A Quantitative Analysis of Electrolyte Exchange in the Salivary Duct

Kate Patterson¹, Marcelo A. Catalán², James E. Melvin²,
David I. Yule³, Edmund J. Crampin⁴, James Sneyd¹

KP: Designed model, carried out simulations and drafted manuscript,
MAC: Designed model,
JEM: Analysis and Interpretation of Data,
DIY: Analysis and Interpretation of Data,
EJC: Designed model and revised draft for important intellectual content,
JS: Designed model and revised draft for important intellectual content.

¹Department of Mathematics, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
²Secretory Mechanisms and Dysfunction Section, Division of Intramural Research, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA
³Department of Pharmacology and Physiology and the Center for Oral Biology, University of Rochester Medical Center, Rochester, NY 14642, USA
⁴Auckland Bioengineering Institute and Department of Engineering Science, The University of Auckland, Private Bag 92019, Auckland, New Zealand

Running head: A quantitative analysis of the salivary duct

Corresponding author:
Kate Patterson,
Department of Mathematics,
The University of Auckland,
Private Bag 92019, Auckland 1142, New Zealand.
Phone: +64 9 373 7599 ext 88777;
Fax: +64 9 373 7457;
Email: k.patterson@math.auckland.ac.nz
Abstract

A healthy salivary gland secretes saliva in two stages. First, acinar cells generate primary saliva, a plasma-like, isotonic fluid high in $Na^+$ and $Cl^-$. In the second stage, the ducts exchange $Na^+$ and $Cl^-$ for $K^+$ and $HCO_3^-$, producing a hypotonic final saliva with no apparent loss in volume. We have developed a tool which aims to understand how the ducts achieve this electrolyte exchange while maintaining the same volume. This tool is part of a larger multi-scale model of the salivary gland and can be used at the duct or gland level, to investigate the effects of genetic and chemical alterations. In this paper, we construct a radially symmetric mathematical model of the mouse salivary gland duct, representing the lumen, the cell and the interstitium. For a given flow and primary saliva composition, we predict the potential differences and the luminal and cytosolic concentrations along a duct. Our model accounts well for experimental data obtained in wild type animals as well as knockouts and chemical inhibitors. Additionally, the luminal membrane potential of the duct cells is predicted to be very depolarized in comparison to acinar cells. We investigate the effects of an electrogenic vs electroneutral anion exchanger in the luminal membrane on concentration and the potential difference across the luminal membrane as well as how impairing the CFTR channel affects other ion transporting mechanisms. Our model suggests the electrogenicity of the anion exchanger has little effect in the submandibular (SMG) duct.

Abbreviations

Additional Information

Three key words: Salivary glands, tubular transport, membrane transport
1 Introduction

Saliva is composed of 99% water, and yet that other one percent helps to maintain oral health by providing an appropriate ecological balance in the mouth [45]. The basic secretory unit of a salivary gland consists of a cluster of acinar cells and a segment of duct (Figure 1). The acinar cells secrete an isotonic primary saliva high in Na\(^+\) and Cl\(^-\), but low in K\(^+\). These cells produce all of the water found in saliva as the duct is considered to be highly impermeable to water [21]. As the fluid travels along the duct, the duct cells exchange Na\(^+\) and Cl\(^-\) for K\(^+\) and HCO\(_3^-\), producing a hypotonic final saliva. This electrolyte exchange creates a final saliva which acts as a buffer in the mouth, neutralizing acids and inhibiting caries progression. This hypotonicity also enhances the ability to taste salty foods and nutrient rich foods [18]. Primary saliva production has been modeled by Gin et al. [16] and Palk et al. [37], however these models do not include any representation of the salivary duct. One aim in studying this system is to provide a quantitative understanding of how hypotonicity is achieved in the mouse submandibular gland duct with no apparent change in saliva volume. It is, to the best of our knowledge, the first quantitative description of a salivary duct and is validated against a variety of experimental data. This model is also a tool which can be used alone, or incorporated into a multi-scale model of the salivary gland, spanning from molecular to the tissue level [37, 31, 47]. Depending on the application, either tool can be used to study genetic and chemical alterations, including, but not limited to: salivary gland disease, knock out studies, and gene therapy through viral vectors.

The pancreas is known to have a similar structure to the salivary gland, and both glands have been used as biological models to investigate secretory epithelium. One important difference between pancreatic ducts and salivary ducts is that while the pancreatic ducts secrete near isotonic fluid, the salivary ducts are impermeable to water and the final saliva is hypo-
tonic. We have combined ideas from both the pancreatic duct cell models [48, 49, 52, 53] and the tubule transport models [51, 11, 22], to constructed a modular, multi-compartment mathematical model of the salivary duct. The ion transporters are displayed in Figure 1. Cl\(^{-}\) uptake occurs via an anion exchanger (AE) [58] located in the BLM and a luminal CFTR channel [19, 6, 57, 55] while efflux occurs through a basolateral ion channel [43] and a luminal AE [58, 46]. Na\(^{+}\) enters the cell via a sodium-bicarbonate co-transporter (pNBC1) in the BLM [17, 30, 39, 1, 4] and through a luminal ENaC ion channel [6, 12, 24]. Na\(^{+}\) leaves the cell through a luminal sodium-bicarbonate co-transporter [17, 28] and via the Na\(^{+}/K\(^{+}\)-ATPase exchanger. HCO\(_3\)\(^{-}\) uptake occurs through the luminal AE and the basolateral pNBC1. The HCO\(_3\)\(^{-}\) efflux mechanisms are a luminal NBC, a basolateral AE and a basolateral HCO\(_3\)\(^{-}\) channel. HCO\(_3\)\(^{-}\) is also allowed to move through the CFTR channel, albeit at a lower conductance than Cl\(^{-}\). Finally, K\(^{+}\) efflux occurs through ion channels (K\(_{\text{Ca}}1.1\) in the LM) and uptake occurs via the Na\(^{+}/K\(^{+}\)-ATPase exchanger. Refer to the Methods for more details.

We validate our model against experimental data. Because of the modular nature of our model, we are able to investigate the effects of an electroneutral versus an electrogenic anion exchanger in the luminal membrane, and the tight junctional conductance for cations. Our model predictions are consistent with the cystic fibrosis transmembrane conductance regulator (CFTR) deletion study by Catalán et al. [6] in which they find that the CFTR deletion also results in a reduction in functional ENaC sodium channels. Additionally, we predict:

1. the luminal membrane potential of the duct must be very depolarized;
2. the CFTR channel is absorbing Cl\(^{-}\) from the lumen while the anion exchanger is secreting Cl\(^{-}\) (contrary to the currently proposed model [5]);
3. the tight junction must have a low permeability for ions.
2 Methods

We employ a radially symmetric advection-diffusion model to a fixed radius ($R_A$) lumen with length $L$, closed at the acinus end. Surrounding the lumen is a ring of epithelia with radial height, $h$. We use the following notation where $x$ is the position along the duct and $t$ is time.

$c$ is one of the following solutes: $\text{Na}^+;, \text{Cl}^-, \text{K}^+; \text{or HCO}_3^-,$

$k$ is a placeholder for $i$, $l$ or $e$ representing the cellular, luminal or interstitial compartment,

$\dagger$ is $A$ or $B$ representing the LM or BLM, respectively,

$[c]_k(x, t)$ is the concentration of the solute $c$ in compartment $k$,

$v(t)$ is the fluid velocity, positive in the direction of increasing $x$,

$N_{\dagger,c}(x)$ is the solute flux per area, across membrane $\dagger$,

$q_{\dagger,c}(x)$ is the osmotic flux per area, across membrane $\dagger$.

We follow the convention that a current or flux is positive when positive ions leave the cell.

