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SECRETION BY THE PAROTID GLAND OF THE SHEEP

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Secretion by the parotid gland of the sheep has been studied in acute preparations and by means of fistulae. The sheep is unusual in having a continuous flow of parotid saliva which increases in amount both with feeding and with cud chewing (Ellenberger & Hofmeister, 1887). As in the ox its parasympathetic nerve supply arrives via the buccal branch of the mandibular nerve as fine branches which go from the anterior border of the masseter muscle with the duct to the gland (Moussu, 1888, 1890), not via the auriculo-temporal nerve. Moussu demonstrated that after section of this nerve the gland continued to secrete and he attributed this continued secretion to a non-nervous mechanism postulated by Colin (1886). Eckhard (1893) who in 1867 (Eckhard, 1867), before Moussu's discovery, had convinced himself that there was no secretomotor nerve to the gland, held the view that it was due to the 'nature' of the gland.

Eckhard (1893), after amplifying these observations and confirming Moussu's observation that stimulating the buccal branch of the mandibular nerve (henceforth referred to as Moussu's nerve) increased secretion, nevertheless stated that section of the nerve did not cause any reduction in the rapidity of secretion by the gland. This view has not been refuted, and is often quoted with the reservation that it requires confirmation (Langley, 1898, in Schafer's Physiology text-book; Babkin, 1944). It is associated with statements that salivary secretion in the ruminant is not stopped by atropine. However, the reduction by atropine of normal secretion to a lower rate is recognized by Babichev, Perstnov & Kulesco (1930) as indicating a biphasic secretomotor mechanism. In this paper we have endeavoured to establish with certainty the effect of the parasympathetic nerve on the gland.

Another controversial matter is the effect of stimulation of the sympathetic fibres to the parotid upon its secretory activity. Eckhard (1869) attacked the

presentation by Wittich (1867) of incomplete evidence that 'die ja auch von Eckhard beobachtete anfängliche Beschleunigung des Speichelausflusses nach Reizung des Nerven entspricht in Wahrheit einer Steigerung des Secretionsdruckes *und* einer Mehrung des Secrets'. Eckhard denied that secretion increases. The source of the controversy, as far as can be judged from their descriptions of surgical procedure, was that, both being ignorant of the route of the parasympathetic nerve supply, Wittich preserved it and Eckhard divided it. Eckhard's (1869) report of low maximal secretory pressure in the sheep's parotid has not been further investigated.

Because we were using parotid fistulae in sheep to produce a long-term subtraction of Na^+ from the body it was of interest to try to clear up these points, by means of the experiments reported below.

METHODS

The sheep used in the thirty-two acute experiments from which the data in this paper were produced were either Merinos or Merino-crossbreds, except on four occasions when Suffolks were used.

Anaesthesia was induced by intravenous pentothal (0·3–0·5 g) and continued after tracheotomy with closed-circuit cyclopropane and oxygen. The animal remained in good condition for 6–8 hr as shown by pulse, respiration, temperature and blood pressure recording. It was occasionally necessary to aspirate the rumen per cannulam because of accumulation of fermentation gases.

The duct of the gland was dissected either just before its entry into the mouth or near its exit from the gland, and a polythene cannula tied into it. When secretomotor effects were sought, the buccal branch of the mandibular nerve was found by cutting the masseter tendon 4 cm above the lower edge of the mandible, retracting the masseter muscle and dissecting the nerve in the loose areolar tissue on the under surface of this muscle.

The cervical sympathetic trunk was found in some animals as a nerve separate from the vagus but when the two nerves occurred as one nerve trunk, dissection was continued caudally until they were found separate and then sufficient length for stimulation was secured.

Vascular isolation of the gland will be described more fully in another paper, but in essence it consisted in undercutting the gland, double tying all vessels irrelevant to the experiment and cutting between these ties.

Nerves were stimulated through platinum electrodes by using a potential divider on the output of a commercial Bell transformer (50 cycles a.c. – 0 to 6 V).

Prolonged injections of pharmacological agents were carried out either by hand, by a motor-driven syringe calibrated against pressure equivalent to arterial pressure, or by a mercury pump modified to give a measurement of the amount injected.

Salivary flow was recorded in almost all instances by collecting the saliva in graduated cylinders.

The adrenaline and noradrenaline used were manufactured by Burroughs Wellcome and Stearns respectively.

RESULTS

(I) *Parasympathetic nerve supply*

(A) *Continuous secretion*

The flow of saliva was studied in sixteen anaesthetized animals. The average results were: right side 0·88 ml./min; left side 0·64 ml./min; t value = 0·73, i.e. no significant difference. After checking that the two sides were secreting at approximately the same rate, the motor nerve (Moussu's nerve) was sectioned

on one side on eleven occasions and a collection made. Average results: uncut side 1·33 ml./min; cut side 0·35 ml./min; $t = 4{\cdot}08$, so that with 10 degrees of freedom $0{\cdot}001 < P < 0{\cdot}01$. From these results it is clear that the secretion is partly due to secretomotor nervous influences (Fig. 1), but there is a residual secretion which was observed to continue for up to 10 hr under anaesthesia.

This secretion continued after section of the cervical sympathetic, facial and auriculotemporal nerves. The rate of secretion of the recently denervated gland was not altered by large doses of atropine (4 mg) given into the carotid artery supplying the gland.

In order to determine whether this continuous secretion was due to a mechanism proceeding from the abdominal viscera, two sheep were eviscerated, i.e. the abdominal alimentary canal, the spleen and pancreas were removed. Despite the operative trauma and lower blood pressure (45 mm Hg rising to 75 mm Hg), secretion from the gland was still going on at the basal rate 2 hr. later.

In view of the preceding results, in each of two sheep a gland was isolated from the circulatory system except for the carotid artery and external jugular vein. The gland was excised and then transferred to a Dale-Schuster pump delivering Ringer–Tyrode solution with 5% Dextran in one case, and Ringer–Tyrode solution with 5% Dextran and 10% sheep's red blood cells in the other. The liquid was equilibrated with 95% O_2 and 5% CO_2. On both occasions, despite a gradual falling-off of perfusion volume per minute, the secretion continued for a period of 2 hr and the composition remained typical of sheep's parotid saliva. Emmelin (1953) has shown a similar result with the sublingual glands of cats. From these experiments there is no result contrary to Eckhard's (1893) conclusion that the continuous secretion of the denervated gland is not excited by outside influences, but is due to the 'nature' of the gland.

