J. Physiol. (1958) 144, 108–122

THE IONIC BALANCES AND TRANSFERENCES OF THE SHEEP'S PAROTID GLAND DURING MAXIMAL STIMULATION

By D. A. COATS, D. A. DENTON and R. D. WRIGHT

From the Department of Physiology, University of Melbourne, Australia

(*Received* 8 *May* 1958)

In a previous study (Coats & Wright, 1957), which aimed to establish the relationship between the composition of parotid saliva and the rate of salivary secretion, it was shown that there were two distinct phases of salivary composition following stimulation of the motor nerve. The start-up phase (Phase I), as it was defined, lasted for the first 2 min. It was characterized by a rise, a fall, and a subsequent further rise of sodium concentration and a roughly reciprocal change in the salivary potassium concentration, these changes being relative to the concentrations of the ions prevailing under the immediately preceding conditions of basal (denervated) parotid secretion. The anions bicarbonate, chloride and phosphate also showed concentration changes during Phase I, a fall in phosphate being reciprocally related to a combined increase of chloride and bicarbonate concentrations. Following this initial period it was found that for each ion the composition of the saliva was related to the rate of secretion, and this stage of the secretory process was defined as the equilibrium stage or Phase II.

The finding by Burgen (1956) and Coats & Wright (1957) that immediately following motor nerve stimulation the venous potassium concentration was increased above the arterial, together with Lundberg's (1955) observations on potential changes in the submaxillary gland of the cat, suggested that Phase I of salivary secretion involved changes in the composition of the parotid gland itself. For these reasons it seemed of importance to investigate in detail the electrolyte state of the gland during its changing activity from basal (denervated) secretion through its period of readjustment (Phase I salivary flow) to the equilibrium state (Phase II salivary flow), a study involving simultaneous estimation of the glands' arterial inflow and salivary and venous outflow of electrolytes.

METHODS

The data presented were obtained from seven Merino sheep. Each animal, before use, was kept indoors in a cage for 5–7 days and was given 25 g of sodium bicarbonate dissolved in 1 l. of tap water by intraruminal tube 2 days before the experiment, to avoid the possibility of minor grades of sodium depletion (Denton, 1956; Coats & Wright, 1957). In all cases the last urine specimen before experimental use contained sodium at a concentration of 100–200 m-equiv/l. indicating sodium repletion.

Anaesthesia was induced by intravenous sodium thiopentone (0·3–0·5 g) and was continued, after tracheotomy, by tracheal tube with closed-circuit cyclopropane and oxygen. In all instances the animal remained in good condition throughout the course of the experiment.

Cannulation of the parotid duct, the preparation of the parasympathetic nerve (Moussu's nerve) for stimulation, and vascular isolation of the parotid gland have all previously been described (Coats, Denton, Goding & Wright, 1956). In all instances the sympathetic trunk was divided in the neck during the course of the preparation.

Stimulation of Moussu's nerve was carried out by means of a platinum cathode inserted into a chamber of Ringer–Tyrode solution surrounding the nerve. An indifferent brass electrode inserted into the rectum was used to complete the circuit. A square-wave electronic stimulator was used and maximum stimulation was attained with a voltage of 8–12 V, a pulse duration of 6 msec and a frequency of 22 pulses per second. For any one episode stimulation was continuous and saliva and venous blood were collected for consecutive periods of 10, 10, 20, 40 and 40 sec and in some instances a further 2 min. In three experiments further blood and saliva samples were taken at periods of up to 42 min after the beginning of stimulation.

In order to obtain volumes of saliva and venous blood adequate for chemical analysis during short period stimulation, the gland was rested for at least 5 min after each stimulation and was then restimulated at the same intensity and frequency, with pooling of the saliva and venous blood obtained from the relevant periods of each episode. In order to avoid the possibility of sampling error, saliva and venous blood were collected throughout the entire extent of any stimulation period and the chemical analyses were performed on a portion of the total sample. As previously described, the cannula dead space was estimated and allowance for this was made in timing the collection of all samples.

The chemical methods used were those described by Denton (1956).

RESULTS

Rate of flow

The rate of salivary production was measured by collecting into a graduated tube the saliva produced in each period. Venous outflow from the gland was measured directly by occluding the jugular vein (which carried only the venous drainage from the isolated gland) and simultaneously releasing a cannula previously inserted into a side tributary between the parotid gland and the point of jugular occlusion. Arterial inflow was calculated as the sum of salivary and venous outflows. This calculation is subject to two sources of error: it presupposes that during the course of stimulation the water content of the gland remained constant and that there was no lymphatic drainage from the gland. Over the period of time involved and with the rates of venous outflow observed, the first of these sources of error is negligible, but the second would cause all estimates of arterial inflow to be low.