Using standard conservation arguments, we derive differential equations for concentration, cell volume and luminal fluid velocity.

$$\frac{\partial [c]_l}{\partial t} = \frac{2N_{A,c}}{R_A} + D \frac{\partial^2 [c]_l}{\partial x^2} - v \frac{\partial [c]_l}{\partial x},$$  \hspace{1cm} (1)$$

$$\frac{d([c]_l w)}{dt} = -2\pi((R_A + h)N_{B,c} + R_A N_{A,c}),$$  \hspace{1cm} (2)$$

$$\frac{dw}{dt} = -2\pi((R_A + h)q_B - R_A q_A), \quad \text{and}$$

$$\frac{\partial v}{\partial x} = 0.$$  \hspace{1cm} (3)$$

For clarity, the variable dependence, $(x, t)$, has been suppressed. Assuming a cylindrical lumen, $[c]_l$ depends on the solute transport across the LM, and the diffusion and fluid flow along the duct. We assume a continuum epithelium model for the cells. $R_A + h$ is the distance from the center of the lumen to the basolateral membrane. We solve for $h$ using the formula $h = \sqrt{w/\pi + R_A^2} - R_A$, allowing the cell height to change as water flows
across the basolateral membrane. The cellular concentration, \([c_i]\), and the cross sectional area, \(w = \pi(2hR_A + h^2)\), are then calculated from the radial ionic \((N_{i,c})\) and osmotic \((q_i)\) fluxes, respectively. This model does not allow diffusion or transport between cells.

We assume that the LM is rigid; essentially impermeable to water \((q_A = 0)\); has a fixed radius; and that saliva is an incompressible fluid. As a result, the fluid velocity in the lumen will be equal to the primary saliva flow rate, \(v_0\), at every point along the duct (uniform in \(x\)).

**Boundary conditions.** We set the concentrations at \(x = 0\) to the primary saliva composition determined experimentally by Mangos et al. [34]. We do not have a primary saliva concentration for \(\text{HCO}_3^-\), but will assume that saliva is electroneutral and that the charge is carried by \(\text{Na}^+\), \(\text{Cl}^-\), \(\text{K}^+\) and \(\text{HCO}_3^-\). We then use electroneutrality to determine the primary saliva bicarbonate concentration: \([\text{HCO}_3^-]_l = [\text{Na}^+]_l + [\text{K}^+]_l - [\text{Cl}^-]_l\). Based on the observations of Martin and Young [35] and Mangos et al. [34], this is a reasonable assumption.

For our second boundary condition, we assume that for a sufficiently long duct, \(x = L\), the concentration in the lumen, at the end of the duct, will attain some steady state. This steady assumption is supported by experimental measurements (see Figure 5 in [34]). The saliva composition is then calculated on a more realistic length, \(x = L_f\). We found \(L = 2.5\) mm to satisfy the steady state assumption and estimated \(L_f = 1\) mm from Flint [15]. By solving the system in this manner, the concentration profile on the region of interest is not artificially affected by the steady assumption at the end of the duct.

**Numerical simulations.** To solve this system of differential equations, we employ a second order, backward differentiation formula with a first order, upwind scheme. For the very first two time steps in a simulation, we calculate one based on the initial condition, and the second using a single step of a first order backward Euler, central difference method. We use the steady state values obtained under a \(1\mu\text{m min}^{-1}\) flow and unstimulated primary saliva
concentrations as the initial condition along the duct. In Table 1 we list the primary saliva concentration.

For these numerical methods, the duct is partitioned spatially into segments of length \( \Delta x \) so that \([c]_{l,0}\) is the concentration at \(x_0\) (this is primary saliva), and \([c]_{l,n}\) is the \(n\)th partition along the duct. The cytosolic concentrations are computed using finite differences for each cell of width \( \Delta x \) at each time step. These computations have been programmed and computed in Matlab.

Luminal, cellular and interstitial transport. The flux of ions traveling from the cell via ion channels, exchangers and co-transporters is represented by \( N_{\dagger,c} \). We represent the current through an ion channel by \( I_{\dagger,c} \) and the flux from an exchanger or a co-transporter is represented by \( J_{\dagger,*}^{(\sigma)} \); \( \sigma \) will distinguish an electroneutral, (1), from an electrogenic, (2), flux; and \(*\) is a place holder for the ions involved in the flux. The various ion transport mechanisms are shown in Figure 1. The individual currents and fluxes are described in more detail below.

Thus \( N_{\dagger,c} \) represents the number of moles of ions traveling from the cell per area and has units \( \text{mol s}^{-1} \mu\text{m}^{-2} \).

Thus \( N_{\dagger,c} \) represents the number of moles of ions traveling from the cell per area and has units \( \text{mol s}^{-1} \mu\text{m}^{-2} \).

\[
N_{A,[\text{Na}]} = \frac{I_{A,\text{Na}}}{z_{\text{Na}} F} + J_{A,\text{NaHCO}_3}^{(1)},
\]

\[
N_{A,[\text{Cl}]} = \frac{I_{A,\text{Cl}}}{z_{\text{Cl}} F} + J_{A,\text{ClHCO}_3}^{(\sigma)},
\]

\[
N_{A,[\text{K}]} = \frac{I_{A,\text{K}}}{z_{\text{K}} F},
\]

\[
N_{A,[\text{HCO}_3]} = \frac{I_{A,\text{HCO}_3}}{z_{\text{HCO}_3} F} - \sigma J_{A,\text{ClHCO}_3}^{(\sigma)} + J_{A,\text{NaHCO}_3}^{(1)},
\]

\[
N_{B,[\text{Na}]} = \frac{I_{B,\text{Na}}}{z_{\text{Na}} F} + 3J_{B,\text{NaK}} + J_{B,\text{NaHCO}_3}^{(2)},
\]

\[
N_{B,[\text{Cl}]} = \frac{I_{B,\text{Cl}}}{z_{\text{Cl}} F} + J_{B,\text{ClHCO}_3}^{(1)},
\]

\[
N_{B,[\text{K}]} = \frac{I_{B,\text{K}}}{z_{\text{K}} F} - 2J_{B,\text{NaK}},
\]

\[
N_{B,[\text{HCO}_3]} = \frac{I_{B,\text{HCO}_3}}{z_{\text{HCO}_3} F} - J_{B,\text{ClHCO}_3}^{(1)} + 2J_{B,\text{NaHCO}_3}^{(2)}.
\]
The BLM has permeability $L_B$ and we represent the osmotic flux across the BLM as:

$$q_B = RT L_B \left( ([\text{Na}^+]_e + [\text{Cl}^-]_e + [\text{K}^+]_e + [\text{HCO}_3^-]_e - \left( [\text{Na}^+]_i + [\text{Cl}^-]_i + [\text{K}^+]_i + [\text{HCO}_3^-]_i + \frac{\chi}{w} \right) \right),$$

where $\chi/w$ is the concentration of impermeable ions and $RT$ is the gas constant and temperature. Because the duct is nearly impermeable to water [45], we set the LM osmotic flux to zero, $q_A = 0$.

