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Secretion of saliva by the rabbit mandibular gland *in vitro*: the role of anions

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Salivary glands form their secretions by first elaborating an isotonic plasma-like primary fluid in the endpieces and then modifying the composition of this secretion during its passage along the gland duct system. We have studied the role of extracellular anions in both primary secretion and ductal modification with a recently developed technique for isolation and perfusion of the rabbit mandibular gland.

Neither of the major extracellular anions (Cl^- or HCO_3^-) is essential for primary fluid secretion. HCO_3^- can be removed altogether and replaced with Cl^- without diminution in secretory rate, provided that extracellular pH is maintained at 7.4, and its replacement with acetate actually enhances secretion. Complete replacement of Cl^- with Br^- also enhances secretion and replacement with I^-, NO_3^-, $CH_3SO_4^-$ or isethionate supports secretion but at progressively diminishing rates. Our data do not yet allow us to distinguish between an electroneutral Na^+–Cl^- cotransport model or a double countertransport (Na^+–H^+ plus Cl^-–HCO_3^-) model as the basis of primary salivary secretion, or to propose any more suitable alternative model.

With respect to ductal modification of the primary saliva, HCO_3^- omission inhibits ductal Na^+ absorption (i.e. salivary Na^+ concentration rises). This inhibition is probably related to an effect of pH on the postulated Na^+–H^+ exchange mechanism in the luminal duct membrane since it can also be induced by lowering perfusate pH, and reversed by substitution of perfusate HCO_3^- with acetate (which enters saliva) but not HEPES (which does not enter the saliva). Substitution of perfusate Cl^- with other anions seems not to inhibit ductal Na^+ and K^+ transport markedly.

Introduction

From a perusal of the papers making up this symposium, it is apparent that the secretion of proteins and other macromolecules can readily be studied by using a variety of *in vitro* preparations (namely gland pieces, dispersed acini or secretory endpieces, and isolated cells) in which discharge of the secretory product into the incubation medium is monitored. The secretion of fluid and electrolytes, however, is not so easily studied since the secretory products cannot readily be distinguished from the same substances already present in the bathing medium. In addition, while there are good morphological correlates for protein secretion, there are none for fluid secretion (Young & van Lennep 1978).

The matter is further complicated since the fluid finally discharged by a gland to the exterior is formed in a two-stage process involving the participation of both the secretory endpieces and the excurrent ducts. Both types of epithelium may secrete fluid, as in the pancreas, where two distinct juices are mixed (Case *et al.* 1980*b*), or the endpieces may be secretory and the ducts absorptive, as in salivary glands (Young & van Lennep 1979) and sweat glands (Sato 1977),

where the role of the ducts is not to secrete fluid but only to modify the electrolyte composition of the primary saliva formed by the endpieces. The properties of the salivary ductal epithelium can readily be studied in isolation by duct microperfusion (Young *et al.* 1967; Knauf 1972) but those of the endpieces can only be gauged indirectly, since the secreted fluid must enter at least the first part of the duct system before it can be sampled by micropuncture and analysed. Nevertheless, our greatly improved understanding of ductal function, arising from duct perfusion studies, has made it much easier to interpret the primary secretory events from the composition of the final saliva emerging from the glandular main duct. To take advantage of this, we have recently developed a technique in which the mandibular gland of the rabbit can be isolated and perfused arterially *in vitro* (Case *et al.* 1980*a*) in order to study fluid and electrolyte secretion under conditions in which the extracellular fluid composition can be varied widely and yet be closely controlled.

Many epithelial organs, both absorptive, such as kidney proximal tubule, intestine and gall bladder, and secretory, such as choroid plexus, pancreatic ducts and pancreatic and salivary endpieces, transport salt and water 'isotonically', i.e. in the absence of substantial transepithelial osmotic gradients (see Giebisch *et al.* (1979) for numerous references). It has come to be widely accepted that such transport involves a process of secondary active transport in which the dissipative flux of one substance (usually Na^+) is coupled to the flux of a second solute, which is thereby concentrated in the cytosol against an electrochemical gradient, by means of a cotransport protein located either in the basolateral or apical plasma membrane. If the permeability properties of the apical and basolateral plasma membranes differ with respect to this secondarily transported solute, it may then diffuse selectively across the opposite plasma membrane and thus undergo transepithelial transport. Cotransport proteins have frequently been invoked to account for the transepithelial movement of anions such as bile salts (in hepatocytes), amino acids (in kidney proximal tubule and small intestine), Cl^- (in kidney tubules, intestine and gall bladder) and HCO_3^- (in pancreatic ducts, choroid plexus and ciliary body). Since salivary glands, in common with most other exocrine glands, form isotonic primary secretions (Young & van Lennep 1979) it seems more than possible that the underlying transport mechanisms may depend on the presence of cotransport proteins for the coupling of Na^+ flux to an anion flux, as in other isotonic-transporting epithelia. The present study was undertaken, therefore, to determine to what extent salivary secretion is dependent on the extracellular anion composition.

Methods

Details of the method employed for isolation and perfusion of the rabbit mandibular gland have been published elsewhere (Case *et al.* 1980*a*) and only a brief description is provided here. Mandibular glands (mean mass of contralateral non-perfused glands: 694 mg, s.d. ±92) were removed surgically from male, albino rabbits, anaesthetized with urethane. The glands were perfused arterially at 4 ml/min with a nutrient salt solution (table 1) warmed to 37 °C. Saliva was collected via a cannula tied in the main excretory duct, in tared vessels, over timed intervals; volume was equated to mass. Venous effluent was collected as required from a cannula placed in the anterior facial vein.