Salivary and arterial flows

Following maximal motor nerve stimulation salivary flow rose to 5·80 ± 1·09 ml./min in the first collection period and fell progressively to achieve a steady flow of 2·76 ± 0·98 ml./min after continuous stimulation for 2 min. Arterial inflow, however, in all instances except one (A.E. 70) was maximal after stimulation of the gland for 2 min when the mean flow was 40·7 ± 6·9 ml./min. During the first 2 min of gland stimulation, arterial inflow was initially 36·1 ± 6·2 ml./min, and then fell after 20–40 sec to 31·7 ± 5·7 ml./min before rising progressively to achieve a steady flow of 40·7 ± 6·9 ml./min some time after stimulation for 2 min. In most instances (five out of seven) the arterial inflow was ten times the salivary outflow about 80 sec after the commencement of stimulation, and in the steady state the arterial inflow was about fourteen times the salivary flow. These relationships are shown in Fig. 1.

Because of this fairly constant pattern of arterial and salivary flows following stimulation of the gland, it was decided to test the hypothesis that salivary flow and composition, involving the dispersal of intraglandular substance, was in some way related to and dependent on the rate of arterial inflow. In order to do this it was necessary to modify the pattern of arterial flow during stimulation of the gland. The infusion of bradykinin is known to increase blood flow through tissues (Hilton & Lewis, 1955*a*, *b*). Augmented blood flow was induced in two animals by infusing bradykinin, at rates of 300, 480 and 675 μg/min, into the arterial blood perfusing the gland. Bradykinin infusion alone resulted in an increase of blood flow through the gland from 8–10 ml./min (basal flow) to 19, 38, and 14 ml./min, respectively. While the bradykinin was still running the glands were maximally stimulated and there occurred a further increase in blood flow to 50, 61 and 49 ml./min, respectively, during the first 10 sec of stimulation. Fig. 2 shows that while bradykinin completely altered the pattern of blood flow through the maximally stimulated gland and caused the initial blood flow to be the highest recorded, it was quite without effect on the pattern of salivary flow. The concurrent infusion of bradykinin did not significantly alter the composition of the saliva produced during maximal stimulation of the gland. It would seem that although both salivary flow and blood flow followed a fairly constant pattern during stimulation of the gland, the salivary flow is nevertheless a prime function of the stimulation and is not a function of the stimulation-induced hyperaemia. Bradykinin alone in these experiments did not cause a maximal blood flow through the gland.

Composition of saliva and blood

Throughout the course of maximal stimulation of the gland the salivary concentrations of sodium, potassium, chloride, bicarbonate and phosphate altered in the manner previously reported for Phase I and Phase II salivary

flows (Coats & Wright, 1957). It was found that during the course of stimulation venous concentrations of these ions were significantly different from the arterial concentrations. This difference occurred during the first 2 min of stimulation (corresponding to the Phase I salivary flow) and thereafter, while stimulation continued, the venous concentrations more closely approached or became equal to the concentrations in the arterial inflow.

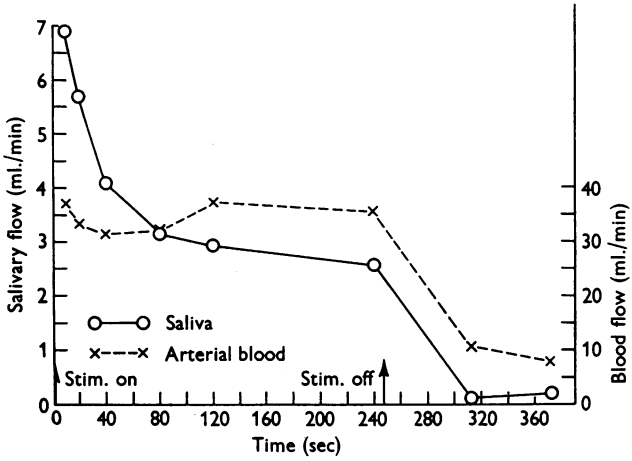

Fig. 1. The relationship between the rates of arterial inflow and salivary outflow. Eighty seconds after the commencement of maximal stimulation of the gland, arterial inflow was ten times salivary flow and in the steady state (Phase II) it was fourteen times salivary flow.

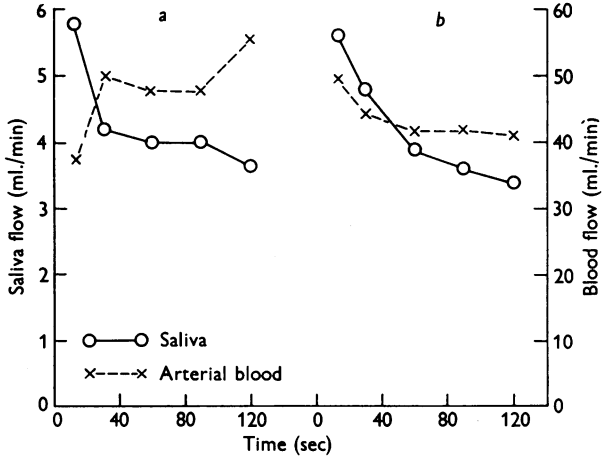

Fig. 2. Salivary and arterial flows during the first 120 sec of maximal gland stimulation (*a*) without bradykinin and (*b*) in the course of bradykinin infusion at 300 μg/min. The infusion of bradykinin has completely altered the pattern of arterial inflow without influencing the rate of salivary secretion.