**Membrane potential difference.** The potential difference as measured from the exterior of the cell to the cytoplasm is represented as follows:

$$C_m \frac{dV_A}{dt} = -I_{A,\text{Na}} - I_{A,\text{K}} - I_{A,\text{Cl}} - I_{A,\text{HCO}_3} - F(\sigma - 1)J_{A,\text{CHCO}_3}^\sigma,$$  

(5)

$$C_m \frac{dV_B}{dt} = -I_{B,\text{Na}} - I_{B,\text{K}} - I_{B,\text{Cl}} - I_{B,\text{HCO}_3} - F( J_{B,\text{NaK}} + J_{B,\text{NaHCO}_3}^{(2)} )$$  

(6)

for the LM and BLM, respectively. $C_m$ is the cell capacitance per membrane area in F $\mu$m$^{-2}$. As the cell capacitance for a duct cell is quite small (4-12 pF Zeng et al. [57] and personal communication with the authors of Catalán et al. [6]), we will solve equations (5)-(6) using a quasi-steady state approximation. This approximation assumes that the potential difference adjusts to changes in concentration immediately, while the ion fluxes occur more slowly. As such, we set $dV_A/dt = 0$ and $dV_B/dt = 0$ and solve for $V_A$ and $V_B$ algebraically.

**Ion channels.** We model transmembrane ion currents, $I_{\dagger,c}$, as:

$$I_{\dagger,c} = g_{\dagger,c} (V_{\dagger} - V_{\dagger,c})$$  

where

$$V_{\dagger,c} = \frac{RT}{z_c F} \log \left( \frac{[c]_k}{[c]_i} \right).$$

For the LM, $\dagger = A$ and $k = l$; for the BLM, $\dagger = B$ and $k = e$. $z_c$ is the valence of the ion $c$, $g_{\dagger,c}$ is the conductance per membrane area, and $V_{\dagger,c}$ is the Nernst potential for the channel. As is standard, $R$, $T$, and $F$ are the universal gas constant, the temperature and Faraday’s
constant, respectively.

We use the whole cell, ENaC- and CFTR-mediated current-voltage data from Catalán et al. [6] to estimate the conductances for the sodium and chloride channels (ENaC and CFTR, respectively) given in Table 2. The CFTR channel is known to be permeable to other anions such as HCO$_3^-$ [40, 41, 29]; we use the permeability ratio from Poulsen et al. [40] to determine HCO$_3^-$ conductance through the CFTR channel.

Table 2 about here.

The only potassium channel reported in the luminal membrane is the outward rectifying, maxi-K channel [36]. To our knowledge, whole cell, K$_{Ca1.1}$-mediated current-voltage data has not been measured for a duct cell. We approximated the potassium channel conductance using the whole cell cation I-V plot for a granular duct cell published by Nakamoto et al. [36], Table 2.

The basolateral membrane contains an ion channel for each ion. These conductances have been chosen to approximate the potential difference and cytosolic concentrations as listed in Table 1.

**Exchangers and co-transporters.** We derive a general three-state model similar to Palk et al. (see [37], Appendix E). We assume the exchanger/co-transporter has two distinct conformations, $I$ and $O$ where $I$ is the exchanger/co-transporter with ions bound and $O$ is unbound. We assume that the ions bind and release simultaneously. Using $C_1$ and $C_2$ to represent the ions, the three-state model can be described as

\[
O + C_1 \xrightleftharpoons[k_1^-]{k_1^+] I \xrightleftharpoons[k_2^-]{k_2^+} O + C_2.
\]

From this model we derive a system of differential equations and determine the turnover rate at steady state to be:

\[
J^{(\sigma)}_{i,*} = \frac{g_{i,*} (k_1^- k_2^- C_1 - k_2^+ k_1^+ C_2)}{k_1^- C_1 + k_2^+ C_2 + k_1^+ C_2 + k_1^-}
\]
The constant $\sigma$ represents the stoichiometry of the exchanger/co-transporter. If it is electroneutral, $\sigma = 1$, otherwise $\sigma = 2$. This general formulation of the turnover rate will be applied below to an electroneutral AE, an electrogenic AE and the luminal NBC.

For $J_{A,\text{ClHCO}_3}^{(1)}$, the electroneutral AE in the LM we use equation (9) with $C_1 = [\text{Cl}^-]_l[\text{HCO}_3^-]_l$, $C_2 = [\text{Cl}^-]_l[\text{HCO}_3^-]_l$, $k_1^+ = 0.02 \text{s}^{-1} \text{mM}^{-2}$, $k_1^- = 0.1 \text{s}^{-1}$, $k_2^+ = 0.0067 \text{s}^{-1}$, and $k_2^- = 0.018 \text{s}^{-1} \text{mM}^{-2}$.

For the basolateral anion exchanger, $J_{B,\text{ClHCO}_3}^{(1)}$, we assume the same form and rate constants as in $J_{A,\text{ClHCO}_3}^{(1)}$, replacing luminal concentrations with interstitial concentrations, $[\text{Cl}^-]_e$, $[\text{HCO}_3^-]_e$ and the density with $g_{B,\text{ClHCO}_3}$.

The electrogenic anion exchanger (stoichiometry of $1\text{Cl}^-:2\text{HCO}_3^-$) is represented by equation (9) with $C_1 = [\text{Cl}^-]_l2[\text{HCO}_3^-]_l$ and $C_2 = [\text{Cl}^-]_l2[\text{HCO}_3^-]_l$. To keep the focus on the electrogenic contribution, we held the rate constants and densities the same as the electroneutral exchanger, only introducing the electrogenic step: $k_1^+ = 0.02 \exp\left(\frac{F V_A}{RT}\right) \text{s}^{-1} \text{mM}^{-3}$, $k_1^- = 0.1 \exp\left(\frac{2F V_A}{RT}\right) \text{s}^{-1}$, $k_2^+ = 0.0067 \text{s}^{-1}$, and $k_2^- = 0.018 \text{s}^{-1} \text{mM}^{-3}$.

For the NBC in the LM we use equation (9) with $C_1 = [\text{Na}^+]_l[\text{HCO}_3^-]_l$, $C_2 = [\text{Na}^+]_l[\text{HCO}_3^-]_l$, $k_1^+ = 0.005 \text{s}^{-1} \text{mM}^{-2}$, $k_1^- = 0.03 \text{s}^{-1}$, $k_2^+ = 0.002 \text{s}^{-1}$, and $k_2^- = 0.08 \text{s}^{-1} \text{mM}^{-2}$. The rate constants and densities were fit to attain mouse SMG final saliva.

While we could design a similar three state electrogenic model for the pNBC found in the BLM, we instead implement the carefully derived, previously tested, pNBC turnover rate from Sohma et al. [49] for the basolateral pNBC, $J_{B,\text{NaHCO}_3}^{(2)}$. We use their parameters, and assume the stoichiometry is $1\text{Na}^+:2\text{HCO}_3^-$. The density, $g_{B,\text{NaHCO}_3}$, is fit to this model.

To represent $\text{Na}^+\text{-K}^+\text{-ATPase}$ exchanger, we implement a simplified model, see Appendix F in Palk et al. [37] for further details. The density, $g_{B,\text{NaK}}$, is fit to this model.

**Saliva composition.** Stimulation of acinar cells, by applying an agonist such as carbachol (CCh), increases fluid production and slightly changes the primary saliva composition [34]. We have made the assumption that carbachol concentration positively correlates to the fluid flow. To model stimulation, we increase $v_0$ from 1 $\mu$L min$^{-1}$ to 8 $\mu$L min$^{-1}$ for 0.3 $\mu$M CCh.
+ 5 µM isoproterenol (IPR) and to 20 µL min⁻¹ for 0.5 µM CCh. In addition, we alter the concentration of the primary saliva, or equivalently, the boundary condition at the beginning of the duct, as shown in Table 1.

We convert the flow rate from µL min⁻¹ to µm s⁻¹ assuming the average SMG has a mass of 67 mg, that there are $2.26 \times 10^7$ acinar cells per 100 mg [37], the duct has a radius of 3 µm, and that an acinus has 6 cells.

Table 3 about here.