Secretion was evoked by addition of acetylcholine chloride to the perfusate, usually in a concentration of 0.8 μmol/l, a dose known to evoke a maximum fluid secretory response, but occasionally in a concentration of 80 μmol/l, referred to hereafter as a 'supramaximal' dose,

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which is known to evoke saliva with higher HCO_3^- concentrations but without further increasing the fluid output (Case *et al.* 1980*a*).

One of two experimental protocols was followed. In one (protocol S), each gland was perfused continuously with a single perfusion fluid, and saliva was collected in successive samples for the duration of the experiment (usually 2 h). In this protocol, the averaged responses of one

TABLE 1. COMPOSITION (MILLIMOLES PER LITRE) OF THE ELECTROLYTE SOLUTION USED FOR PERFUSION OF THE ISOLATED RABBIT MANDIBULAR GLAND†

Na^+	146.0	Cl^-	127.3‡
K^+	4.3	HCO_3^-	25.0§
Mg^{2+}	1.0	SO_4^{2-}	1.0
Ca^{2+}	1.5	PO_4^{3-}	1.0
glucose	5.0	ACh–Cl^-‖	0.0008

† Equilibrated with 5% CO_2 in O_2. In protocol M, employed for the experiments depicted in figures 4 and 9, the Ca^{2+} concentration was 2.5 mmol/l and PO_4^{2-} was 1.5 mmol/l.

‡ In some experiments (figures 4 and 9), Cl^- was replaced with Br^-, I^-, NO_3^-, $CH_3SO_4^-$ or isethionate; Cl^- introduced with acetylcholine remained, however.

§ In some experiments (figures 1, 2, 3, 5 and 8), HCO_3^- was replaced with Cl^-, acetate, HEPES, sulphamerazine or sulphisoxazole. In such cases the solutions were equilibrated with 100% O_2.

‖ Acetylcholine chloride.

group of glands could be compared with the responses of other groups, perfused with different solutions. In the other protocol (M), the glands were first perfused with HCO_3^--buffered nutrient saline (table 1, the 'control' perfusate) until the secretory rate had fallen to its plateau level (figure 1). Thereafter the perfusate composition was changed periodically so that 20 min test perfusions alternated with 20 min control perfusions. In this protocol, the secretory response to a given test perfusion could be expressed as a percentage of the control response, averaged from the collection periods immediately before and after the test period. This protocol allowed elimination of variability within glands but had the disadvantage of confining investigation to the plateau phase of the secretory response.

In experiments carried out to determine the effect of inhibition of carbonic anhydrase on salivary secretion, acetazolamide (Sigma) or methazolamide (American Cyanamid Co.) was added to the vascular perfusate in a concentration of 10^{-4} mol/l. The concentrations of Na^+, K^+, Cl^- and HCO_3^- were determined in all samples by standard methods (Case *et al.* 1980*a*). In acetate perfusion experiments, acetate was sometimes determined directly, by using acetate kinase (Holz & Bergmeyer 1974), but in most cases it was estimated as the so-called 'residual ion' concentration: $[Na^+ + K^+ - Cl^- - HCO_3^-]$.

RESULTS AND DISCUSSION

It is now widely accepted that saliva is formed by a two-stage process in which an isotonic primary fluid with constant, plasma-like electrolyte composition is first formed by the secretory endpieces (and/or intercalated ducts) and then modified in the gland excurrent duct system by reabsorption of Na^+ and Cl^- and secretion of K^+ and HCO_3^- (Young & van Lennep 1979). The ducts, which are very impermeable to water, render the saliva hypotonic since they reabsorb Na^+ and Cl^- more rapidly than they secrete K^+ and HCO_3^-. Hence it follows that the salivary secretory rate closely reflects the secretory activity of the endpieces, whereas the

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salivary electrolyte composition is determined largely by the activity of the excurrent ducts. It is convenient here for us to separate these two processes, discussing endpiece fluid secretion first and ductal electrolyte transport subsequently.

Fluid secretion

Role of extracellular HCO_3^-

The isolated mandibular gland does not secrete appreciably in the absence of exogenous stimulation (Case *et al.* 1980*a*). Its secretory response when perfused with HCO_3^--buffered saline (table 1) containing acetylcholine (0.8 μmol/l) is depicted in figure 1. Initially, the secretory rate was about 150 μl/min (i.e. about 216 μl/min per gram gland wet mass), a rate equal to

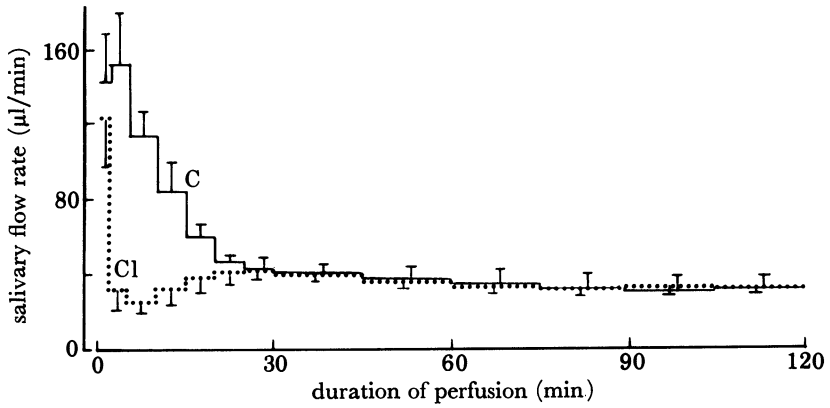

FIGURE 1. The secretory response of the isolated mandibular gland of the rabbit perfused with a HCO_3^-–CO_2 buffered nutrient physiological salt solution (C) (for composition, see table 1) or a similar solution (Cl) in which all HCO_3^- was replaced with Cl^- and exogenous CO_2 with O_2. Each horizontal bar is the mean of observations in four glands ± s.e.m. Secretion was evoked by continuous infusion of acetylcholine (0.8 μmol/l) (data from Case *et al.* 1981).