(B) *Parasympathetic stimulation*

Stimulation of Moussu's nerve to undisturbed glands was carried out on twelve occasions. During stimulation the secretion was greatly increased in amount. Means: unstimulated, 0·88 ml./min; stimulated, 5·23 ml./min; $t = 6{\cdot}52$ with 11 D.F.; $P < 0{\cdot}001$ (Fig. 1). The latent interval was very short (1–2 sec) and the rapidity of secretion gradually decreased despite continuous moving of the electrodes to prevent polarization (Fig. 2). This procedure was carried out with vascularly isolated glands on twelve other sheep with a similar result.

Acetylcholine injected directly into the artery gave an increase in amount of secretion of the same order as stimulation of Moussu's nerve. Atropine (4 mg) given over 2 min intra-arterially completely prevented the secretomotor effect, in contrast to its complete failure to influence the residual

secretion. Pilocarpine (10 mg in 2 min given intra-arterially) caused a great and prolonged increase in secretion from the gland (from 0·4 to 2·6 ml./min) and this was inhibited by atropine. Eserine intra-arterially (0·5 mg) may increase secretion from the denervated gland (Feldberg & Guimarais, 1935), but did not increase the effect of maximal secretomotor stimulation.

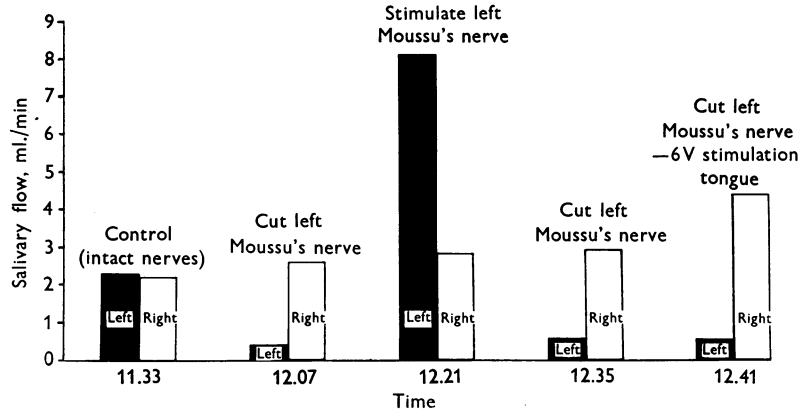

Fig. 1. The salivary minute volume from the right and left parotid glands: (*a*) with nerves intact; (*b*) after cutting Moussu's nerve on the left side; (*c*) during stimulation of Moussu's nerve on the left side; (*d*) control (left Moussu's nerve cut); (*e*) stimulating back of tongue (6 V).

(II) *Cervical sympathetic nerve*

(A) *Effect of cervical sympathetic stimulation on motor nerve response*

If, during secretomotor stimulation, the peripheral end of the cervical sympathetic trunk was stimulated, there was a marked reduction in the flow of saliva (Czermak, 1857). On cessation of sympathetic stimulation there was, after a latent interval of 15–30 sec, a return to the level expected at that time of stimulation (Fig. 2). If secretomotor stimulation was started immediately after stopping stimulation of the cervical sympathetic to a quiescent gland this 'after discharge' effect was well demonstrated as a reduction in the amount secreted in the first half minute (Fig. 3). Noradrenaline (4×10^{-4}) injected at 0·5 ml./min into the ipsilateral carotid artery caused a similar effect in two animals. There was an irreversible inhibition in another sheep: 2 hr later not even basal flow was occurring and neither secretomotor stimulation nor intra-arterial acetylcholine produced any increase in flow. Adrenaline in the same dose also gave reversible reduction in flow on two occasions, but on one occasion gave no effect in an animal responsive to noradrenaline.

It was important to determine whether the effects of noradrenaline and of stimulation of the cervical sympathetic were due to a direct action on the secretory cells or were due solely to a vascular effect, and the following experiment was therefore carried out. All tributaries of the external jugular vein

except those coming from the gland were cut and the carotid branches above the digastric muscle were divided leaving only the branches to the gland, i.e. the superficial temporal artery, posterior auricular artery, transverse facial

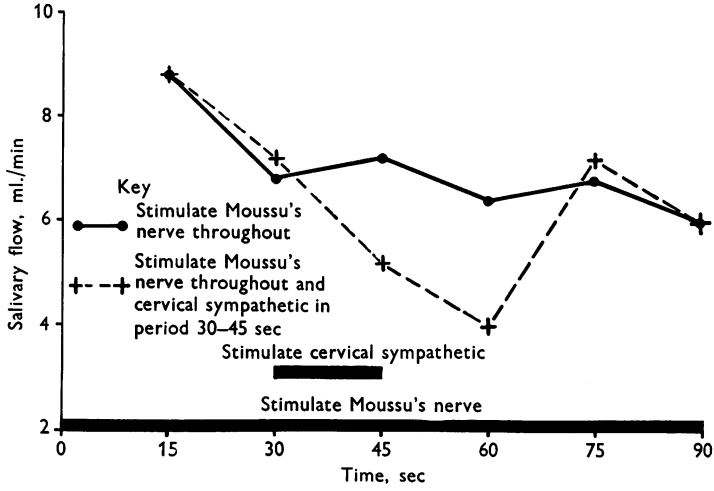

Fig. 2. Solid curve—gradual decrease in the minute volume of saliva during prolonged stimulation of Moussu's nerve. Broken curve—effect of concurrent cervical sympathetic stimulation during the course of Moussu's nerve stimulation.