To compare our results to Nakamoto et al. [36] and Catalán et al. [6], we compute the luminal solute concentrations on a duct of length $L = 2.5$ mm for 10 minutes under unstimulated conditions to allow the system to reach a steady state. We then stimulate the gland for 10 minutes, and begin collecting the final saliva. After 10 minutes we stop stimulating the gland. For the Nakamoto et al. [36] experiments, the saliva collection period is complete; for the Catalán et al. [6] experiments, we continue collecting unstimulated saliva for a further 10 minutes.

Concentrations in the collected final saliva are estimated by computing the average at $x = L_f$ over the collection period.

$$[c]_f = \frac{\int_0^T [c]_l(L_f, t)v\pi R_A^2 dt}{\int_0^T v\pi R_A^2 dt},$$

where $[c]_f$ represents the final saliva concentration for solute $c$; $t$ is from 0 to $T$, and $T = 10$ or $T = 20$ minutes depending on the experimental collection time; $[c]_l(L_f, t)$ is the concentration of the solute at $x = L_f$; $v$ is the fluid velocity; and $R_A$ is the radius of the duct. The choice of $L_f$ (determined in the Boundary Conditions, above) will affect the final saliva composition; the smaller the value of $L_f$, the less duct available for electrolyte exchange,
resulting in higher NaCl and lower K in the final saliva. Table 4 lists the WT final saliva composition at $L_f = 1.0$ mm and at $L_f \pm 10\%$.

Table 4 about here.

### 3 Results

**Wild type model.** In an early model of the salivary duct, we modeled the AE as the primary mechanism for absorbing chloride ions while the CFTR channel recycled Cl$^-$ back into the lumen. This is the traditional model of chloride transport in the duct, (see for example Boron and Boulpaep [5]). In the wild type model, we could obtain the appropriate final saliva concentrations if we included an active potassium flux. However we were unable to capture the observed increase in chloride concentration associated with a CFTR channel deletion or block. Once the CFTR channel was compromised, modeled by a severe decrease in conductance, the model predicted a decrease in final saliva chloride, contrary to the data (Figure 3B). Due to this result, we determined that the CFTR channel must be absorbing chloride. Therefore, the potential difference across the luminal membrane must be depolarized enough to allow for chloride absorption until final saliva chloride concentrations are attained. Further, for the CFTR channel and the AE to reach a steady state, the AE must be moving chloride from the cell to the lumen at low luminal chloride concentrations.

With a luminal potential difference of about $-20$ mV in the wild type model, a potassium channel is sufficient to attain final saliva potassium composition. Further, any CFTR channel dysfunction results in increased final saliva chloride, as desired.

For our parameter values, we incorporate channel conductances from data whenever possible, and fit the remaining parameters, Table 2. This model predicts final saliva composition under two different experimental conditions: 0.3 $\mu$M CCh + 5 $\mu$M isoproterenol (IPR) [6] (Figures 2 and 3) and 0.5 $\mu$M CCh [36] (Figure 4). Additionally, the model predicts that the
cytosolic concentrations are essentially constant along the length of the duct. At steady state \((v = 1 \ \mu \text{L min}^{-1})\) the maximal changes along the duct are 0.1, 0.3, 0.3, and 0.2 mM, for \([\text{Na}^+]_i, [\text{Cl}^-]_i, [\text{K}^+]_i,\) and \([\text{HCO}_3^-]_i,\) respectively.

We have tested the sensitivity of our parameter choices by comparing the steady state final saliva resulting from changing one parameter by \(\pm 10\%\) to the wild type steady state saliva composition. The parameters tested were the densities and conductances in Table 2 as well as each of the rate constants in the three-state AE and NBC (see Methods). The ENaC conductance proved to be the most sensitive resulting in a maximal change of around 11\%. All other parameters resulted in changes \(\leq 7\%\).

**Channel blocks and deletions.** To validate our model, we compute the final saliva composition and compare to the results of Catalán et al. [6] and Nakamoto et al. [36]. In these papers they investigate how various channel deletions and blockers affect the mouse SMG duct by measuring the final saliva composition of the gland. A channel deletion or block can be modeled by a reduction in the conductance. Since we do not know how strongly each deletion or chemical block affects the channel, we determine the reduction in conductance by fitting our model to the data. The conductance parameters we used to fit the model to the data are listed in Table 2.

In the bar charts depicting final saliva ion composition (Figures 2-4), the solid bars represent the model while the striped bars are data from Catalán et al. [6] and Nakamoto et al. [36]. We represent the following, in order from left to right: sodium, potassium, chloride, bicarbonate and the osmolality. The wild type or control experiment is represented by a white bar for the model, or a sparsely striped bar for the data, while the blocked or deleted channel experiment is represented by a shaded bar for the model and a densely striped bar for the data. When the bicarbonate or osmolarity data was not published, as in Catalán et al. [6], we have approximated the bicarbonate value under the assumption that \([\text{HCO}_3^-]_l = [\text{Na}^+]_l + [\text{K}^+]_l - [\text{Cl}^-]_l,\) i.e. that the luminal composition is electroneutral. However, our approximation may be slightly
Catalán et al. [6] focus on the deletion and block of the CFTR channel and the block of the ENaC channel. These are the primary chloride and sodium channels found in the luminal membrane of the salivary duct cells. We show their ex-vivo results in Figures 2, 3 and 4B.

**ENaC channel block.** Amiloride is known to block the ENaC sodium channel, resulting in an increased NaCl concentration in final saliva. We model this chemical block by reducing the value of $g_{Na^+}$, the ENaC channel conductance, by 80%. Our model accurately captures the experimental findings, Figure 2A.

**CFTR channel block.** We next consider the chemical block of the CFTR channel. In the experiment, Catalán et al. [6] use a highly selective CFTR channel inhibitor, CFTR$_{inh}$-172. We find a reduction in the conductance to 15% of the wild type CFTR channel conductance provides a good fit the CFTR blocked channel data, Figure 2B.

![Figure 2 about here.](image)

Our model, consistent with the data, finds an increase in luminal chloride, and a slight increase in sodium. Our model also predicts an increase in potassium. This potassium increase is due to the luminal membrane becoming even more depolarized, an event which does not seem to happen in the mouse model (compare potassium concentrations from the model and data in Figure 2B).

**CFTR mutation.** For both blocked channels, our model is in good agreement with the data. The final dataset we consider from the Catalán et al. [6] paper is a CFTR mutation. Interestingly, the final sodium concentration in the data is much higher in the CFTR mutation (Figure 3) when compared to the CFTR channel block (see Figure 2B).

In Figures 3 and 4B we compare several variations of our model to the CFTR ΔF508 dataset from Catalán et al. [6]. We first present the model with the CFTR channel conductance greatly reduced to represent the cystic fibrosis (CF) mouse model carrying the ΔF508
mutation in the CFTR channel. As Figure 3A shows, we are unable to accurately capture the sodium concentration for the CF mouse model. Note that for ease of comparison, we reduce the conductance by the same value we used in the CFTR channel block in Figure 2B, where we were able to represent the data accurately.