or greater than that encountered *in vivo* during pharmacological or direct nerve stimulation (Martin *et al.* 1973; Smaje 1973). With continued stimulation, the secretory rate declined over a period of 20–30 min to reach a plateau of about 40 μl/min which was then maintained for 2 h or more. This tachyphylaxis is not an artefact of the *in vitro* preparation: it is seen also *in vivo* and the gland, *in vitro*, will recover its initial responsiveness to acetylcholine after a 30 min period without stimulation (Case *et al.* 1980*a*).

The effect of removing extracellular HCO_3^- is also shown in figure 1. Although the initial secretory rate was high, it fell within 1–5 min to about 25 μl/min and then rose to reach the same plateau (40 μl/min) as seen in control studies. Replacement of extracellular HCO_3^- with Cl^-, leaving only 1 mM phosphate as buffer, caused the pH of the now inadequately buffered extracellular fluid to fall, so that we recorded a mean pH of 6.85 (s.d. ± 0.02) in the venous effluent with a HCO_3^--concentration of 0.08 mmol/l (s.d. ± 0.13). Inevitably, therefore, the intracellular pH must also have fallen.

To distinguish between pH-related and direct effects of HCO_3^- omission, we performed additional experiments in which HCO_3^- was replaced by other buffer anions including the hydrophilic HEPES (10 mmol/l, a concentration giving buffer capacity comparable with our HCO_3^--buffered control fluid), the lipophilic sulphonamides, sulphamerazine (4.0 mmol/l) and

sulphisoxazole (12.5 mmol/l), and acetate (25 mmol/l), although acetate has negligible buffering capacity at pH 7.4. The results are summarized in figures 2 and 3.

HEPES restored the extracellular pH fully. Thus the pH in the venous effluent was 7.4 (s.d. ± 0.04) although the HCO_3^- concentration remained less than 1 mmol/l. As can be seen in figure 2, HEPES also restored the fluid secretory rate to normal. Since under these conditions,

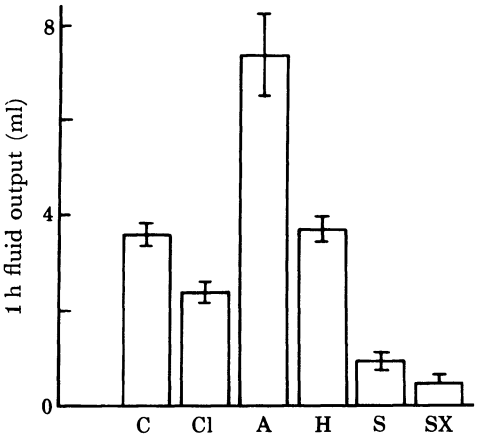

FIGURE 2. The secretory response of the isolated mandibular gland of the rabbit, expressed as the total volume of fluid secreted during 1 h from the onset of acetylcholine infusion, for glands perfused with a HCO_3^-–CO_2 buffered nutrient physiological salt solution (C), or similar solutions in which HCO_3^- was replaced with either 25 mM Cl^- (Cl), or 25 mM acetate (A), or 10 mM HEPES (H), or 4.0 mM sulphamerazine (S) or 12.5 mM sulphisoxazole (SX). Each bar represents the mean of 4–6 studies ± s.e.m. (data from Case *et al.* 1981).

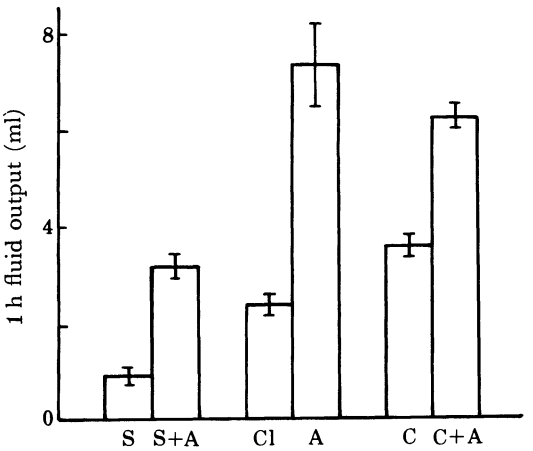

FIGURE 3. The effect of acetate on the secretory response of the isolated mandibular gland of the rabbit. The bars labelled C, Cl and S represent experiments with control, HCO_3^--free and sulphamerazine-buffered solutions (cf. figure 2), while the bars labelled C + A, A, and S + A represent experiments in which 25 mM acetate was added to these perfusates at the expense of Cl^-. Each bar represents the mean of 4–6 experiments ± s.e.m.

intracellular HCO_3^- concentration must have been drastically reduced, and because the zwitterionic HEPES is most unlikely to have been able to replace HCO_3^-–CO_2 as a buffer system for carrying protons across the plasma membrane, it seems inevitable that intracellular pH must still have fallen to some extent during HEPES perfusion. Hence we conclude that fluid secretion is affected by changes in extracellular pH and that HCO_3^- ions *per se* play no essential role in the secretory process.