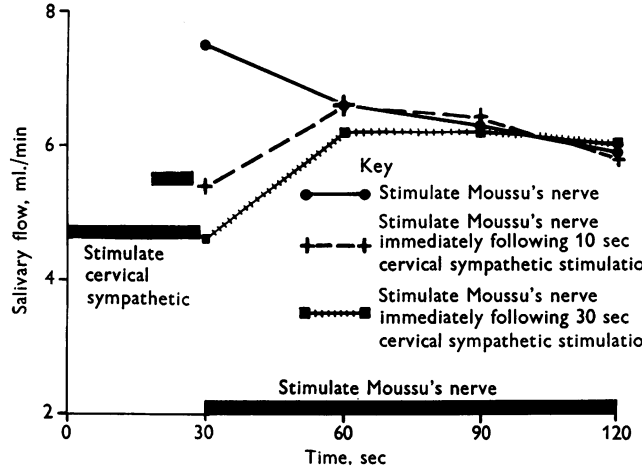

Fig. 3. The effect of immediately preceding cervical sympathetic stimulation upon the salivary flow response to Moussu's nerve stimulation.

artery at the anterior border of the gland, and internal maxillary arteries were divided. Moussu's nerve and the cervical sympathetic were prepared for stimulation. The stump of the main facial vein (a large vein in the sheep) was

then cannulated and the mouth of the cannula was passed through the bifurcatory valve into the external jugular vein thus making a T tube out of the veins. A resilient polythene tube was put on the cannula and clipped. The animal was then given 10,000 units of heparin intravenously. To measure the venous outflow from the gland a clip was placed on the jugular vein just distal to the cannula and the polythene tube was released; the flow was measured against time.

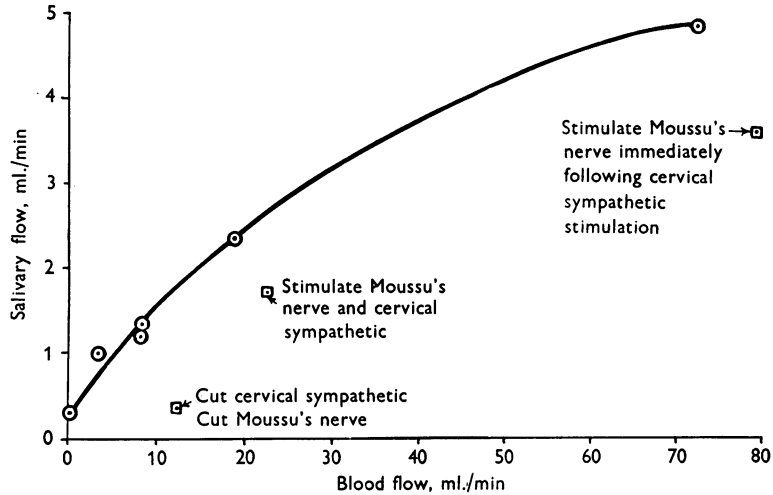

Fig. 4. The relationship between salivary flow induced by Moussu's nerve stimulation and blood flow when the latter is reduced by graded constriction of the arterial supply to the gland. Neither basal salivary flow nor reduced salivary secretion induced by simultaneous or immediately preceding cervical sympathetic stimulation fall on this curve.

In order to determine the relationship between maximal secretomotor flow and blood flow, a bulldog clip with an adjustable stop was used to produce a graded constriction of the carotid artery just proximal to the vascularly isolated gland. Then with a mercury manometer recording the blood pressure in the arterial supply to the gland, the stop was adjusted to give a number of stages in reduction of blood flow. Fig. 4 illustrates the effect of this mechanical adjustment of blood flow upon maximal secretomotor effect (stimulation of Moussu's nerve). By comparing blood and salivary flows on a gland during simultaneous stimulation of Moussu's nerve and the sympathetic, it was possible to see whether the blood flow/secretory flow ratio fell on the curve. In the experiments shown in Fig. 4 it falls below the 'mechanical' value.

In two other similar experiments the points on the blood-salivary flow curve were more scattered and the point for stimulation of the sympathetic fell within the scatter.

On another occasion the sympathetic was first stimulated concurrently with Moussu's nerve, and measurements made of blood and salivary flows. Then with stimulation of Moussu's nerve proceeding, the clamp on the artery was adjusted to give the same blood flow as during stimulation of the sympathetic, and the resultant salivary flow observed (Table 1).

With blood flows of 4·5 and 4·75 ml./min respectively the salivary flows were 1·0 ml./min for sympathetic stimulation and 0·65 ml./min for mechanical restriction of blood flow.

TABLE 1. Comparison of effects of sympathetic stimulation and of mechanical restriction of blood flow on maximum rate of salivary secretion

Time	Procedure	Blood flow (ml./min)	Saliva (ml./min)
1508	Gland unstimulated	11	0·25
1526	Moussu's nerve stimulated	42	3·7
1542	Moussu's nerve stimulated + sympathetic stimulation (5 min)	4·5	1·0
1611	Artery constricted with Moussu's nerve stimulated	4·75	0·65
1627	Moussu's nerve stimulated	21	2·0

TABLE 2. Comparison of effects of noradrenaline and of mechanical restriction of blood flow on maximum rate of salivary secretion

Time	Duration (min)	Procedure	B.P. in gland field (mm Hg)	Blood flow (ml./min)	Salivary flow (ml./min)
1551	1½	Moussu's nerve stimulation	72	58	2·0
1552½	2½	Moussu's nerve stimulation + noradrenaline 1 ml./min (1:50,000)	87–108	44	0·48
1555	3	Moussu's nerve stimulation (immediately after noradrenaline)	80–60	48	0·27
1631	1	Moussu's nerve stimulation	—	—	5·0
1641	3	Moussu's nerve stimulation-constricted artery	40–20	5	2·1
1650	2	Moussu's nerve stimulation-constricted artery	70	31	3·8
1659	2	Moussu's nerve stimulation-constricted artery	40	18	2·5
1714	2	Moussu's nerve stimulation	46	52	3·0

In a similar experiment, a comparison was made of the effects on salivary flow of intra-arterial injection of noradrenaline and mechanical restriction of blood flow. Noradrenaline caused a restriction of salivary flow out of all proportion to the degree of vascular constriction (Table 2).

These results indicate that while there is a clear-cut difference between the effects on salivary flow of mechanical restriction of blood flow and injection of noradrenaline, there is no significant disparity in the case of sympathetic stimulation. It seems probable that the effect of sympathetic stimulation is due simply to vasoconstriction (Heidenhain, 1868; Langley, 1878).