Blood sodium and potassium. During the first 2–3 min of stimulation (Phase I) the whole-blood sodium concentrations in the venous effluent were constantly lower (1–15 m-equiv/l.) than in the arterial inflow, while whole-blood potassium concentrations in the venous effluent were constantly higher (up to 2·7 m-equiv/l.). During the ensuing 2–4 min (Phase II) this relation held for sodium but the concentration of potassium in the venous effluent closely approached, or was equal to, the arterial concentration.

Blood chloride, bicarbonate and phosphate. Whole-blood chloride and bicarbonate, and plasma phosphate, concentrations were also constantly and significantly different in venous effluent from their concentrations in the arterial inflow during the course of stimulation of the glands. The venous chloride concentrations were up to 10 m-equiv/l. higher, while the bicarbonate concentrations were up to 14 m-equiv/l. lower and the phosphate concentrations were 1–4 m-equiv/l. lower. As with the cations, the anion arteriovenous concentration differences were most marked in the first 2 min of stimulation and became smaller when the 'steady-state' or Phase II salivary flow had become established.

Particular interest attaches to the constant finding of a higher concentration of potassium in the venous effluent than in the arterial inflow, because of the high concentration of this ion in the stimulated saliva relative to that in the blood. The animals used in these experiments all had 'high-sodium, low-potassium' erythrocytes, and the highest whole-blood potassium concentration recorded was 11·0 m-equiv/l., whereas the highest salivary potassium concentration was 18·2 m-equiv/l. This is clear evidence confirming the previously reported findings of Burgen (1956) and the present authors that stimulation of the gland is associated with a considerable loss of glandular potassium both to the saliva and to the venous effluent.

Ionic balance of the parotid gland during stimulation

From the data obtained concerning the rate and electrolyte composition of the arterial inflow to the stimulated gland, and the rate and electrolyte composition of its salivary and venous outflows, it was possible to calculate the degree to which during stimulation it contributed from its own substance or drew from the blood perfusing it for the electrolyte components of its saliva.

Sodium. In six out of the seven experiments (all except A.E. 70) the first 10 sec of maximal stimulation was associated with a release of glandular sodium at the rate of 147 ± 78 μequiv/min. The resultant negative balance, however, was usually of short duration, so that within 40–80 sec of the commencement of stimulation the gland was in equilibrium and its combined salivary and venous output no longer exceeded its arterial delivery. In two animals, however (A.E. 63, 64), there was a further period of negative sodium balance between 120 and 240 sec before final equilibrium was achieved. The form of

the initial sodium loss and the subsequent achievement of equilibrium is illustrated in Fig. 3.

Potassium. Of the electrolytes studied in this context potassium was the most consistent in its behaviour. During the first 10 sec of stimulation there occurred a glandular loss at the rate of 85 ± 30 μequiv/min, and in successive

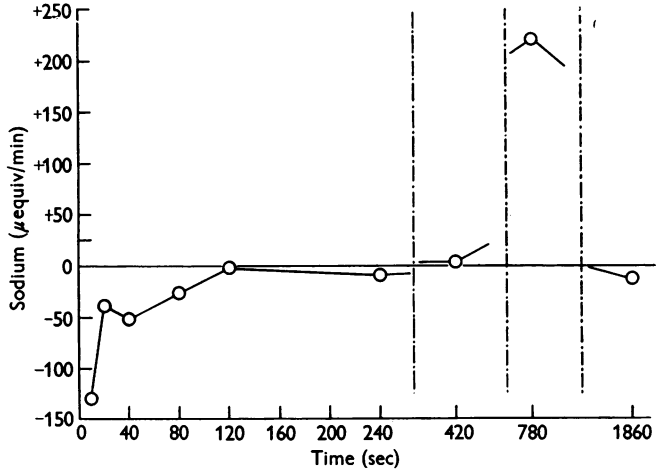

Fig. 3. The glandular balance of sodium at various times up to 31 min during maximal stimulation. During the first 10 sec of stimulation the gland is losing sodium at a rate of 130 μequiv/min, but equilibrium has been achieved by 120 sec and there is evidence of glandular accumulation of sodium at 13 min.