Interpretation of our findings with lipid-soluble buffers (figures 2 and 3) is more difficult. All three buffer salts gave rise to fluid secretory responses resembling the control response depicted in figure 1 rather than that seen with Cl^- replacement of HCO_3^-, i.e. the tachyphylactic fall to a plateau secretory rate was quite regular. However, both sulphamerazine and sulphisoxazole reduced the fluid output markedly (by 74 and 87%, respectively), whereas acetate more than doubled it. The extracellular pHs recorded in the venous effluent were: sulphamerazine, 7.25 (s.d. $\pm$0.09); sulphisoxazole, 7.17 (s.d. $\pm$0.07); acetate, 7.06 (s.d. $\pm$0.067). All three buffers, being lipid-soluble in the protonated form, would have controlled intracellular pH more effectively than HEPES. However, the two sulphonamides may also have acted as direct uncouplers of mitochondrial metabolism (McLachlan & Dilger 1980), thereby reducing the secretory response. The stimulatory effect of acetate, though currently under investigation, remains unexplained. It is probably not related to pH changes (despite a report that acetate in these concentrations can elevate cytosol pH in the pancreas (Swanson & Solomon 1975)), since the effect persisted when acetate was added to fluids containing HCO_3^-–CO_2 or sulphamerazine buffer (figure 3). At present we are inclined to believe that acetate is simply providing an alternative source of metabolic substrate for a secretory system in which the rate of cellular glucose uptake is normally the rate-limiting step. We have some data to suggest that the effect may not be seen in other species such as the rat (I. Novak & J. A. Young, unpublished 1981). Regardless of the true explanation for the effects of the three lipid-soluble buffers, our findings reinforce the conclusion that HCO_3^- is not essential for fluid secretion by the rabbit mandibular gland. This is in complete contrast to secretion by the secretin-stimulated pancreas, where there exists a linear relation between secretory rate and perfusate HCO_3^- concentration over the range 0–25 mmol/l (Case *et al.* 1970).

Role of pH

To study the effect on secretion of varying perfusate pH more carefully, we used acetate-buffered perfusion fluids equilibrated with 100% O_2, since it proved difficult to control the extracellular p_{CO_2} adequately when working with HCO_3^-–CO_2 buffer systems. At a perfusate pH of 6.0 all secretion ceased, but as pH was increased from 6.2 to 7.8, secretory rates increased in approximately linear fashion, rising from 12.5% of the 'control' (pH = 7.4) value at pH 6.2, to 130% at pH 7.8 (figure 4). In view of its low pK_a value (4.76), acetate is ineffective as a buffer much above a pH of 6.8, thereby allowing metabolically derived CO_2 to lower the perfusate pH (e.g. the pH of the venous effluent during perfusion at a nominal pH of 7.4 was 7.06, s.d. $\pm$0.067). Therefore, had true extracellular pH been plotted in figure 4, the curve would have been even steeper. Despite the fact that the sulphonamide buffers inhibited secretion (figure 2), the same dependence of secretory rate on perfusate pH was demonstrable when these buffers were used. For example, with sulphamerazine buffer (pK_a = 7.1; perfusate made up to have a constant concentration of undissociated weak acid of 1.34 mmol/l), the secretory rate at pH 6.5 was 29% of the control (pH = 7.4) value, and it rose to 119% at pH 7.8. This effect of pH alteration, in contrast to the effect of HCO_3^- depletion, is similar to that seen in the pancreas (cf. figure 4). However, unlike these secretory epithelia, absorptive epithelia such as frog skin and turtle bladder show a much less marked pH-dependence (Funder *et al.* 1967; Gentile & Brodsky 1969).

Also shown in figure 4 are the results of experiments in which methazolamide (10^{-4} mol/l) was added to the gland perfusate. No statistically significant change in secretory rate was

observed during perfusion with either acetate or HCO_3^-. Since this concentration of methazolamide can be expected to have inhibited most of the carbonic anhydrase activity (Maren 1967), we conclude that the CO_2 hydration–dehydration reaction is not rate-limiting in the primary formation of saliva. Of course, this does not exclude the possibility that secretion is driven by a process involving Na^+–H^+ exchange, with subsequent HCO_3^-–Cl^- exchange (see below), but it makes it seem rather unlikely, particularly since the drug did have marked effects on salivary HCO_3^- secretion (see below and Case *et al.* 1981).