Catalán et al. [6] also note the difference in sodium concentration between the CF mouse model and the CFTR channel block. They find that the whole cell ENaC-mediated sodium inward current decreases and the amount of $\alpha$-ENaC protein, a protein necessary for ENaC functionality [3], is reduced in the mouse model carrying the $\Delta F508$ mutation. We reduce the ENaC channel density by 60%, in addition to the CFTR reduction, and find we are able to accurately capture the sodium concentrations reported in the CF mouse model [6], Figure 3B.

\[ K_{Ca,1.1} \text{ channel deletion.} \] Nakamoto et al. [36] find the $K_{Ca,1.1}$ channel is the primary pathway involved in $K^+$ efflux. In Figure 4A we compare our model to the data published in Nakamoto et al. [36]. This data comes from a gland stimulated with 0.5 $\mu$M CCh [36], whereas the Catalán et al. [6] experiments use 0.3 $\mu$M CCh and IPR. The higher level of agonist suggests a faster flow rate, resulting in less NaCl absorption. However, as Romanenko et al. [43] showed, when IPR and CCh are applied, the final saliva chloride concentrations are considerably lower than when IPR is not included, presumably because CFTR is activated by IPR. In order to obtain the experimental results, the $K_{Ca,1.1}$ deletion is represented here as a reduction to 1% of the wild type $K_{Ca,1.1}$ channel conductance. We have also reduced the CFTR conductance by 50% to account for the different agonist response.

\[ \text{Cl}^-/\text{HCO}_3^- \text{ exchanger.} \] Zhao et al. [58] demonstrated the existence of a chloride-bicarbonate exchanger in the luminal and basolateral membranes of duct cells. Shcheynikov et al. [46]
found the electrogenic chloride-bicarbonate exchanger, SLC26A6, to be responsible for the majority of bicarbonate transport in the LM of parotid duct cells. However Shcheynikov et al. [46] also show immunolocalization of SLC26A4, an AE thought to be electroneutral [13], to the LM of submandibular ducts.

We compare the effect of an electrogenic (1Cl\(^{-}\):2HCO\(_{3}^{-}\)) versus an electroneutral AE. Then we investigate the dependence of the AE activity on the CFTR channel. In Figure 5A, we compare the luminal concentration profiles along the first 1.0 mm of the duct (at steady state) produced by the model with an electrogenic vs an electroneutral AE in the LM. In the right panel, the electrogenic AE, \(J_{\text{ClHCO}_3}^{(2)}\), is active while the left panel has the same parameter values except the AE is electroneutral, \(J_{\text{ClHCO}_3}^{(1)}\). We have not refit any parameters to obtain this data. Some differences exist along the duct, but nothing which clearly suggests which stoichiometry should be used in the duct. At 1.0 mm, the electrogenic exchanger results a decrease of 1.3 mM Na\(^{+}\), 6.5 mM K\(^{+}\) and 13.7 mM HCO\(_{3}^{-}\) in the lumen, while the Cl\(^{-}\) increases 5.9 mM. The potential difference across the LM is also decreased 3.5 mV. We have adopted the electroneutral AE for the figures in this paper.

Figure 5 about here.

The CFTR channel activity has also been tied to the activity of the anion exchanger [50, 46]. In Figure 4B, we have reduced the electroneutral anion exchanger density, \(g_{\text{ClHCO}_3}\) to 60% (black bars) to 40% (gray bars) and to 20% (white filled bars) of the wild type value; all other parameters are as in Figure 3B. As the AE activity is reduced, the final saliva Cl\(^{-}\) and HCO\(_{3}^{-}\) are affected, but not greatly.

**Duct luminal membrane is depolarized.** When the CFTR channel is mutated or blocked, the chloride concentration found in final saliva is greatly increased [6, 19]. To investigate the requirements this places on the luminal membrane potential, consider the luminal Cl\(^{-}\) current, equation (7). To obtain the desired final saliva concentrations, the CFTR channel
current must be positive until \([\text{Cl}^-]_l \approx 38 \text{ mM} \) [34, 6]. Using equation (7) and \([\text{Cl}^-]_l = 38\), and \([\text{Cl}^-]_i = 25 \text{ mM} \) [56] with the restriction that the current must be positive, we estimate \(V_A \geq -11 \text{ mV}\). This seems very depolarized, but by reducing \([\text{Cl}^-]_l\) to 18 mM, we can decrease this bound to \(V_A > -20 \text{ mV}\). We are unaware of any experimental results reporting a salivary duct luminal potential difference.

Tight junction has low permeability to cations. We explored the possibility of allowing cations to move across the tight junction, by implementing the following current:

\[
I_{t,c} = g_t \left( V_A - V_B \right) - \frac{RT}{z_K F} \log \left( \frac{[c]_l}{[c]_c} \right),
\]

(11)

where \(g_t\) is the tight junctional conductance; and \(c\) is either \(\text{Na}^+\) or \(\text{K}^+\). We find we can attain the final saliva measured by Catalán et al. [6] and Nakamoto et al. [36] when the tight junctional conductance is \(g_t = 0.1 \times g_{A,K^+}\). This value of \(g_t\) correlates to a resistance of 70,000 \(-250,000 \Omega/\text{cm}^2\), consistent with other tight epithelia [2]. However, if the tight junctional conductance is increased to \(g_t = g_{A,K^+}\), the resulting final saliva is high in \(\text{Na}^+\) (99 \(-115 \text{ mM}\)) and low in \(\text{K}^+\)(24 \(-25 \text{ mM}\)), when simulating the Catalán et al. [6] and Nakamoto et al. [36] experiments, respectively.

For the simulations presented in this paper, we have not included any tight junctional current. We have observed through simulations that we can include a tight junctional current, but it must be at least an order of magnitude smaller than the \(K_{Ca1.1}\) cannell conductance. In addition, the duct is considered to be a very tight epithelium, also suggesting there is essentially no tight junctional flow.

4 Discussion

There are three major salivary glands in most animals, the parotid, the sublingual and the sub-mandibular (or submaxillary) glands. The parotid gland produces a serous, watery secretion.
and is the largest of the glands. The other two glands produce a serous and mucous solution. Whenever possible, we used data from the SMG from the mouse. We choose the SMG gland because the majority of the experimental data on the salivary ducts seem to come from this gland. One possible reason for this preference may be because the NaCl absorption is more extreme in comparison to the parotid gland [34, 33, 32, 54].

We chose the mouse model for a similar reason. Due to the high density of the CFTR channel in the salivary ducts, researchers often use salivary ducts to study cystic fibrosis. Because of the mouse cystic fibrosis model system [55], many of the recent salivary duct experiments have been performed on the mouse model [6, 36, 7, 20, 26, 56].

However, mouse SMG duct is not necessarily a good model for all salivary gland ducts in all animal models. Young and Schneyer [54] reviewed the various animal models and their differences. At maximal rates of pilocarpine stimulated secretion, rat SMG saliva is reported to have 5 mM Na\(^+\) and 35 mM K\(^+\) while the rat parotid saliva has 115 mM Na\(^+\) and 10 mM K\(^+\) and finally, monkey SMG saliva has 90 mM Na\(^+\) and 40 mM K\(^+\). Just considering these three, the variation from animal to animal in the same gland, or gland to gland in the same animal is so large, one model cannot hope to describe all possible scenarios. However, with the model that we have developed, it is straightforward to change conductances and densities or to introduce/remove an ion transporting mechanism. It is in this way that we can use this model as a tool and apply it to other projects. For example, one avenue of particular interest is studying the effects of genes introduced using viral vectors, such as ductal aquaporins, [10]. In general, the structure remains the same, so the only changes necessary are the primary saliva compositions and the ion transporting mechanisms found along the duct.

**Comparisons to other epithelial models.** To the best of our knowledge, there does not exist another model of a salivary duct or salivary duct cell. However, there are many other models of tubules with ion transport across the membrane; for some examples see Weinstein [51], Dias et al. [11], Keener and Sneyd [22]. There are also other models of exocrine gland
duct cells, some examples of which are Sohma et al. [48, 49], Whitcomb and Ermentrout [52], Yamaguchi et al. [53].

Our model, in construction, is similar to other tubule transport models in that we have some flow along a tube, and ion flux across the membrane. In particular, the ascending loop of Henle in the kidney is water impermeable and ion absorbing. However, the set of ion transporting mechanisms known to exist in duct cells differs from any of the tubule models we are aware of.