(B) *Effect of cervical sympathetic stimulation on continuous salivary flow from the denervated gland*

(i) *Stimulation of short duration.* On stimulating the peripheral portion of the ipsilateral cervical sympathetic trunk in an animal with Moussu's nerve cut there was, after 3–4 sec, a rapid transient increase in the flow of saliva from the cannula (8–45 drops). The response lasted from 10 to 40 sec, and it was proportional to the time elapsing since a previous stimulation. In an instance where sympathetic stimulation produced a gush of twenty drops, 2 min delay was required for a maximal and half a minute for a minimal subsequent response. The effect was associated with a decrease of blood flow through the gland. In one instance four control blood-flow readings were 11·5, 11, 11 and 10·5 ml./min respectively. During the first minute of stimulation the blood flow fell to 2·5 ml./min. Generally the first cervical sympathetic stimulation produced approximately ten more drops than on subsequent occasions when each sympathetic stimulation produced a remarkably constant number of drops. The effect of cervical sympathetic stimulation with Moussu's nerve cut was not changed by intra-carotid administration of eserine (cf. Secker, 1936). Gentle irrigation of the duct system for 3 min with 1 mg of dihydroergotamine in 2 ml. of saliva greatly diminished the effect, whereas irrigation with the same volume of saliva had no effect.

There was, however, no increase in the total amount of saliva collected over a 30 min period with stimulation for 20 sec of every 2 min; on three occasions with different sheep the slowing of flow following each stimulation almost exactly compensated for the transient increase, e.g. control, 11·1 ml.; stimulation, 11·9 ml.; control, 12·5 ml. The effect of sympathetic stimulation in this experiment was shown by the amount of saliva collected during stimulation, 5·9 ml., during a total period of 300 sec, and that collected in the intervals, 6 ml., during 1500 sec. Fig. 5 presents the data of an experiment of this type as a cumulative drop record. A similar gush of drops was produced when either 1 ml. of noradrenaline 1/40,000 or 1 ml. of adrenaline 1/40,000 was injected intra-arterially.

This effect might be due to contraction of the ducts, of the acini, or of vessels 'compressing' the gland; or it might be due to an actual change in the secretomotor mechanism.

In order to see whether it was due to contraction of the extra-glandular duct, the cannula was connected to a vertical 3 mm bore glass tube and with the column at 15 cm of saliva a rapid rise and fall of 10 cm in the column occurred on stimulating the sympathetic nerve. After 2 min rest a finger was applied firmly to the skin so as to block the duct at its exit from the gland. On stimulating the cervical sympathetic not a trace of the original effect was now

observed. This was also the case when noradrenaline or adrenaline was injected into the carotid artery.

In order to investigate the possibility of the effect being due to a vascular change, the isolated artery to the gland was clamped and the venous outflow observed. When blood flow had stopped, the sympathetic was stimulated with a resulting gush of saliva but no outflow of blood from the vein. This experiment was repeated after compression of the gland and duct so as to expel as much saliva as possible before cervical sympathetic stimulation. There was a gush of four to six drops of saliva but no outflow from the vein. The venous outflow from a resting gland without arterial compression did not show any unequivocal transient increase when the sympathetic was stimulated.

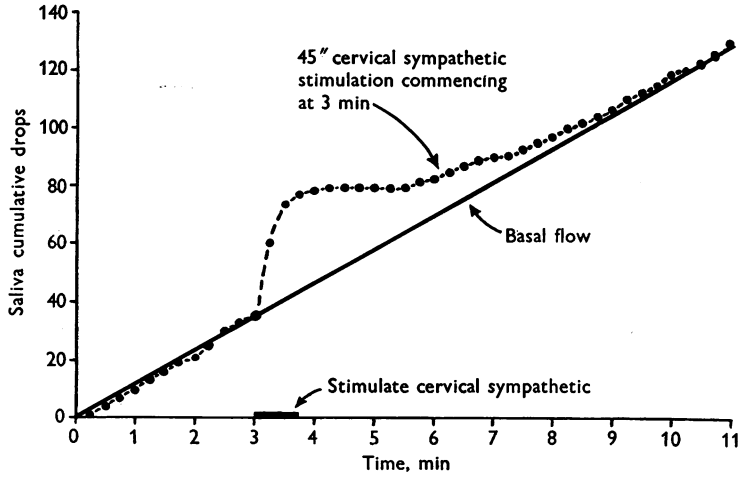

Fig. 5. The effect of cervical sympathetic stimulation upon the basal flow of saliva in a gland with Moussu's nerve divided. The gush of saliva obtained on sympathetic stimulation is compensated for in the subsequent pause.

In order to examine whether sympathetic stimulation influenced the character of the secretion, experiments were designed to discover whether the saliva appearing during stimulation differed in composition from that obtained without stimulation.

After cutting Moussu's nerve to an otherwise undisturbed gland, a short cannula was tied into the duct at its exit from the gland. The gland and duct were then 'milked' and the number of drops of saliva which could be obtained was observed. In one instance, five drops could be obtained by squeezing the gland, and the cannula 'dead space' was another two drops. Then six test-tubes, into each of which 10 ml. of distilled water had been pipetted, were made ready. Two drops of saliva were allowed to fall into the first as a control, and then the cervical sympathetic was stimulated for one minute. During this

time thirty-five drops were ejected at a greater than basal frequency. There was the usual post-stimulation compensatory pause so that the end result was that fifty-three drops fell in 5 min 10 sec. The drops 20 and 21, 30 and 31, 34 and 35, 40 and 41, and 46 and 47 were collected into the test-tubes. These solutions were analysed by flame photometry to see if there was any change in $Na^+:K^+$ ratio (Table 3).