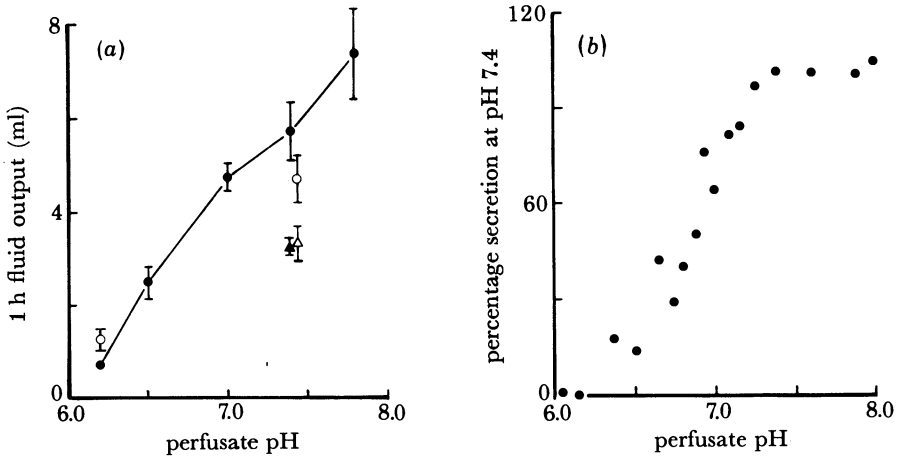

FIGURE 4. The effect of perfusate pH on fluid secretion by the perfused rabbit mandibular gland (*a*) and the perfused cat pancreas (*b*). For the cat pancreas the data represent observations in four experiments during perfusion with an acetate solution (from Case *et al.* 1979). For the rabbit mandibular gland the means (±s.e.m.) for 4–8 experiments are shown (from Case *et al.* 1981). Filled circles (●) depict data obtained during perfusion with acetate solutions while the filled triangle (▲) depicts results of experiments with a control, HCO_3^--buffered solution. Open circles (○) and the open triangle (△) show experiments with similar perfusion fluids which also contained methazolamide (10^{-4} mol/l).

Role of non-buffer anions

In figure 5 are shown the results of experiments designed to study the role of the non-buffer extracellular anion, Cl^-. In these experiments, the extracellular buffer was always HCO_3^-–CO_2, and Cl^- was replaced with another anion. Replacement with Br^- increased secretory rate to about 122% of control levels, and replacement with I^- and NO_3^- reduced it respectively to 74 and 58%; even $CH_3SO_4^-$ and isethionate, considered to be predominantly extracellular anions, supported secretion at 41% of control rates. Similar observations have been made on the perfused cat pancreas (figure 5) except that in this gland, replacement with NO_3^- had little inhibitory effect (Case *et al.* 1979). One is forced to conclude that salivary secretion is quite non-specific with respect to what extracellular anions are present, and that neither Cl^- nor HCO_3^- plays an essential role in secretion. A comparable non-specificity with respect to anions has been reported for the bullfrog gastric mucosa (Hogben 1965) and rabbit gall bladder (Whitlock & Wheeler 1967). Simultaneous replacement of both Cl^- and HCO_3^- with $CH_3SO_4^-$, however, abolished secretion (J. A. Young & E. Favaloro, unpublished 1981).

Models for fluid secretion

On the basis of numerous micropuncture studies on the major salivary glands of various species (Young *et al.* 1980), it is now generally accepted that salivary glands secrete an isotonic primary fluid having an electrolyte composition not greatly different from that of a plasma ultrafiltrate. Although only few data are available on transepithelial voltages in secretory endpieces, it seems safe to conclude that the endpiece epithelium is of the leaky type, transporting large volumes of fluid isotonically. It is widely held (although certainly not proved) that the bulk of the salts and water transported under such circumstances passes via paracellular channels across the junctional complexes, but it seems certain that an active transcellular flux of at least one ion species must occur.

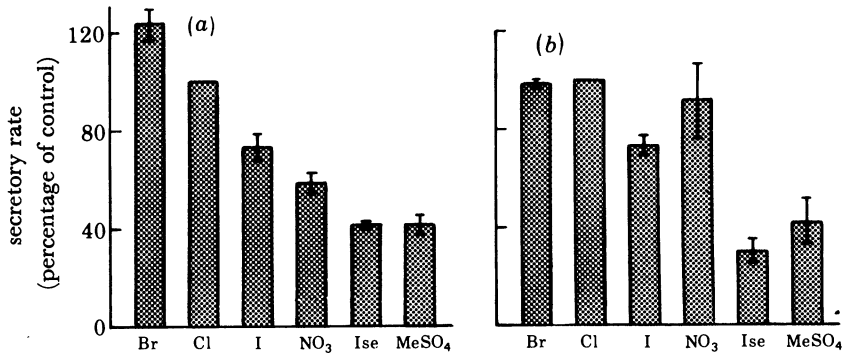

FIGURE 5. The effect of replacing Cl^- with other anions on secretion by the perfused rabbit mandibular gland (*a*) (data from Case & Hunter 1981) and the perfused cat pancreas (*b*) (data from Case *et al.* 1979). The glands were perfused according to protocol M (see Methods) with a nutrient physiological bicarbonate saline and stimulated to secrete with acetylcholine (mandibular gland) or secretin (pancreas). In control perfusions (Cl), the perfusate contained 127.3 mM Cl^- (mandibular gland) or 134.3 mM Cl^- (pancreas), 1.5 mM PO_4^{3-} and 25 mM HCO_3^- as anions; in test perfusions, the HCO_3^- and PO_4^{3-} were left unchanged but Cl^- was replaced by Br^-, I^-, NO_3^-, isethionate (Ise) or methylsulphate ($MeSO_4$). Each bar depicts the mean of 4–11 studies ± s.e.m.

Evidence concerning the nature of this process in salivary glands is particularly scant but, by analogy with what is known about isotonic transport across epithelia such as small intestine and kidney proximal tubule, two alternative models can be considered. One model involves a tightly coupled electroneutral Na^+–Cl^- cotransport protein located in the basal plasma membrane of the secretory cell (Frizzell *et al.* 1979). It is postulated that the diffusion of Na^+ into the cytosol, down the electrochemical gradient established by the Na^+, K^+-ATPase in the basolateral plasma membrane, concentrates Cl^- in the cytosol against an electrochemical gradient ('secondary active transport'). Cl^- then moves passively into the endpiece lumen across the apical cell membrane by an undefined mechanism. This ion flow is thought to initiate a secretion process (electrostatically and osmotically) in which the bulk of the secreted ions and water enter the lumen by a paracellular route. While such a secretory mechanism is thermodynamically sound (Cook & Young 1981), it remains to be explained how the junctional complexes can be adequately permeable to ions and water and yet maintain a high reflexion coefficient to the secreted ion (Hill 1980).