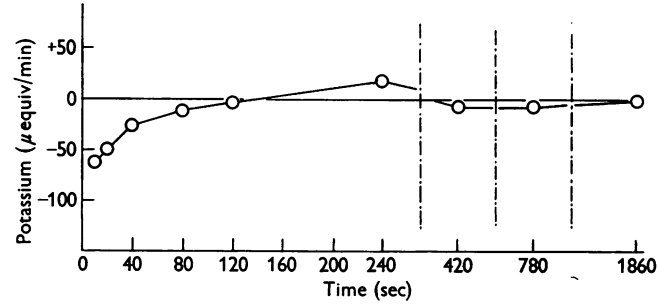

Fig. 4. The glandular balance of potassium at various times up to 31 min during maximal stimulation. During the first 10 sec of stimulation the gland is losing potassium at a rate of 63 μequiv/min, but equilibrium has been achieved by 120 sec and is thereafter maintained.

collections during the first 120–240 sec of stimulation this glandular loss reduced progressively until equilibrium was achieved. The initial 'explosive' release of potassium from glandular substance was less than in the case of sodium, but the duration of glandular loss before equilibrium was established was considerably longer. The form of the initial negative potassium balance during stimulation and its gradual decline towards equilibrium is illustrated in Fig. 4.

Chloride. In every case the first 10 sec stimulation of the gland was associated with a glandular accumulation of chloride. The subsequent behaviour of this ion as stimulation continued was variable but, in all cases except A.E. 68 (in which the total deficit was 13 μequiv), a continuing accumulation within the gland occurred, so that after stimulation for 2 min the mean chloride gain was 198 ± 54 μequiv. The form of this continuing chloride accumulation in the gland during the course of stimulation is illustrated in Fig. 5.

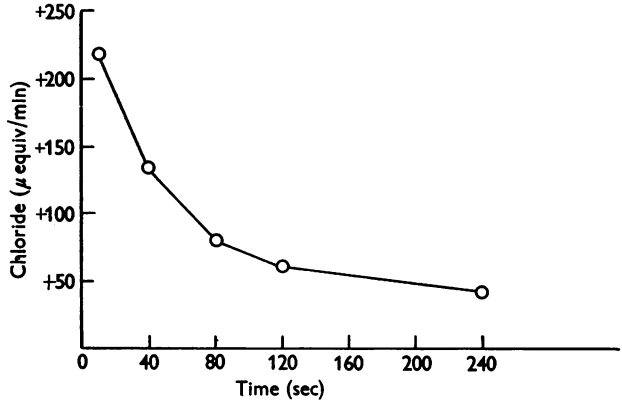

Fig. 5. The glandular balance of chloride at various times up to 240 sec during maxima lstimulation. There is a continuing accumulation of chloride at rates falling progressively from 224 μequiv/min at 10 sec to 42 μequiv/min at 240 sec.

Bicarbonate. Of the ions studied by this balance technique bicarbonate presents the greatest difficulty in interpretation, the reason being that it is the only one in which glandular production is possible, to compensate for an external input-output deficit. Assuming an average arteriovenous oxygen difference during stimulation of the gland of 6 ml./100 ml. blood, a respiratory quotient of 0·8 and an average blood flow of 35 ml./min, glandular metabolic activity would result in the production of bicarbonate at the rate of 75 μequiv/min:

$$\text{Blood flow} = 35 \text{ ml./min},$$

$$\text{oxygen used} = \frac{6 \times 35}{100} \text{ ml./min},$$

$$CO_2 \text{ produced} = \frac{0{\cdot}8 \times 6 \times 35}{100} \text{ ml./min},$$

$$22{\cdot}4 \text{ ml. } CO_2 = 1000 \ \mu\text{equiv bicarbonate},$$

hence,
$$CO_2 \text{ produced} = \frac{0{\cdot}8 \times 6 \times 35 \times 1000}{22{\cdot}4 \times 100} \ \mu\text{equiv/min}$$

$$= 75 \ \mu\text{equiv/min}.$$

For this reason any external negative bicarbonate balance of less than 75 μequiv/min may be apparent rather than real.

In all experiments there was an initial (apparent) bicarbonate loss of 270 ± 103 μequiv/min. This initial large loss, however, was followed within 40–80 sec by a steady state in which there was either no apparent further loss (A.E. 63, 68, 70) or a loss in the region of 100 μequiv/min (A.E. 62, 64, 65, 69). In view of the considerations above concerning the possibility of metabolic bicarbonate production these two situations could represent equilibrium in the absence and presence respectively of metabolic production by the gland.