A second experiment, also designed to permit comparison of the composition of basal saliva and the saliva produced by sympathetic stimulation, was performed. Basal saliva was collected in a beaker for analysis. The cervical sympathetic was then stimulated for 30 sec and saliva obtained from the cannula during this 30 sec and for $4\frac{1}{2}$ min subsequently was collected in

TABLE 3. Na and K concentrations in saliva collected before, during and after sympathetic stimulation. The ratios Na/K are accurate, but the absolute values are only approximate due to the technique of collection.

	m-equiv/l.		
Episode	Na^+	K^+	Ratio: Na/K
(1) Control	153	5·2	29·5
(2) Drops 20 and 21	151	6·6	22·9
(3) Drops 30 and 31	152	7·3	20·8
(4) Drops 34 and 35	147	8·7	16·9
(5) Drops 40 and 41	98	18·5	5·3
(6) Drops 46 and 47	106	22·5	4·7

TABLE 4. Na and K concentrations in saliva before, during and after sympathetic stimulation

	m-equiv/l.		
Episode	Na^+	K^+	Ratio: Na/K
(1) Pre-stimulation	185	3·8	48·7
(2) Drops 1–15	179	4·6	38·9
(3) Drops 16–25	182	5·0	36·4
(4) Drops 26–30	188	6·3	29·8
(5) Drops 31–35	189	12·5	15·1
(6) Drops 36–40	177	15·9	11·1
(7) Remainder	170	7·5	22·7
(8) Post-stimulation	182	3·8	47·9

several portions. Drops 1–15, 16–25, 26–30, 31–35, 36–40, and the remainder (to the end of the 5 min period) were collected separately. This whole procedure was performed nine times in order that the various volumes should be adequate for analysis. Fifteen minutes after the end of the experiment a further control collection of basal saliva was made. The results of the analysis are shown in Table 4. There was a remarkable constancy in the number of drops produced in each 'gush' during sympathetic stimulation (33, 31, 30, 31, 29, 30, 30, 31 and 29), and in each total 5 min period (50, 52, 48, 52, 51, 50, 51, 54 and 52).

These two experiments show a rise of K^+, relative to Na^+, in the saliva collected during sympathetic stimulation, and a clear-cut rise of K^+, and a fall

of Na^+, concentration in the saliva occupying the duct system at the cessation of stimulation. The results also suggest that the volume of the duct system or 'dead space' was greater than that indicated by the milking procedure.

In another three sheep the sympathetic was stimulated and the number of drops estimated to be in the dead space was collected in one container. Then the remainder of the drops arising during sympathetic stimulation was collected in another container. Any drops appearing between the end of this collection and the time for repetition of stimulation were collected in the first container, and this procedure was repeated until sufficient material was obtained for analysis of Na^+, K^+ PO^{2-}, i.e. approx. 4 ml. The results were:

		Na^+	K^+	Na/K
Experiment I	Interval	157	27·1	5·8
	Stimulation	177	13·2	13·4
Experiment II	Interval	154	36·8	4·2
	Stimulation	172	25	6·8
Experiment III	Interval	145	38·1	3·8
	Stimulation	157	27·1	5·8

These results are consistent with the findings shown in Tables 3 and 4.

The possibility that these differences were the result of diminished blood flow due to vasoconstriction was tested by producing comparable reduction of blood flow by constriction of the artery. The result was

Experiment	Saliva flow (ml./min)	Blood flow (ml./min)	m-equiv/litre Na^+	m-equiv/litre K^+	Ratio Na/K
Control (15 min)	0·27	22	156	22·4	7·0
Clamped artery (10 min)	0·15	2·5	149	18·7	8·0

Thus during mechanical arterial constriction, the ionic change was not the same as that during sympathetic stimulation.

The question of contraction of intra-glandular ducts remained. For this investigation a vertical glass tube was attached to the cannula and the rise due to basal secretion from a gland with Moussu's nerve cut was plotted against time. Then the pressure was reduced to atmospheric and as the column rose stimulation of the cervical sympathetic was started. The effect is seen in Figs. 6 and 7. A sharp initial rise was maintained during stimulation of the sympathetic and was followed by a fall to approximate base-line, followed by a secondary slower rise. Then after a period of flattening the column returned to its former rate of rise. It seems likely that the secondary rise was due to dilatation of the blood vessels of the gland; it did not occur if the artery to the gland was clamped when the 'ebb wave' reached bottom, nor did it occur when the sympathetic response was elicited in a gland with no blood flow (Figs. 7 and 8).

It is well known that both the sympathetic and parasympathetic are secretomotor for the submandibular glands of the cat and dog. Langley (1889)

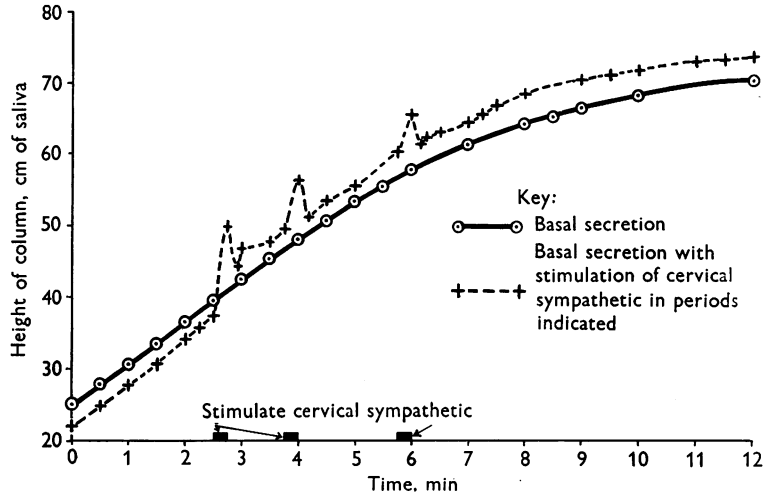

Fig. 6. Rise of the salivary column supported by basal secretion of the gland with Moussu's nerve divided, compared with the rise of the column coincident with three episodes each of 15 sec cervical sympathetic stimulation.