The exocrine duct cells models [48, 49, 53, 52] are all of pancreatic cells. They represent ion transport mechanisms in the LM and BLM, and as a result have a system of differential equations for the cytosolic and luminal concentrations. The major differences between the pancreatic models and our model are (i) a water permeable versus impermeable membrane, (ii) that the final pancreatic juice is near isotonic and high in NaHCO$_3$ whereas final saliva is hypotonic and low in NaCl, and (iii) that they model a single cell while we model a section of duct.

**CFTR channel and membrane potential.** Although the CFTR channel has been modeled as a Cl$^-$ secreting mechanism in the pancreas, we find the CFTR channel in the duct must be Cl$^-$ absorbing. In particular, if Cl$^-$ ions are being secreted by the CFTR channel, when the channel is blocked or deleted, the concentration in the lumen will decrease. However, that is not what has been observed in the SMG duct, [6, 19]. In further support of this, Ishibashi et al. [19] injected either CFTR-siRNA or CFTR$_{inh}$-172 into the duct lumen and reported an increase in final saliva Cl$^-$ in both experiments.

Because it has been previously proposed that the CFTR channel and the anion exchanger are reversed, where Cl$^-$ is secreted through the CFTR and absorbed through the anion exchanger [5], we investigated our model under these conditions. For the CFTR channel to secrete Cl$^-$, the membrane potential difference must be larger. With this change several problems arose. First, we were unable to find an ion with enough charge difference across
the luminal membrane to set the potential difference. Furthermore, any impairment of the CFTR channel resulted in a decrease, rather than increase as seen in the block and deletion data [6], in the final saliva Cl\(^-\) concentration. Even if we set the potential difference artificially, we were unable to find a set of valid parameters in which the impaired CFTR channel resulted in an increase in final saliva chloride.

In Ko et al. [23], using a trans-epithelial voltage and the basolateral membrane potential measurements from the literature, they estimate the luminal membrane potential for a pancreatic cell to be \(-20\) to \(-30\) mV. However, we are not aware of similar measurements in the salivary duct. With the final saliva being low in sodium and chloride while high in potassium, the membrane potential could vary greatly from a pancreatic cell. In addition, the trans-epithelial voltage reported for the salivary duct ranges greatly, from \(-71\) mV in rat to \(-13\) mV in rabbit [14] and to the best of our knowledge, no basolateral membrane potential has been reported. Given the available evidence, we predict that the luminal membrane of the duct will be depolarized.

Another variation on the behavior of the CFTR channel has been suggested by Park et al. [38]. They have observed that the ratio of HCO\(_3\) \(^-\) permeability to Cl\(^-\) permeability, through the CFTR channel, can change. They propose that in the proximal duct, the CFTR channel is primarily a Cl\(^-\) channel, but smoothly changes to primarily a HCO\(_3\) \(^-\) channel by the distal duct. In our model we are able to attain reasonable values for bicarbonate concentration in the final saliva with constant CFTR conductance along the duct, and hence do not find it necessary to consider variation of the current carried by CFTR in this manner.

**Anion exchanger.** We have investigated either an electroneutral or electrogenic (1Cl\(^-\) : 2HCO\(_3\)^-) anion exchanger. To the best of our knowledge, the electrogenic SLC26a6 has only been found in the parotid ducts, not the SMG ducts. Here, we aim to model the SMG duct, but we include the electrogenic exchanger to understand the affect, if any, the electrogenic exchanger may have. In particular, we wanted to see how the exchanger affected the membrane poten-
tial, and if the direction of Cl\(^-\) through the CFTR channel could be reversed (Cl\(^-\) secreting) while still achieving an increased Cl\(^-\) concentration in the case of a blocked or deleted CFTR channel. Having explored all of the deletions and blocks, using the exact same parameters and conditions except for the AE representation, we found the differences in the final saliva concentration to be so near the experimental error that the two models are essentially the same. It is possible that in the parotid ducts the electrogenic AE has a more obvious contribution to the final saliva, but for the mouse SMG, the electrogenicity of the anion exchanger has little effect on the overall behavior of the duct.

We have fit the rate constants as well as the densities of the ion exchangers and co-transporters to the data. For each parameter (rate constants, conductances, densities) we have tested the sensitivity by calculating the final saliva obtained when the parameter is altered by \(\pm 10\%\). The most sensitive parameter is the ENaC conductance which results in a change as large as \(10\%\) in final saliva sodium. All other parameters result in \(\leq 6\%\) change.

If we set the membrane potential artificially so that the CFTR channel is secreting, we can find parameter values which generate realistic SMG saliva concentrations. However, we are unable to capture the experimental results in the case where the CFTR channel is blocked or deleted. Even when we reduce the anion exchanger activity by 40, 60 or 80 percent, if the CFTR channel is secreting, the CFTR channel deletion causes a decrease in final saliva Cl\(^-\), opposite to the experimental observations. For the parameter values listed in Table 2, the CFTR channel absorbs Cl\(^-\) into the cell, and the anion exchanger secretes Cl\(^-\) into the lumen at steady state.

We investigated reducing the anion exchanger flux in the luminal membrane for the CFTR \(\Delta F508\) mutation, but not for the CFTR channel block. Wang et al. [50] found that the chemical block for CFTR, CFTR\(_{inh}\)-172, did not inhibit Cl\(^-\)/HCO\(_3^\-\) exchange activity in wild type pancreatic ducts. However, Wang et al. [50] and Shcheynikov et al. [46] both report siRNA against \(CFTR\) inhibits/alters Cl\(^-\)/HCO\(_3^\-\) exchange activity. Our model suggests that the an-
ion exchanger does not appear to contribute significantly to alterations in final saliva due to CFTR channel dysfunction.

**Bicarbonate.** Bicarbonate is an essential buffer for pH in saliva. However, it is difficult to measure directly. As such, concentrations of bicarbonate along the duct are unknown. However, because the salivary gland is thought of as a bicarbonate secreting epithelium, we felt the inclusion of bicarbonate as a variable in the final saliva composition was useful. Here we have provided one scenario, based on the available data.

We have made an assumption that final saliva is electroneutral. Fortuitously, our model \([\text{HCO}_3^-]_l\) is relatively constant as flow rate is increased, which looks quite similar to the mouse SMG displayed in Young and Schneyer [54] (Figure 5B). In addition, we have found the luminal bicarbonate to be rather robust as we changed the anion exchanger from electroneutral, as described by Lee et al. [27], to electrogenic, as found in the parotid [23, 46].

**Cellular and luminal pH.** We realize the our pH assumption may result in a coarse approximation of the duct behavior. pH is obviously an important component which cannot be disregarded entirely. In particular, understanding the transporters which control pH are likely key to understanding the mechanisms that affect caries progression. Unfortunately, we are not aware of sufficient data being available to constrain these fluxes in the model.

As a first attempt at a salivary duct model, we have assumed that the changes in cellular and luminal pH are not large enough to have a significant affect on the mechanisms represented in this model. This simplification helps us to reduce the complexity of the salivary duct and create a reasonable first approximation. In both compartments pH is, in reality, affected by sodium proton exchangers (NHE) (not represented in the model) which in turn may alter both the bicarbonate and the sodium concentrations in the model. Further, It has been shown that the parallel activity of the apical NHE and the AE can mediate NaCl absorption in some epithelia (for example [25]). However, Catalán et al. [6] found that blocking the ENaC channel with lower concentrations of amiloride nearly abolished the NaCl reabsorption, sug-
suggested that this channel is the primary Na\textsuperscript{+} influx mechanism. In addition, they found that the \textit{Nhe2-/-} and \textit{Nhe3-/-} mice did not have impaired NaCl reabsorption. This may indicate that NHE is not necessary for Na\textsuperscript{+} absorption, or that the NHE is inhibited when the gland is stimulated, or it could be that other mechanisms are compensating for the loss of NHE in the Catalán et al. [6] knockout experiments. Obviously, this is an area which will require further investigation and modeling. Due to the success of our simulations capturing the wild type behavior as well as the observed results associated with several impaired or knocked out transporters, we are optimistic that this simplified form will provide insight.

