus stimulation of secretion rate in a Na^+-depleted sheep caused the salivary Na^+/K^+ ratio to rise, but in no instance did it cause the ratio to approach normal or anywhere near this.

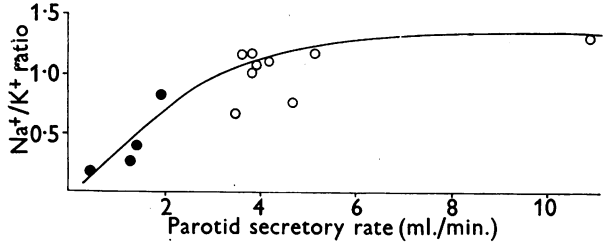

Fig. 8. P.F. 7, Na^+-depleted: relation between salivary Na^+/K^+ ratio and secretion rate, during maximal secretomotor stimulation of the parotid by intracarotid ACh; ●, control; ○, during ACh infusion.

DISCUSSION

The experimental procedures aimed at altering the secretion rate and blood supply of the parotid gland. It seemed important to interpretation that this was done in conscious, trained animals which were undisturbed by the procedures (Pavlov, 1897; Verney, 1947). Whereas this variation caused little change in salivary Na^+/K^+ ratio of a sheep in normal Na^+ balance, it had a significant effect on the salivary Na^+/K^+ ratio of a Na^+-depleted sheep. Over a limited range of secretion rate there was a linear relation between secretion rate and salivary Na^+/K^+ ratio. The experiments did not differentiate whether this 'local factor', involved in the fall in Na^+/K^+ ratio in Na^+ depletion, was the secretomotor mechanism itself, the parotid blood flow, or the amount of Na^+ presented to the secretory cells. However, in relation to interpretation of the water-depletion experiments (see p. 227) in so far as they bear on the Na^+ load to the parotid cells, ACh stimulation adequate to cause secretion rates of 4–10 ml./min probably increased the blood flow, and hence

the Na^+ load, very considerably. This was very likely in the experiment in which 4 M-NaCl was infused during the ACh infusion.

Therefore, considered in conjunction with the effect of the carotid sinus reflex, the Na^+-depleted sheep were given sustained periods during which the blood pressure, parotid blood flow and Na^+ load to secreting cells were probably considerably greater than those usually pertaining under basal conditions, when the animal was in normal Na^+ balance and secreting saliva of normal Na^+/K^+ ratio. However, in no instance did this large haemodynamic change cause the salivary Na^+/K^+ ratio to change to normal or approach anywhere near normal (Table 4 and Fig. 7). It seems that the change in salivary Na^+/K^+ ratio seen in Na^+ depletion is not a simple and direct consequence of a reduction in the amount of Na^+ per unit time presented to the secretory cells. This contention is further supported by the results of McDonald & Denton (1956) when, during ACh infusion, 4 M-NaCl was infused at 1·6 ml./min and the Na^+ concentration of the blood perfusing the parotid gland was found to rise by 18 m-equiv/l.: during this 4 M-NaCl infusion there was little change in the salivary Na^+/K^+ ratio.

Whereas interpretation of the above experiments has involved the likely assumption that a large measured increase of secretion rate was associated with an increased parotid blood flow (Langley, 1898; Coats *et al.* 1956), in the case of the experiments on carotid loop occlusion it was determined directly that a large reduction of cranial blood pressure did occur. The close relation over very short time intervals of secretion rate and the pressure of the ipsilateral occluding cuff suggested that the reduction of secretion rate was directly caused by the reduced blood flow rather than was it secondary to reduction of parasympathetic motor impulses to the gland. It might be argued in the case of P.F. 7 that the reduction of secretion was caused by a sympathetic effect secondary to the reduced cerebral blood pressure and blood flow. However, no gush of saliva characteristic of sympathetic stimulation of the parotid gland was seen when the occlusion was first made, and clearly in P.F. 16 (Fig. 4) this possibility was eliminated.

The capacity of the sheep to withstand bilateral carotid occlusion is probably a consequence of the large occipito-vertebral anastomoses which are easily demonstrable by dissection. The diminished ability to do so during Na^+ depletion was interesting collateral evidence of the circulatory effects of negative external Na^+ balance of 500–900 m-equiv (Denton 1956*b*). On the evidence available, it cannot be determined whether the inferior ability of P.F. 16 to withstand bilateral occlusion was a matter of individual variation or contingent on the extirpation of the left superior cervical ganglion and ligation of the left occipital artery. The fall of blood pressure which occurred during Na^+ depletion was probably significant, though not large.