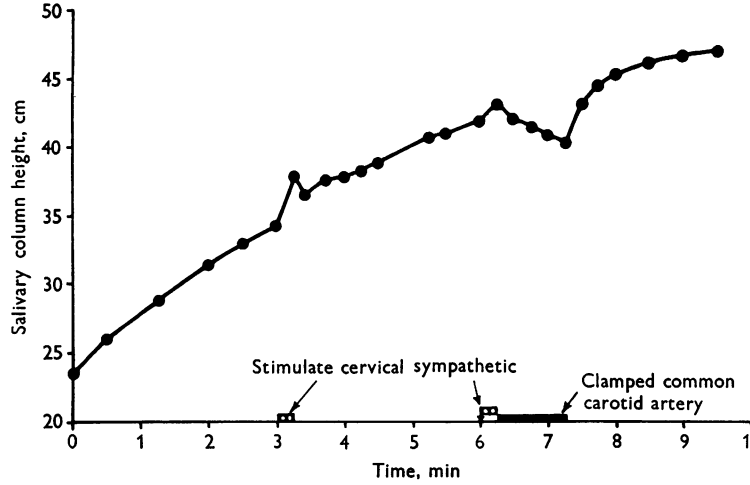

Fig. 7. The effect on the height of the salivary column (supported by basal secretion of the gland with Moussu's nerve divided) of (*a*) 15 sec cervical sympathetic stimulation, and (*b*) 15 sec cervical sympathetic stimulation immediately followed by clamping of the common carotid artery for 1 min.

has described the augmentor action of the after-discharge effect of the parasympathetic upon the response to sympathetic stimulation. It was not possible to demonstrate such augmentation in the sheep's parotid (Fig. 9).

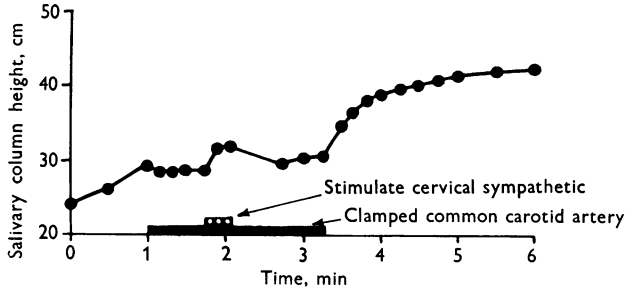

Fig. 8. The effect on the height of the salivary column (supported by basal secretion of the gland with Moussu's nerve divided) of clamping the common carotid artery and superimposing on this 20 sec cervical sympathetic stimulation.

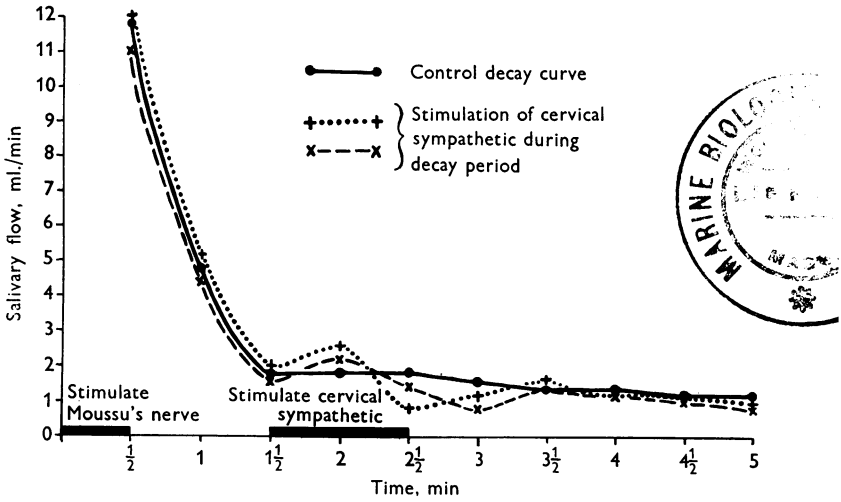

Fig. 9. The effect on the decay curve of salivary flow following 30 sec Moussu's nerve stimulation of one minute's cervical sympathetic stimulation. The salivary gush is seen, but it is immediately followed by a compensatory pause.

This was also the case with submaximal stimulation of Moussu's nerve using maximal intensity but a submaximal frequency of 2 or 5 per sec (a frequency of 12 per sec gives a maximal response from the gland).

(ii) *Prolonged stimulation of the cervical sympathetic.* Stimulation of the cervical sympathetic to the gland with Moussu's nerve cut for a period of 10 min usually resulted in a reduction of saliva production (Fig. 10). This was also the case on stimulating the cervical sympathetic for 10 min in a gland with

Moussu's nerve intact. Chemical analyses in this latter instance showed alterations in the same direction as in the experiments with short-term sympathetic stimulation.

Episode	m-equiv/l. Na^+	m-equiv/l. K^+	Ratio Na/K
Control (6·1 ml. in 8 min)	182	5·0	36·4
Stim. cervical sympathetic (2·3 ml. in 10 min)	182	16·0	11·4
Post-period control (5·8 ml. in 10 min)	175	17·5	10·0

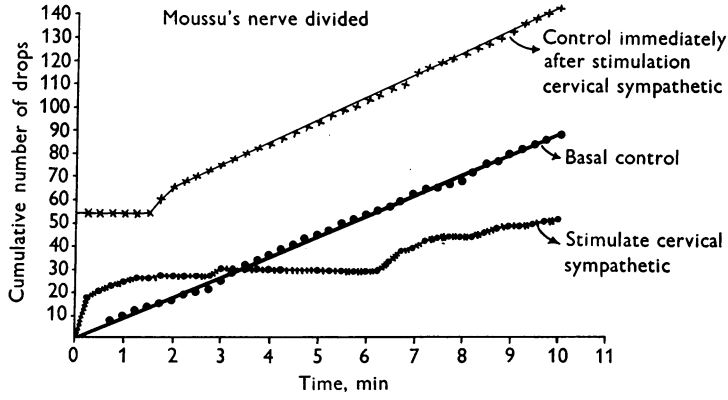

Fig. 10. The effect of 10 min continuous cervical sympathetic stimulation, and 10 min of the immediate post-stimulation phase, compared with the basal secretion of the parotid gland. After the initial gush of saliva occurring at the commencement of stimulation there is an overall reduction so that the total amount in 10 min is reduced. 1½ min after the cessation of sympathetic stimulation the secretion rapidly returns to the basal rate.