The alternative model, first described by Turnberg *et al.* (1970), which we shall call the

'double countertransport' system, is no better able to resolve the dilemma of paracellular flow, but may be more applicable to salivary glands since it has been invoked to explain isotonic transport by other epithelia lacking Cl^- specificity (Heintze & Petersen 1980; Liedtke & Hopfer 1980). The model requires a basal Na^+–H^+ countertransport exchange by means of which passive Na^+ influx gives rise to secondary active accumulation of HCO_3^- in the cytosol. In turn, the HCO_3^- is envisaged as diffusing back into the interstitium via a Cl^-–HCO_3^- exchange carrier, also located in the basolateral plasma membrane, which causes Cl^- to accumulate in the cytosol and diffuse across the luminal plasma membrane. The nett result is an apparent electroneutral NaCl secretion. A similar Na^+–H^+ countertransport process has been postulated

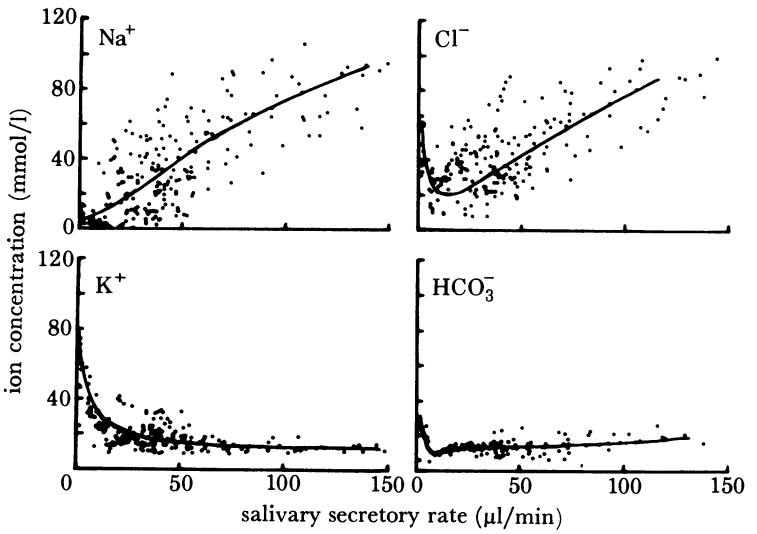

FIGURE 6. The electrolyte excretion patterns of the isolated mandibular gland of the rabbit, perfused with nutrient physiological bicarbonate–saline (table 1). The data are from studies on 14 glands and include samples collected before and during stimulation with acetylcholine (0.8 µmol/l). The gland masses averaged 694 ± 92 mg (s.d.).

in the pancreas, except that HCO_3^- is thought then to cross the luminal plasma membrane to initiate secretion (see Case *et al.* 1979). One might predict that such a system would be sensitive to carbonic anhydrase inhibition, or SITS interaction with the Cl^-–HCO_3^- exchanger, whereas a Na^+–Cl^- system might be sensitive to furosemide (Frizzell *et al.* 1979).

Since the double countertransport system inevitably involves the recycling of CO_2 (or H_2CO_3) across the basolateral plasma membrane, one would anticipate that only lipid-soluble buffers would be effective in replacing HCO_3^-–CO_2. Our experiments with HEPES and with carbonic anhydrase inhibitors argue against the operation of such a system in the mandibular gland, although the possibility remains that metabolically derived CO_2, hydrating at its uncatalysed rate, is adequate to support secretion. On the other hand, Na^+–Cl^- cotransport processes are described as being specific for Cl^- (Frizzell *et al.* 1979) which argues against simple Na^+–Cl^- cotransport as the basis of secretion in the mandibular gland. The sensitivity to extracellular pH change may favour the double countertransport model but careful studies are required to differentiate between intracellular and extracellular pH change.

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Ductal electrolyte transport

Normal ductal function

The electrolyte excretion patterns for the perfused rabbit mandibular gland, stimulated with acetylcholine (0.8 μmol/l), are shown in figure 6. Concentrations of Na^+ were below 10 mmol/l at the lowest secretory rates and rose sigmoidally as secretory rate increased to maximum values of about 100 mmol/l. In contrast, K^+ concentrations were highest (*ca.* 80 mmol/l) at low secretory rates and fell to approach an asymptote of 8–10 mmol/l. The pattern for Cl^- resembled that for Na^+, except that concentrations rose again at very low secretory rates; the HCO_3^- pattern tended to follow that for K^+. In general terms these excretion curves resemble those reported for other salivary glands and are typical for the whole class of hypotonic-secreting glands (Young & van Lennep 1979). When due allowance is made for the mode of stimulation