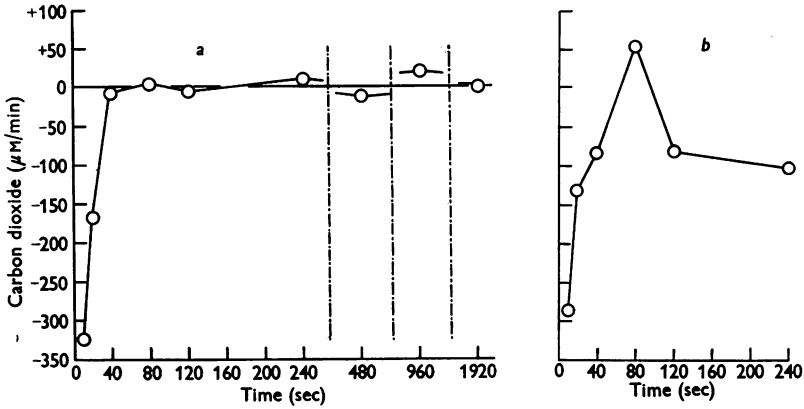

Fig. 6*a*. The glandular balance of carbon dioxide at various times up to 32 min during maxima stimulation. There is a large initial loss of carbon dioxide from the gland but equilibrium has been achieved by 40 sec and is thereafter maintained. There is no evidence of carbon dioxide production in this gland. *b*. The glandular balance of carbon dioxide in a gland in which the initial loss is of the same magnitude as in Fig. 6*a*, but in which a steady state is achieved with an apparent input deficit of 92 μequiv/min. This may be interpreted as evidence of metabolic carbon dioxide production in this gland.

The two types of balance found are illustrated in Figs. 6*a*, *b* (in which the 'steady state' is achieved with an apparent input deficiency of 92 μequiv/min). The general form of the bicarbonate balance curves with an initial large loss and a rapid achievement of equilibrium is very similar to the form of the sodium balance curves.

Phosphate. For technical reasons phosphate analyses were performed on arterial and venous plasma, whereas for the other ions studied the analyses were performed on whole blood. For that reason the phosphate balance of the gland throughout the course of stimulation must be presented with less assurance than the balances of the other ions. Differences in the haematocrit values of arterial and venous blood and the possibility of movement of phosphate between red cells and plasma, either during the course of the passage of the blood through the gland or during the interval between blood sampling

and plasma separation, may all impose limits upon the validity of the phosphate balances. It is probable that the errors introduced by this technique were small relative to the glandular depletion of phosphate which occurred but their possible presence should be appreciated.

In all cases there was an initial loss during the first 10 sec of stimulation of 72 ± 37 μequiv/min. This loss became progressively smaller during subsequent collection periods until equilibrium was achieved between 120 and 240 sec after the beginning of stimulation. The initial 'explosive' release of phosphate from gland substance was smaller than in the case of bicarbonate (even allowing for the complicating factor of possible bicarbonate production), but the time necessary for the establishment of an equilibrium state was considerably longer. The form of the phosphate-balance curve is shown in Fig. 7.

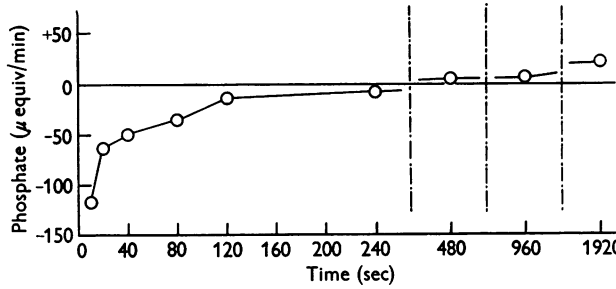

Fig. 7. The glandular balance of phosphate at various times up to 32 min during maximal stimulation. During the first 10 sec of stimulation the gland is losing phosphate at a rate of 118 μequiv/min, but equilibrium has been achieved by 120–240 sec and is thereafter maintained.

It is similar to the potassium curve (Fig. 4) with an initial loss smaller than in the case of sodium or bicarbonate but a longer period before the establishment of equilibrium.

Cumulative balances. Cumulative balance studies on the glands in these experiments revealed that during the course of the first 2 min of maximal stimulation there was always a glandular loss of potassium and bicarbonate (although a bicarbonate correction for metabolic production should probably be applied in some cases). In all cases except one there was also a sodium loss, a phosphate loss and a chloride accumulation in the gland. These cumulative 2 min balances are presented in Table 1.

Where the total cation loss is greater than the total measured anion loss endogenous bicarbonate production can probably be invoked to explain the discrepancy. In three experiments, however (A.E. 62, 63, 69), cation loss is associated with an apparent accumulation of anion. In these cases the total cation loss is of greater magnitude and the disturbance in ionic state may well have been sufficient to alter significantly the pH and anion equivalance of intracellular protein and organic phosphate.