There is also some indirect evidence on the effect of reduction of glandular

blood flow in a Na^+-depleted sheep. It has been shown that after an initial gush of saliva, protracted stimulation of the cervical sympathetic reduces parotid secretion rate and blood flow (Coats *et al.* 1956). As already indicated, removing a sheep from its cage and securing it on its side upon a table resulted in a very great reduction of salivary flow: secretion sometimes ceased completely for 10–30 min. This effect, which caused a large reduction of Na^+/K^+ ratio of the saliva of a Na^+-depleted sheep but little change when the sheep was in normal Na^+ balance, was immediately reversed by intracarotid infusion of ACh. The effect was greatly reduced if the ipsilateral superior cervical ganglion were removed, or if the animal were trained by frequent repetition of the procedure. It does, however, occur in a bilaterally adrenalectomized sheep (Denton, unpublished). Whereas a Na^+-depleted sheep, while in the cage, secreted saliva at 1·0 ml./min with Na^+/K^+ ratio of $80/120 = 0{\cdot}6$, upon being placed on the table the secretion dropped to 0·1 ml./min, and the composition changed to a Na^+/K^+ ratio of $10/190 = 0{\cdot}05$. This effect was mediated via the cervical sympathetic, and the reduction of saliva flow and Na^+/K^+ ratio was probably associated with a reduction of glandular blood flow (Coats *et al.* 1956).

The experiments here, and similar observations on P.F. 17, indicated that division of the carotid sinus nerve, and division of the ipsilateral cervical sympathetic and removal of the superior cervical ganglion, had no influence on the response of the parotid gland to Na^+ depletion.

In conclusion, while these experiments do not reveal the specific mechanism, they show that a local glandular factor is involved in the parotid response to Na^+ depletion. Like the secretion of the adrenal cortex (Goding & Denton, 1956), it is a contributory condition in a set of conditions jointly sufficient and severally necessary for the response to occur.

SUMMARY

1. A carotid sinus reflex has been demonstrated in the sheep.

2. Sheep with permanent unilateral parotid fistulae were depleted of large amounts of Na^+ by withholding the daily Na^+ supplement. The characteristic fall of the salivary Na^+/K^+ ratio caused by Na^+ depletion was unaffected by division of the ipsilateral carotid sinus nerve, the ipsilateral cervical sympathetic, and removal of the ipsilateral superior cervical ganglion.

3. Na^+ depletion caused a fall of systemic blood pressure.

4. Infusion of ACh into the carotid loop on the same side as the parotid fistula caused the salivary secretion rate to rise to 2–11 ml./min. This did not affect the salivary Na^+/K^+ ratio of a sheep in normal Na^+ balance, but caused a significant though comparatively small rise in the salivary Na^+/K^+ of a Na^+-depleted sheep. The increase produced was a function of the degree of

Na^+ depletion existing, being greatest when the sheep was most depleted of Na^+. The salivary Na^+/K^+ ratio remained far below normal.

5. A rise of systemic blood pressure of 10–50 mm Hg was produced in normal and in Na^+-depleted sheep by complete occlusion of the contralateral (innervated) carotid artery. This caused an increase of secretion rate and salivary Na^+/K^+ ratio of a Na^+-depleted sheep, but when it was done during ipsilateral intracarotid ACh infusion no change occurred further than that caused by the infusion.

6. The blood pressure in the ipsilateral (denervated) carotid loop of a Na^+-depleted sheep was measured directly by inserting into the artery a needle connected to a mercury manometer. Complete occlusion of the contralateral (innervated) carotid loop, and compression of the ipsilateral loop caudal to the needle by a cuff inflated to 80–120 mm Hg, caused the mean blood pressure measured at the needle to fall from 80–90 to 33–40 mm Hg. This reduction of blood flow to the head was associated with a large decrease of salivary secretion rate and of the Na^+/K^+ ratio. Release of either loop caused the blood pressure, secretion rate, and salivary Na^+/K^+ ratio to rise again rapidly.

7. An apparently linear relation between secretion rate and salivary Na^+ and K^+ concentrations of Na^+-depleted sheep was shown over a secretion range of 0·1–3·0 ml./min. However, this line became parallel to the secretion-rate axis when secretion was greatly stimulated by intracarotid ACh infusion.

8. These experiments indicated that a local factor is involved in the change in salivary Na^+/K^+ ratio which occurs during Na^+ depletion. The results do not indicate the precise nature of this factor. The major cause of this striking parotid behaviour in response to Na^+ depletion lies elsewhere.

It is a pleasure to thank Dr I. R. McDonald and Professor R. D. Wright for their helpful advice on the experiments and the manuscript. Three results in Table 4 were obtained during collaboration on Dr McDonald's investigation of the delayed response of the parotid gland to ipsilateral intracarotid infusion of 4 M-NaCl. I also wish to thank Dr D. A. Coats for administering the anaesthetics and Miss Magda Reich and Mr B. Dyzenhaus for valuable assistance.

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