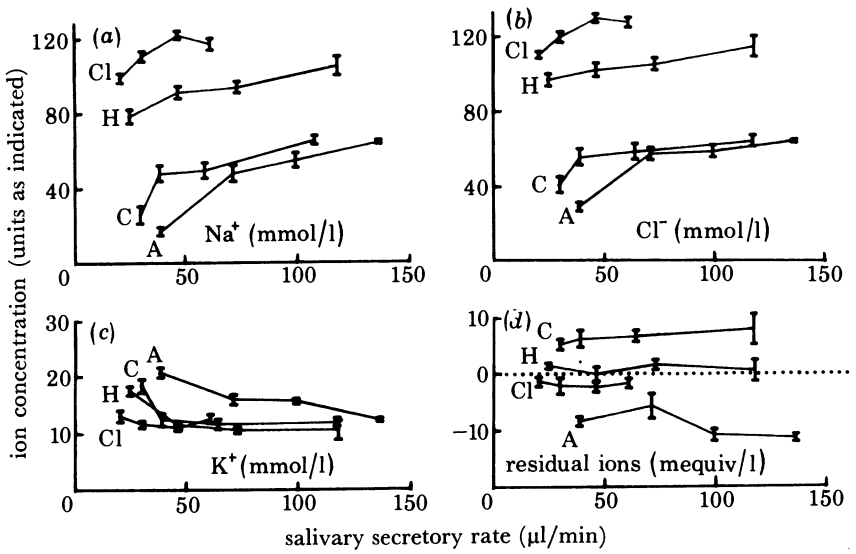

FIGURE 7. The electrolyte excretion patterns of the isolated perfused rabbit mandibular gland for the same experiments as are depicted in figure 2. (*a*) Sodium; (*b*) chloride; (*c*) potassium; (*d*) residual ions. Each point is the mean of 8–15 samples from 4–6 glands (±s.e.m.). The symbols C, Cl, A and H are as defined in the legend to figure 2. Residual ions have been calculated as $[Na^+ + K^+ - Cl^- - HCO_3^-]$; a negative sum indicates an anion residuum, a positive sum a cation residuum (from Case *et al.* 1981).

and the exact composition of the extracellular fluid, the patterns resemble closely those encountered in the rabbit mandibular gland *in vivo* (Endre & Young 1981). As pointed out above, the patterns arise as the consequence of ductal modification of the primary fluid and indicate that the ducts reabsorb Na^+ and Cl^- extensively while simultaneously secreting K^+. In this particular gland it is probable that the ducts ordinarily reabsorb HCO_3^-, but secretion can be observed under some circumstances and is the rule rather than the exception in many other species (Young *et al.* 1980; Martin *et al.* 1973).

Role of HCO_3^-

The effects on ductal electrolyte transport of removal of HCO_3^- from the gland perfusate and its replacement with Cl^-, HEPES or acetate, can be assessed from the data presented in figure 7. In order to facilitate comparisons of the electrolyte excretion curves obtained from each

experimental series, the data have been grouped according to secretory rate into four bins, each containing approximately equal numbers of samples. The mean salivary electrolyte concentration for each bin (±s.e.m.) was then plotted against the corresponding mean flow rate.

Removal of HCO_3^- without substitution with another buffer led to a dramatic increase in salivary Na^+ concentration of about 55–75 mmol/l, indicative of a marked inhibition of ductal Na^+ absorption, accompanied by a parallel increase in salivary Cl^- concentration (figure 7). Restoration of the extracellular pH with HEPES did not restore Na^+ and Cl^- concentrations to normal, but replacement of HCO_3^- with acetate was fully effective; indeed, a small stimulation of ductal Na^+ transport was apparent (figure 7). Ductal K^+ secretion, as reflected by salivary K^+ concentrations, seemed not to be affected by removal of HCO_3^- and, consequently, addition of HEPES had no effect. However, acetate increased salivary K^+ concentrations by about 5–6 mmol/l.

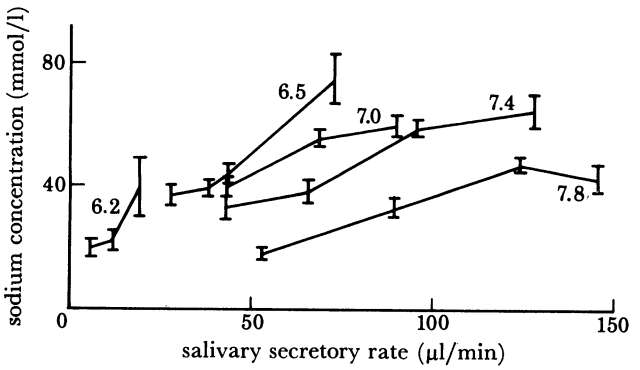

FIGURE 8. The effect of extracellular pH on the Na^+ excretion curves of the perfused rabbit mandibular gland; data are from the same experiments as are depicted in figure 4. Each point is the mean of 8–15 samples from 5–10 glands (±s.e.m.) (data from Case *et al.* 1981).

Although more difficult to interpret, the effect of each perfusion fluid on the salivary concentration of residual ions, $[Na^+ + K^+ - Cl^- - HCO_3^-]$, is also of interest (figure 7). During perfusion with HCO_3^--buffered fluid, there was always a cationic residuum of about 5 mequiv/l, presumably due largely to the presence of Ca^{2+} and Mg^{2+}. Removal of HCO_3^- converted this residuum to a slight anionic excess (*ca.* 2 mequiv/l), which might indicate a reduced salivary content of divalent cation or, more likely, an increase in the salivary content of an organic anion such as lactate (cf. sweat (Sato 1977)). Since HEPES failed to increase this anion residuum further, it can be inferred that it failed to enter the saliva, whereas the sharp increase in residual anions (to *ca.* 10 mequiv/l) seen during acetate perfusion, suggests that this anion entered the saliva in appreciable concentrations. To check this, we measured acetate directly with an enzymatic method: in 24 samples from four glands the mean salivary acetate concentration was 14.0 mmol/l (s.e.m. ±0.9, $n = 24$), exceeding the calculated residual anion concentration by 4.5 ± 1.4 mequiv/l (doubtless due to the presence of divalent cations, as in control perfusion). The measured acetate concentration did not differ significantly from the HCO_3^- concentration found in saliva during control perfusion (14.0 ± 0.4 mmol/l, $n = 116$).