These results form a basis for consideration of the effect of short-term stimulation of the cervical sympathetic upon basal secretion by the parotid. The lobules of the gland are contained in relatively inextensible capsules. The components within this capsule are the acini and the ductules with their salivary contents, and numerous blood vessels. In steady conditions the salivary outflow would be that produced by the acini. Expansion of the blood vessels would produce a transient increase in salivary outflow as would contraction of the acini or ducts. A transient reduction in salivary flow would result from vascular constriction or dilatation of salivary channels. It has been shown that with stimulation of the cervical sympathetic trunk to a parotid with Moussu's nerve cut the total salivary flow for the period of stimulation and a prolonged subsequent period was the same as when stimulation of this nerve was not carried out. The simplest explanation of the facts (particularly Table 4 and Fig. 5) is that stimulation has not altered the rate of water transference to the duct at any stage, but has altered the composition of the saliva. At the same time it has caused a transient decrease in the capacity of the intra-glandular duct system. On stimulation of the cervical sympathetic the vessels to the gland almost certainly constrict with the

reduced blood flow. The gush of saliva resulting from stimulation is therefore not likely to be due to vasodilatation. The sharp gradient of the rise and fall in the manometric experiments is very suggestive of a contractile mechanism rather than of increased secretion. The myoepithelial tissue of the intercalated ducts is the most likely source of this effect (Silver, 1954; Kay, 1954). The effect of dihydroergotamine introduced into the duct system supports this contention.

That the vascular distension affects the pressure relationships is shown by the fact that arterial clamping prevents the positive wave in the post-stimulatory period. Vasoconstriction probably is indicated by the result of prolonged stimulation of the sympathetic. On the figures available, the changed salivary composition on sympathetic stimulation cannot with certainty be ascribed to any mechanism other than extreme vasoconstriction of the arterioles to the acini themselves.

(III) *Reflex stimulation of salivary secretion*

For these experiments both ducts were cannulated and usually both glands left innervated. A control collection was taken in these experiments and a steady rate of dropping was required before any stimuli were tried.

Stimulation of the central end of the cut vagus gave on all three occasions a clear-cut increase in secretion per unit time, e.g. 0·6 ml./min increasing to 1·25 ml./min (Oehl, 1864; Clark & Weiss, 1954). Cessation of artificial respiration in apnoeic animals also uniformly caused increased secretion, e.g. 1·0 ml./min increasing to 4·5 ml./min (Langley, 1898). In these circumstances the concentration of cyclopropane would remain approximately constant but the concentration of CO_2 would increase while the O_2 concentration would fall. Increasing CO_2 to 10% in the respired mixture gave no response nor did reducing O_2 pressure until the animal was very cyanotic. It would appear that the cause of increased secretion in this experiment is combined anoxia and increased CO_2. This effect of combined CO_2 excess and oxygen lack in increasing salivary flow has also been demonstrated in the submandibular glands of dogs (Eddy, 1929).

The following procedures gave an increased secretion on some occasions but not on others: acetic acid pH 3·0 on the tongue or acetic acid vapour into the nostril; bottle brushing of the oesophagus (Clark & Weiss, 1954); sucrose 20% solution in the mouth (0·6 ml./min increasing to 1·4 ml./min); faradic stimulation of the tongue at 6 V and 50 c/s (Fig. 1).

A series of likely procedures gave no response. These consisted of: HCl at pH 2 and 3 on the tongue; sodium acetate 100–200–300 m-equiv/l. on the tongue; chloromycetin powder (very bitter) on the tongue; faradic stimulation of the buccal mucosa; excitation of chewing by inflating a rubber object in the sheep's mouth; ruminal puncture, inflation and deflation; 150 ml. of

0·1 N-HCl injected into the rumen; stimulation of the distal end of the cut vagus; faradic stimulation of the ruminal mucosa; acetylcholine 1:50,000 injected into the coeliac axis or the superior mesenteric artery so as to produce violent ruminal and intestinal movements.

(IV) *Secretion pressure*

When a vertical tube was connected to the cannula and Moussu's nerve was stimulated, it was found that the column rose to 136 cm of saliva (100 mm Hg) while the blood pressure (mean of the Hg manometer) was 88 mm Hg. From this level the column suddenly receded and a sialoma rapidly developed in the upper portion of the gland. On two other occasions similar pressures were developed (108 and 60 mm Hg cannula pressure, and 75 and 58 mm Hg blood pressure respectively). That secretion finally stopped because the blood vessels of the acini were compressed by the expansion of the acini was indicated by two lines of evidence.

Stimulation of Moussu's nerve was discontinued at various levels of secretory pressure and noradrenaline was injected into the carotid artery. The 'gush' effect was obtained until the intracannular pressure was approximately at the level of arterial pressure: at higher levels it did not occur but stimulation of the cervical sympathetic still gave a rise in the cannula, e.g. with cannula pressure 73 mm Hg and blood pressure 60 mm Hg. Evidently intra-arterial noradrenaline did not reach the acini, while they were still capable of responding to the chemical mediator liberated from nerve endings in their neighbourhood. At this stage it was also shown that while stimulation of Moussu's nerve still gave a rise of pressure, intra-arterial acetylcholine was ineffective, e.g. with blood pressure 70 mm Hg and secretory pressure 85 mm Hg. For this demonstration it was important that the sensitivity of the gland to acetylcholine should be tested. In one sheep the gland, at normal secretory pressure, responded to 1 ml. of $1:4\times10^{7}$ of acetylcholine injected intra-arterially in 47 sec. This gland responded to intra-arterial acetylcholine $1:4\times10^{4}$ with blood pressure 74 mm Hg and cannula pressure 85 mm Hg, but not to $1:5\times10^{5}$ or $1:2\times10^{7}$ at these pressures.

A second line of approach employed a technique developed in Prof. E. B. Verney's laboratory at Cambridge for the purpose of marking vascular areas in the brain.

Winsor and Newton's artist's water colour 'Cadmium Yellow' was ground in normal saline in the proportion of 1 wet colour to 4 of normal saline. When fully mixed it was centrifuged for a short period (1 min–centrifuge rose to 1000 r.p.m.); the supernatant fluid then showed fine particles 1–2μ in diameter in even dispersion and Brownian movement. Mixed with blood this dispersion did not aggregate, lake or cause rouleaux formation. In transmitted light the granules appeared dark, by reflected light bright yellow.

It has been found by Verney and one of us (D. A. D.) that when short injections of this material were made into the carotid artery of a dog under pentobarbitone anaesthesia, retinoscopy revealed that the dispersion swept rapidly through the retinal vessels and cleared immediately. Further, if this material (in a concentration of 20% solids in 3% gum-acacia saline) were infused for 6 sec at a rate of 0·6 ml./sec into a common carotid artery there was no disturbance of the systemic arterial pressure. If the animal were thereupon suddenly killed and sections made of the celloidin-embedded brain, the dispersion was found in the cerebral capillaries. If, on the other hand, the animal were killed 8 sec after the end of the infusion, the brain was found to be free of dispersion, and particles could then be seen in sections of the lungs. This dispersion would therefore appear to be a relatively inert marker of capillary beds.