\textbf{Flow rate.} We have used data from Nakamoto et al. [36] and Catalán et al. [6] to validate our model. Both report on ex-vivo experiments where glands are stimulated by carbachol, but the concentrations of carbachol differ between the papers. Surprisingly, Catalán et al. [6] report a higher flow rate with 0.3 \textmu M CCh than Nakamoto et al. [36] with 0.5 \textmu M CCh. The flow rate differences may be explained by the changes in protocol since both the CCh concentration and the temperature during the experiments were changed; The Nakamoto et al. [36] data was collected at room temperature while the Catalán et al. [6] data was collected at 37\degree C. In addition, Catalán et al. [6] also apply IPR during stimulation. However, since it is well known that increasing carbachol increases the flow rate, we have assumed a maximal flow rate of 8 \textmu L min\textsuperscript{-1} for the Catalán et al. [6] data, and a maximal flow rate of 20 \textmu L min\textsuperscript{-1} for the Nakamoto et al. [36] data. To convert from a whole gland flow rate to an acinar flow rate (the input flow, \(v_0\), for this model) the rate must be converted from \textmu L min\textsuperscript{-1} to \mu m s\textsuperscript{-1}. As such, in addition to the glandular flow rate, we must have an estimate for the number of acinar cells per gland, the number of acinar cells per acinus and the radius of the duct. For simplicity we have assumed fixed values for all parameters except the flow rate, and represent all stimulation by changes in the primary saliva and flow rate.

The final saliva composition is also dependent on the flow rate. At a low flow rate, the \([K^+]_l\) is high and the \([Na^+]_l\) and \([Cl^-]_l\) are low. As the flow rate is increased, the concentr-
tions adjust: $[K^+]_l$ decreases while $[Na^+]_l$ and $[Cl^-]_l$ increase (Figure 5B) before reaching a steady state similar to primary saliva. This is the same behavior reported by Mangos et al. [34] in the mouse parotid gland.

Strangely they do not observe the same behavior in the mouse SMG. Instead all three ion concentrations, $[Na^+]_l$, $[Cl^-]_l$, and $[K^+]_l$ decrease before reaching a plateau. This difference could result from the choice of agonist, pilocarpine, used by Mangos et al. [34]. Romanenko et al. [44] showed that the SMG and the parotid gland have significantly different responses to pilocarpine.

**Future directions.** The duct has been represented here as a radially symmetric tube of length $L$. We do not represent any ductal branching or change in radius. It would be straightforward to include a changing radius, though Schneyer et al. [45] states that the luminal diameter of any part of the ductal network, excluding the excretory duct, averages 4 μm. Our aim has been to represent a basic secretory unit. In the future we would like to continue this model to include branching, but for simplicity we have restricted our initial model to an unbranched duct.

In our model, we have assumed that pH does not affect these ion transporters. An important next step would be to explore the effects of modeling the pH along the duct. We expect pH to have an effect on bicarbonate due to buffering, but pH may also affect the membrane potential and the sodium concentration, through the ENaC channel [42].
Acknowledgements

KP thanks Ted B. Begenisich at the University of Rochester and Ivo Siekmann at the University of Auckland for their comments on an earlier version of this article. This work was supported by the National Institutes of Health (NIH) Grant R01-DE19245.
A.1 References


[46] Shcheynikov N, Yang D, Wang Y, Zeng W, Karniski LP, So I, Wall SM, Muallem S. The Slc26a4 transporter functions as an electroneutral Cl\textsuperscript{−}/I\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchanger: role of Slc26a4 and Slc26a6 in I\textsuperscript{−} and HCO\textsubscript{3}\textsuperscript{−} secretion and in regulation of CFTR in the parotid duct. *J Physiol*, 586, 3813–24, 2008.


Figure 1: Basic secretory unit. A basic secretory unit consists of a cluster of acinar cells and a section of duct. Each acinar cell could be modeled as in Palk et al. [37] or Gin et al. [16]. In this paper we describe how to model the duct. Above and below are enlarged basolateral and luminal membranes, respectively. The ion pathways are described in further detail in the Introduction as well as the Methods.

Figure 2: Final saliva comparisons between model simulations and data for wild type and chemically blocked channels. A: Application of amiloride blocks the ENaC sodium channel. We represent this as a reduction in the sodium channel conductance ($g_{\text{block Na}^+} = 0.2 \times g_{A,\text{Na}^+}$). B: CFTR channel chemically blocked ($g_{\text{block Cl}^-} = 0.15 \times g_{A,\text{Cl}^-}$).

Figure 3: Model variations against data from the wild type and CFTR$^{\Delta F/\Delta F}$ mouse final saliva. The CFTR$^{\Delta F/\Delta F}$ mouse model has the CFTR channel deleted. A: The CFTR channel conductance is reduced ($g_{\Delta \text{Cl}^-} = 0.15 \times g_{A,\text{Cl}^-}$). Notice how the model sodium concentration for the deletion is unable to capture behavior as measured in the CFTR$^{\Delta F/\Delta F}$ mouse. B: In addition to the CFTR channel reduction performed in A, the ENaC channel conductance is also reduced ($g_{\Delta \text{Na}^+} = 0.4 \times g_{A,\text{Na}^+}$).