The simplest explanation of our findings would be that ductal Na^+ absorption, which is

thought to involve a Na^+–H^+ exchange mechanism located in the luminal plasma membrane (Young & van Lennep 1979), is inhibited by a decrease in pH in the duct lumen, and that HEPES fails to restore transport to normal because it fails to enter the saliva. Acetate, being able to enter the saliva in the lipid-soluble protonated form, is able to replace HCO_3^- as a potential carrier of H^+ across the cell membrane although its poor buffering capacity at high pH must result in some fall in salivary pH; it would be expected to restore Na^+ transport to normal provided that significant reversal of the postulated luminal Na^+–H^+ exchanger does not occur above a pH of about 6.5 or if some acetate uptake mechanism should have increased cytosol pH (cf. Swanson & Solomon 1975). The fact that acetate actually stimulated ductal Na^+ and K^+ transport, just as it stimulates fluid secretion, tempts us to favour a metabolic explanation for both stimulatory effects, which may have masked an inhibitory effect due to acetic acid's having a lower pK_a than carbonic acid.

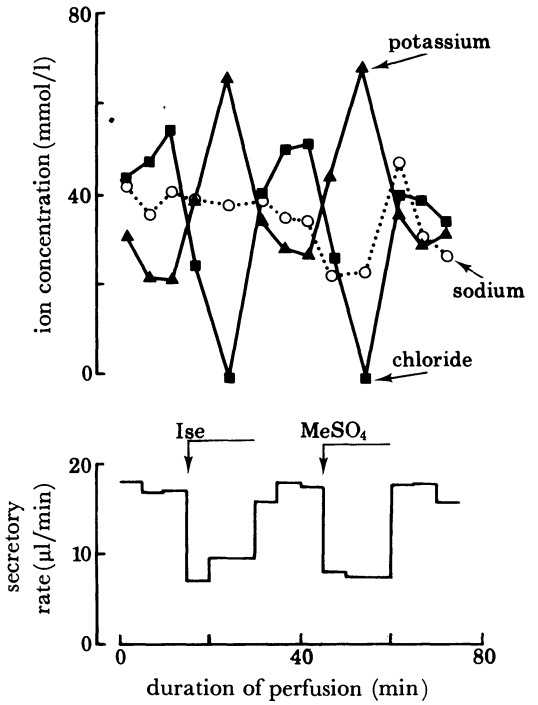

FIGURE 9. The effect of replacing Cl^- with isethionate (Ise) or methylsulphate ($MeSO_4$) on the volume and composition of saliva secreted by the perfused rabbit mandibular gland. The data are from a single experiment conducted according to protocol M (see Methods). Horizontal bars indicate periods during which the Cl^--containing perfusate was replaced with test solutions.

Role of pH

Figure 8 shows the Na^+ excretion curves for the experiments in which the pH of the glandular perfusate was varied from 6.2 to 7.8 (cf. figure 4) by using 25 mM acetate as the extracellular buffer. Salivary Na^+ concentration increased as perfusate pH fell, indicating a progressive inhibition of ductal Na^+ absorption. A similar pattern has been observed with Cl^- concentrations (Case *et al.* 1981). Two possible explanations can be advanced to account for these findings: either the ductal Na^+ absorptive mechanism is directly influenced by extracellular pH or the postulated luminal Na^+–H^+ exchange mechanism may have been inhibited by a progressive lowering of luminal pH, occurring as extracellular pH was reduced. The latter proposal is

unlikely since the salivary pH seems to have remained fairly constant as judged by the HCO_3^- content (Case *et al.* 1981). Similarly, by assuming that acetate is at equilibrium between saliva and interstitium, and taking the pH of the venous effluent as equal to that of the extracellular fluid, we estimate that the salivary pH would only have ranged between 6.39 and 6.86 as the perfusate pH was changed from 6.2 to 7.8 and the venous pH changed from 6.24 to 7.10. For this reason we conclude that extracellular (or intracellular) pH can influence ductal Na^+ transport directly, in addition to whatever influence the availability of lipid-soluble buffers in the duct lumen may have on the hypothetical Na^+–H^+ exchange protein.

Role of non-buffer anions

When extracellular Cl^- is replaced completely with other anions such as $CH_3SO_4^-$, secretion persists, albeit usually at a reduced rate (figure 5). Since micropuncture studies showing what the composition of the primary fluid would be under these conditions are not available, it is difficult to interpret the electrolyte excretion patterns encountered in these experiments. Figure 9 shows a typical experiment (protocol M) in which perfusion with control perfusate was alternated with perfusion with isethionate or $CH_3SO_4^-$. During control periods, the salivary Na^+ concentration was about 40 mmol/l, K^+ about 20 mmol/l, Cl^- about 50 mmol/l, and HCO_3^- about 10 mmol/l. After a change to Cl^--free solutions, when the secretory rate fell to 40%, the Na^+ concentration either remained constant or, as in figure 9, fell even further. Concentrations of K^+ rose, in the example in figure 9 to more than twice control levels. At present we can only conclude that substitution of Cl^- with other anions does not seem to inhibit ductal Na^+ and K^+ transport markedly.

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