For the present purpose the parotid gland was prepared with vascular isolation. Then stimulation of the secretomotor nerve was used to cause the pressure in the duct attached to a mercury manometer to rise above the arterial blood pressure. 10 ml. of cadmium yellow dispersion was then injected into the carotid artery in approximately 20–30 sec, at which time the venous outflow from the gland was heavily marked by the yellow material. The vein, artery and duct were then clamped and tied, and the gland was placed in 20% formalin in alcohol. After fixing for 48 hr the tissues were prepared for frozen sections. Control preparations were made in a similar manner except that the secretory pressure was not raised.

The results are illustrated in Pl. 1. Whereas the normal gland showed an extensive filling of a profuse capillary vasculature, the gland with high secretory pressure showed practically no injection of periacinar vessels, many of which however contained blood corpuscles. Such trapping of corpuscles may occur in the capillaries of a vasculature subjected to an ambient pressure greater than the arterial blood pressure (Wright, 1938). In both circumstances the vessels in the interlobular tissue were filled with the dispersion.

Some notion of the extent of closure of the vasculature in the gland with a secretory pressure higher than blood pressure was obtained by measuring the outflow from a gland under stimulation with and without raised pressure. With a blood pressure of 33 mm Hg and an unimpeded salivary outflow the venous outflow was 13 ml./min; when the secretory outflow was against a pressure of 48 mm Hg and the blood pressure was 40 mm Hg the venous outflow was 5 ml./min. Barcroft (1908) showed that ligature of Wharton's duct greatly reduced or abolished the increased blood flow caused by chorda tympani stimulation.

That the intralobular tissues can support high pressures was shown by having a 120 cm column of Ringer–Tyrode solution in a 3 mm glass tube connected to a fine hypodermic needle by a clipped rubber connexion: when the

needle tip was placed in subcutaneous tissue and the clip released the column fell steadily to 8 cm with obvious distension and oedema of the tissue. On replenishing the tube and placing the tip of the needle in interlobular connective tissue and releasing the clip the column again fell steadily to 15 cm. The interlobular tissue became distended and the lobular parenchyma was thereby defined. On refilling the tube once more and placing the tip in one of the lobules made obvious by the previous experiment the column was maintained, falling only a few centimetres in a period of 5 min.

SUMMARY

The parotid gland of the sheep has been investigated and the following results obtained:

(1) *Basal secretion.* After both cranial and sympathetic neurotomy the gland continues to secrete. No stimulus for this secretion has been found.

(2) *Parasympathetic effects.* It has been confirmed that the secretomotor nerve reaches the gland via the buccal branch of the Vth cranial nerve (Moussu, 1888). Contrary to Eckhard (1893), section of this nerve reduces the flow of secretion. The rate of secretion under stimulation is a function of the blood flow through the gland. The reduction of secretomotor effect by simultaneous stimulation of the sympathetic is associated with a reduction in blood flow.

(3) *Sympathetic effects.* Section of the cervical sympathetic trunk has no effect upon basal or secretomotor secretion. Brief (30 sec) stimulation of the cervical sympathetic causes a transient increase of rate for 8–40 drops. A compensatory decrease in rate follows. It was confirmed that this effect involved contractile elements in the gland, and that the rate of water transfer to the ductules was unchanged. It was shown that the stimulation caused the electrolyte composition of the saliva to change. Prolonged stimulation (10 min) caused a reduction of saliva formation.

(4) *Reflex effects.* In the circumstances of these experiments only stimulation of the central end of the vagus and asphyxia regularly produced increased secretion.

(5) *Secretion pressure.* Secretory pressure can rise above blood pressure. It has been shown that in these circumstances the flow of blood in the periacinar capillaries virtually ceases.

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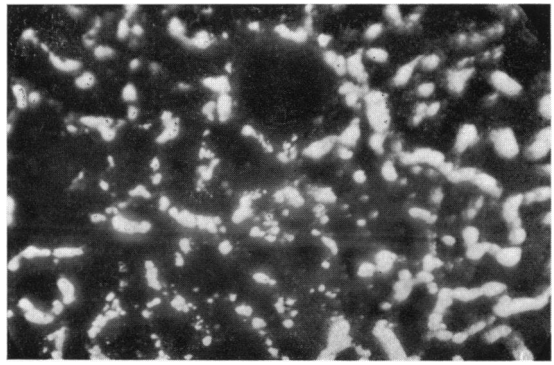

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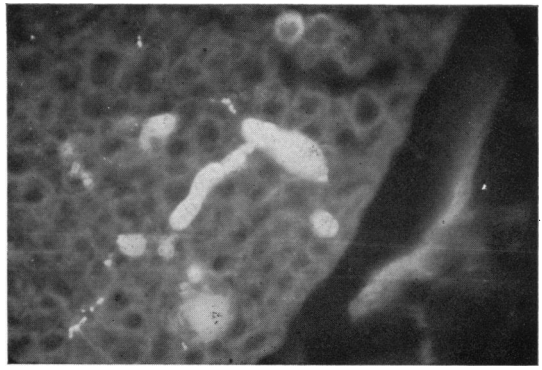

B

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EXPLANATION OF PLATE

A. Photomicrograph of section (40 μ thick) of parotid gland with vessels injected with cadmium yellow and tissues stained with eosin. Blood pressure at time of injection 25 mm Hg, cannula pressure nil. Full injection of a rich capillary network between acini and around an intralobular duct is shown. The section was photographed on Kodachrome using combined epi- and diascopic illumination with a green filter in the diascopic beam; injected vessels appear bright. Magnification 180.

B. Photomicrograph of section (40 μ thick) of parotid gland with vessels injected with cadmium yellow and tissues stained with eosin. Blood pressure at time of injection 35 mm Hg, and cannula pressure 45 mm Hg. An intralobular artery is shown fully injected but only occasional periacinar capillaries are filled. The section was photographed as for Pl. A.