TABLE 1. Cumulative ionic balances after 2 min maximal stimulation of parotid gland (μequiv)

Expt.	Na^+	K^+	Cl^-	CO_2	HPO_4^-	Net gland state: Cation	Net gland state: Anion
A.E. 62	−140·4	−108·9	+116·8	− 71·7	− 15·1	−249·3	+ 30·0
A.E. 63	−129·6	− 71·6	+177·2	− 22·6	−117·5	−201·2	+ 37·1
A.E. 64	−204·3	−109·4	—	−158·6	—	−313·7	—
A.E. 65	− 64·9	− 31·8	− 92·0	−239·5	+ 6·1	− 96·7	−141·4
A.E. 68	+ 9·1	− 64·2	− 12·7	− 90·5	− 80·6	− 55·1	−183·8
A.E. 69	− 53·7	−116·9	+553·9	−124·2	− 51·3	−170·6	+378·4
A.E. 70	+280·8	− 99·4	+261·0	− 55·3	− 28·0	+181·4	+177·7

Gland analysis

It was, of course, not possible to analyse a gland before and after stimulation in order to test the accuracy of the alterations in glandular electrolyte content indicated from the balance studies. In two instances, however, the gland after stimulation was removed and analysed, and compared with the analysis of the opposite gland which had been denervated throughout the course of the experiment but had not been stimulated. It was assumed that the control gland would have approximately the same electrolyte content per unit mass as the experimental gland, and that the comparison would give some check on the deduced balances. In three normal sheep whose right and left unstimulated parotid glands were similarly analysed the paired potassium and chloride contents per unit mass did not differ by more than 5%, and in two of them the sodium content on each side also was within 5%. In the third there was a difference of 10% in the sodium content per unit mass of gland on the two sides. The analyses gave an indication that the stimulated glands had lost potassium and had gained both sodium and chloride and that the magnitude of the deduced potassium loss (200 μequiv) was feasible (analysis deficit for the same gland 164 μequiv when compared with the same mass of the opposite unstimulated gland).

Ionic fluxes across the secretory cell

In view of the rapidity of ionic transference to saliva during maximal stimulation of the gland an attempt was made to determine its order of magnitude. Histological study of the gland showed that the mean outside acinar diameter was of the order of 20 μ and the mean inside acinar diameter was of the order of 5 μ. Analyses of normal glands allow an assumption of an extracellular water content of the gland of 50% of its mass and it was assumed that the specific gravity of the acini did not differ significantly from unity. The maximum transferences of sodium, potassium and chloride and average transferences obtained during Phase II salivary flows are indicated in Table 2.

In computing the transferences it was assumed that each acinus was a sphere, that there was no secretion from the duct system and that the acinus

did not contain any secretory canaliculi. These assumptions should be valid for the external (blood to acinar cell) transfer, but may not be valid for the internal (acinar cell to saliva) transfer. Should the assumptions be invalid the magnitudes of internal transference would be rather less than those indicated.

In the case of potassium the initial explosive release from the gland following stimulation involves dispersal to venous effluent as well as to saliva. The mean magnitude of this potassium flux to venous blood for the first three collection periods following the commencement of stimulation (10, 10 and 20 sec) is indicated in Table 3.

TABLE 2. Electrolyte transferences to saliva (*a*) during peak flow following stimulation and (*b*) during established (Phase II) secretion

Ion	Concn. (m-equiv/l.)	Salivary flow (ml./min)	Transference across external acinar membrane (pmoles/cm²/sec)	Transference across internal acinar membrane (pmoles/cm²/sec)
Na^+ (peak flow)	190	6·9	$2·5 \times 10^3$	$4·0 \times 10^4$
Na^+ (Phase II flow)	182	1·35	$5·0 \times 10^2$	$8·0 \times 10^3$
K^+ (peak flow)	14·3	6·9	$1·9 \times 10^2$	$3·0 \times 10^3$
K^+ (Phase II flow)	9·8	1·35	$2·5 \times 10$	$4·0 \times 10^2$
Cl^- (peak flow)	25	7·2	$3·4 \times 10^2$	$5·4 \times 10^3$
Cl^- (Phase II flow)	22	1·35	$6·0 \times 10$	$9·6 \times 10^2$

TABLE 3. Net potassium flux from acinar cells to venous blood at the beginning of stimulation

Period (sec)	K^+ flux (pmoles/cm²/sec)
0–10	17
10–20	42
20–40	17

DISCUSSION

The denervated parotid gland of the sheep maintains a continuous basal secretion of saliva in the absence of external stimuli. In this phase the electrolyte content of the gland presumably remains constant and the sole source of salivary components must be the perfusing blood. When the gland is stimulated, however, there occurs not only an increase in the rate of production of saliva, but also a complex readjustment of its ionic constituents associated with an alteration in the ionic content of the gland. During this phase of altering secretory activity there is no longer a balance between the arterial inflow of ions and their combined salivary and venous outflows. The present work has shown that the imbalance is characterized by a dispersal of glandular sodium, potassium, phosphate and carbon dioxide and an accumulation of chloride. In all instances the magnitude of the imbalance is greatest during the first 10 sec of stimulation and thereafter gradually declines towards a re-set equilibrium between inflow and outflow. This re-setting of the equilibrium, however, does not imply a restitution of the gland to its basal (denervated and unstimulated) constitution.

The dispersal of glandular substance and the re-setting of equilibrium during stimulation do not occur over the same period of time for all the ions studied. In general there are two main orders of magnitude. At the beginning of stimulation there occurs a sudden and 'explosive' efflux of glandular sodium and bicarbonate with glandular depletion proceeding at the rates of 147 ± 78 and 270 ± 103 μequiv/min, respectively (the apparent bicarbonate depletion may, however, be spuriously high owing to concurrent carbon dioxide production within the gland). These large initial negative balances are of relatively short duration and within a period of 40–80 sec of continuous stimulation equilibrium has been achieved (allowing for the possibility of carbon dioxide production by the gland) with near equality of net arterial input and salivary loss. The second order of intrinsic glandular depletion following stimulation is seen with the ions potassium and phosphate. Here the initial losses are considerably smaller (85 ± 30 and 72 ± 37 μequiv/min, respectively) but the time interval required before equilibrium is achieved is of the order 120–240 sec. Equilibrium, of course, does not involve restitution of the gland to its pre-stimulation ionic composition. In three experiments (A.E. 64, 65, 68) stimulation was maintained for a total period of 32–42 min and the cumulative balances indicated in each instance a total glandular deficit of potassium and excess of sodium compared with the resting state. Whether resting glandular composition is ever regained during continuous stimulation is not known.

The chloride content of the stimulated gland is of interest in that in all experiments except one (A.E. 68) there occurred a glandular accumulation of chloride during stimulation. During the first 10 sec this accumulation was at the rate of 141 ± 62 μequiv/min and at the end of 2 min of stimulation the total chloride accumulation was 198 ± 54 μequiv. One explanation might be that stimulation of the gland resulted in oedema with an increased extracellular chloride content. This, however, would require a concomitant sodium accumulation of some 280 μequiv to maintain extracellular proportions of the two ions, whereas the corresponding mean sodium balance was a deficit of 16 ± 96 μequiv. This must mean that the chloride accumulation was intracellular, a conclusion supported by histological evidence of lack of significant glandular oedema following stimulation.

Consideration of the 'explosive' glandular release of cellular substance at the beginning of stimulation suggests the possibility that the requirements of ions for secretion in saliva at this stage is in excess of arterial delivery. This might particularly be so in the case of potassium and phosphate where the arterial plasma concentrations are considerably below the salivary concentrations and in the first few seconds of stimulation when salivary volume is maximal and arterial inflow is still rising (Fig. 2). Should arterial inflow at the beginning of stimulation impose a limit on the ions from this source available for secretion then glandular contribution of the remainder would be explicable.

This hypothesis was tested by increasing blood flow through the gland by the infusion of bradykinin before stimulation. This resulted in a maximal blood flow associated with the first salivary collection without altering the 'explosive' release of glandular substance or the rate of salivary production. This evidence would indicate that the release of glandular substance into saliva at the beginning of stimulation was a prime function of the stimulation rather than

TABLE 4. Fluxes across various biological membranes (pmoles/cm²/sec)

	Ion	Influx	Efflux	Net flux	Reference
Sepia axon (post-stimulation)	Na^+	32	39	—	Hodgkin & Keynes (1955)
	K^+	21	28	—	
Sepia axon (resting)	Na^+	61	31	—	Keynes (1951)
	K^+	17	58	—	
Desheathed cat nerves					Dainty & Krnjević (1955)
(If whole surface is available for exchange)	Na^+	—	3·9	—	
(If only region near nodes of Ranvier is available for exchange)	Na^+	—	500–2500	—	
Frog skin (short-circuited)	Na^+	514	20	494	Ussing & Zerahn (1951)
Human kidney tubule	Na^+	—	—	3000	Rehberg (1926)
Cat					Lundberg (1958)
Submaxillary gland	K^+	—	30–40	—	
Sublingual gland	K^+	—	400–500	—	
Sheep parotid					This paper
Maximal response	Na^+	—	—	2500 (plasma to cell) 40,000 (cell to duct)	
Steady flow	Na^+	—	—	500 (plasma to cell) 8000 (cell to duct)	
Maximal response	K^+	—	—	190 (plasma to cell) 3000 (cell to duct)	
Steady flow	K^+	—	—	25 (plasma to cell) 400 (cell to duct)	

a reflexion of paucity of external supply. The conclusion is further supported in the case of potassium by the constant finding at the beginning of stimulation of a higher concentration in venous effluent, than in arterial inflow indicating a glandular contribution both to saliva and blood.

The magnitudes of the ionic fluxes across the secretory cell during stimulation are very large compared with those of the fluxes for stimulated Sepia axons (Keynes, 1951; Hodgkin & Keynes, 1955) and for desheathed cat nerves (Dainty & Krnjević, 1955). Ussing & Zerahn (1951) showed a sodium influx across short-circuited frog skin of 1·85 μmoles/cm²/hr (514 pmoles/cm²/sec) and Rehberg (1926) gives a figure of 3000 pmoles/cm²/sec for the net sodium flux

across human renal tabular cells. Table 4 presents a summary of representative fluxes of sodium and potassium taken from the literature compared with the net fluxes found for the sheep's parotid gland. Only the figures of Dainty & Krnjević (1955) for sodium fluxes for the desheathed cat nerve are in any way of comparable magnitude to those here presented and then only if the assumption is made that exchange is limited either to the myelin-free gap or to a 10μ length in the region of the node of Ranvier. The authors give reasons for rejecting this hypothesis of restricted area of exchange. In a recent publication Lundberg (1958) quotes from unpublished work of Krnjević & Lundberg an initial potassium efflux in the submaxillary gland of the cat of 30–40 pmoles/cm^2/sec and in the sublingual gland of about 400–500 pmoles/cm^2/sec.

SUMMARY

1. Stimulation of the parotid gland of the sheep gives rise not only to a great increase in the rate of salivary production, and changes in salivary composition which may be divided into two phases, but also to an 'explosive' release of glandular ionic constituents which appear in the saliva. There is a large negative balance of sodium and bicarbonate which persists for the first 40–80 sec of stimulation before equilibrium is achieved. There is also a smaller negative balance of potassium and phosphate which, however, persists for the first 120–240 sec of stimulation. During the period of glandular depletion of these ions there occurs a glandular accumulation of chloride. The negative balance of potassium is reflected not only in the loss of glandular potassium to saliva but also in its dispersal into the venous effluent from the gland.

2. After the period of glandular imbalance at the beginning of stimulation a new equilibrium is achieved in which there is an equality of arterial input and combined salivary and venous outflows of the ions sodium, potassium, chloride, bicarbonate and phosphate. This new equilibrium is achieved during continuing stimulation without restitution of the gland to its basal (pre-stimulation) composition. After continuous stimulation for a period of 32–42 min, the gland has a higher content of sodium and chloride and a lower content of potassium than the unstimulated gland.

3. The release of glandular content during the initial phase of stimulation is not due to a lack of availability of ions for secretion in the arterial blood perfusing the gland. Increasing the blood flow by the infusion of bradykinin before and during stimulation increases the availability of ions for diversion into the saliva, but fails to modify the 'explosive' dispersal of glandular substance or the composition and rate of production of saliva.

4. Ionic fluxes across the acinar cells are very large when compared with fluxes across nerve, frog skin and renal tubules. The highest fluxes occur during maximal stimulation of the gland, and across the internal (acinus to saliva) acinar membrane the order of magnitude for sodium, potassium and chloride

is $4{\cdot}0 \times 10^4$, $3{\cdot}0 \times 10^3$ and $5{\cdot}4 \times 10^3$ pmoles/cm^2/sec, respectively. During established (Phase II) salivary flow while stimulation continues the corresponding fluxes for the same ions are of the order of $8{\cdot}0 \times 10^3$, $4{\cdot}0 \times 10^2$ and $9{\cdot}6 \times 10^2$ pmoles/cm^2/sec, respectively. In all cases the ionic fluxes across the external (blood to acinus) acinar membrane are smaller by a factor of 16.

The authors wish to express their gratitude to Dr S. M. Hilton and Dr G. P. Lewis of the National Institute for Medical Research, London, for the gift of the purified bradykinin used in some of these experiments. This work was aided by grants from the Australian National Health and Medical Research Council and the Wool Industry Research Fund of the Commonwealth.

REFERENCES

BURGEN, A. S. V. (1956). The secretion of potassium in saliva. *J. Physiol.* **132**, 20–39.

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

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

DAINTY, J. & KRNJEVIĆ, K. (1955). The rate of exchange of ^{24}Na in cat nerves. *J. Physiol.* **128**, 489–503.

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

HILTON, S. M. & LEWIS, G. P. (1955*a*). The cause of the vasodilatation accompanying activity in the submandibular salivary gland. *J. Physiol.* **128**, 235–248.

HILTON, S. M. & LEWIS, G. P. (1955*b*). The mechanism of the functional hyperaemia in the submandibular salivary gland. *J. Physiol.* **129**, 253–271.

HODGKIN, A. L. & KEYNES, R. D. (1955). Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol.* **128**, 28–60.

KEYNES, R. D. (1951). The ionic movements during nervous activity. *J. Physiol.* **114**, 119–150.

LUNDBERG, A. (1955). The electrophysiology of the submaxillary gland of the cat. *Acta physiol. scand.* **35**, 1–25.

LUNDBERG, A. (1958). Electrophysiology of salivary glands. *Physiol. Rev.* **38**, 21–40.

REHBERG, P. B. (1926). Studies on kidney function. II. The excretion of urea and chlorine analysed according to a modified filtration-reabsorption theory. *Biochem. J.* **20**, 461–482.

USSING, H. H. & ZERAHN, K. (1951). Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. scand.* **23**, 110–127.