Figure 4: A. Comparison between our model and the wild type and K$^{\text{Ca}_{1.1}}$ channel deletion final saliva [36]. The K$^{\text{Ca}_{1.1}}$ channel deletion is represented by a reduction in channel conductance, $g_{\Delta \text{K}^+} = 0.01 \times g_{A,\text{K}^+}$. We also reduce the CFTR conductance due to the absence of IPR [36], $g_{\text{No IPR Cl}^-} = 0.5 \times g_{A,\text{Cl}^-}$. A further reduction in the channel conductance will raise the final Cl$^-$ concentration. B. Anion exchanger activity is reduced and compared to the CFTR$^{\Delta F/\Delta F}$ mouse. In addition to the parameter modifications in Figure 3B, the anion exchanger activity is also reduced to 60\%, $g_{\Delta \text{HCO}_3} = g_{A,\text{HCO}_3} \times 0.6$ (in black); to 40\%, $g_{\Delta \text{HCO}_3} = g_{A,\text{HCO}_3} \times 0.4$ (in gray); and to 20\%, $g_{\Delta \text{HCO}_3} = g_{A,\text{HCO}_3} \times 0.2$ (in white). Slight decreases in Cl$^-$ and increases in HCO$_3^-$ occur as the activity is reduced.
Figure 5: A. Luminal concentration and potential difference along the duct for the electrogenic and the electroneutral anion exchanger. The luminal concentration profile from our model has the same behavior as in Figure 5 from Mangos et al. [34]. For these figures, we show the saliva composition spatially along the first 1.0 mm of the wild type salivary duct at steady state under low flow (1 μm min⁻¹), unstimulated conditions. Notice how the bicarbonate profile is nearly constant along the duct. B. Concentration of ions versus flow rate. The circles and squares represent ion concentration at 1.0mm along the duct for the given flow rate. The units are scaled so that a flow rate of one (dashed line) is equal to the flow rate we used when modeling the Catalán et al. [6] data. As the flow rate increases (along the x-axis), it is clear that the Na⁺ and Cl⁻ absorption decreases, resulting more Na⁺ and Cl⁻ in final saliva. Similarly, K⁺ secretion decreases, resulting in less K⁺ in final saliva. This is comparable to Figure 1 in Mangos et al. [34]
<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>Experimental Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytosolic ion concentrations</strong></td>
<td></td>
</tr>
<tr>
<td>$[\text{Na}^+]_i$</td>
<td>14.1 mM</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_i$</td>
<td>18 mM</td>
</tr>
<tr>
<td>$[\text{K}^+]_i$</td>
<td>142 mM</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]_i$</td>
<td>19 mM</td>
</tr>
<tr>
<td>$\chi/w_0$</td>
<td>80 mM</td>
</tr>
<tr>
<td>$w_0$</td>
<td>$2.09 \times 10^{-12}$L</td>
</tr>
<tr>
<td><strong>Primary saliva ($x = 0$) (stimulated)</strong></td>
<td></td>
</tr>
<tr>
<td>$[\text{Na}^+]_l$</td>
<td>157.2 mM</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_l$</td>
<td>122.9 mM</td>
</tr>
<tr>
<td>$[\text{K}^+]_l$</td>
<td>4.9 mM</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]_l$</td>
<td>39.2 mM</td>
</tr>
<tr>
<td><strong>Primary saliva ($x = 0$) (unstimulated)</strong></td>
<td></td>
</tr>
<tr>
<td>$[\text{Na}^+]_l$</td>
<td>150.7 mM</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_l$</td>
<td>124.3 mM</td>
</tr>
<tr>
<td>$[\text{K}^+]_l$</td>
<td>5.6 mM</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]_l$</td>
<td>32 mM</td>
</tr>
<tr>
<td><strong>Interstitial ion concentrations</strong></td>
<td></td>
</tr>
<tr>
<td>$[\text{Na}^+]_e$</td>
<td>140.2 mM</td>
</tr>
<tr>
<td>$[\text{Cl}^-]_e$</td>
<td>102.6 mM</td>
</tr>
<tr>
<td>$[\text{K}^+]_e$</td>
<td>5.3 mM</td>
</tr>
<tr>
<td>$[\text{HCO}_3^-]_e$</td>
<td>24.7 mM</td>
</tr>
<tr>
<td><strong>Potential difference at the start of duct ($x = 0$)</strong></td>
<td></td>
</tr>
<tr>
<td>$V_A$</td>
<td>-36.6 mV</td>
</tr>
<tr>
<td>$V_B$</td>
<td>-57.3 mV</td>
</tr>
<tr>
<td><strong>Initial velocity ($x = 0$)</strong></td>
<td></td>
</tr>
<tr>
<td>$v_0$</td>
<td>1 $\mu$L min$^{-1}$</td>
</tr>
<tr>
<td>$v_0$</td>
<td>8 $\mu$L min$^{-1}$</td>
</tr>
<tr>
<td>$v_0$</td>
<td>20 $\mu$L min$^{-1}$</td>
</tr>
</tbody>
</table>
### Table 2: Conductance parameters

<table>
<thead>
<tr>
<th>Conductance (nS µm$^{-2}$)</th>
<th>Conductance (nS µm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{A,Na^+}$</td>
<td>1.9/$S$</td>
</tr>
<tr>
<td>$g_{A,Cl^-}$</td>
<td>7.7/$S$</td>
</tr>
<tr>
<td>$g_{A,K^+}$</td>
<td>3.0/$S$</td>
</tr>
<tr>
<td>$g_{A,HCO_3^-}$</td>
<td>$\frac{1}{3}g_{Cl^-}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Density ($\times 10^{-14}$ mol µm$^{-2}$)</th>
<th>Density ($\times 10^{-14}$ mol µm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{A,NaHCO_3}$</td>
<td>2.1/$S$</td>
</tr>
<tr>
<td>$g_{A,ClHCO_3}$</td>
<td>2.26/$S$</td>
</tr>
<tr>
<td>$g_{B,NaK}$</td>
<td>0.15/$S$</td>
</tr>
</tbody>
</table>

**Deletion/block conductances (fit to data)**

- $g_{\text{block } Na^+}$: $0.2 \times g_{A,Na^+}$
- $g_{\text{block } Cl^-}$: $0.15 \times g_{A,Cl^-}$
- $g_{\Delta Cl^-}$: $0.15 \times g_{A,Cl^-}$
- $g_{\Delta Na^+}$: $0.4 \times g_{A,Na^+}$
- $g_{\Delta ClHCO_3}$: $r \times g_{A,ClHCO_3}$
- $g_{\Delta K^+}$: $0.01 \times g_{A,K^+}$
- $g_{\text{No IPR } Cl^-}$: $0.5 \times g_{A,Cl^-}$

- ENaC block
- CFTR block
- CFTR $\Delta F/\Delta F$
- ENaC reduction from CFTR $\Delta F/\Delta F$
- CFTR $\Delta F/\Delta F$ dependent reduction, $r = 0.2, 0.4$ or $0.6$
- KCa1.1 deletion lower conductance in absence of IPR
Table 3: Duct parameters

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S$</td>
<td>2123.7 $\mu$m$^2$</td>
</tr>
<tr>
<td>$L$</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>$L_f$</td>
<td>1000 $\mu$m</td>
</tr>
<tr>
<td>$R_A$</td>
<td>3 $\mu$m</td>
</tr>
<tr>
<td>$D$</td>
<td>800 $\mu$m$^2$s$^{-1}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$</td>
</tr>
<tr>
<td>$R$</td>
</tr>
<tr>
<td>$F$</td>
</tr>
<tr>
<td>$L_B$</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Table 4: WT final saliva composition when $L_f$ is 0.9, 1.0 and 1.1 mm.

<table>
<thead>
<tr>
<th></th>
<th>0.9 mm</th>
<th>1.0 mm</th>
<th>1.1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[Na^+]_l$</td>
<td>44.4 mM</td>
<td>39.5 mM</td>
<td>35.6 mM</td>
</tr>
<tr>
<td>$[K^+]_l$</td>
<td>52.7 mM</td>
<td>55.3 mM</td>
<td>57.6 mM</td>
</tr>
<tr>
<td>$[Cl^-]_l$</td>
<td>48.5 mM</td>
<td>46.3 mM</td>
<td>44.6 mM</td>
</tr>
<tr>
<td>$[HCO_3^-]_l$</td>
<td>48.5 mM</td>
<td>48.5 mM</td>
<td>48.5 mM</td>
</tr>
</tbody>
</table>

Following the same method we used to simulate the Catalán et al. [6] data, we find a 10% change in duct length ($L_f = 1.0 \pm 0.1$ mm) has the largest effect on final saliva sodium, resulting in a maximum change of 12.3%, or 4.9 mM.
Table 5: Steady-state solution at $x = 1.0$ mm, $v_0 = 1 \mu$L min$^{-1}$

<table>
<thead>
<tr>
<th>Model</th>
<th>Solution</th>
<th>Experimental Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary saliva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[Na^+]_t$</td>
<td>29.2 mM</td>
<td>4.9 – 30 mM [34]-[6]</td>
</tr>
<tr>
<td>$[K^+]_t$</td>
<td>61.3 mM</td>
<td>46.3 mEq/L [34]</td>
</tr>
<tr>
<td>$[Cl^-]_t$</td>
<td>42.5 mM</td>
<td>37.8 mEq/L [34]</td>
</tr>
<tr>
<td>$[HCO_3^-]_t$</td>
<td>47.9 mM</td>
<td></td>
</tr>
</tbody>
</table>

Cytosolic ion concentrations

<table>
<thead>
<tr>
<th>Model</th>
<th>Solution</th>
<th>Experimental Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[Na^+]_i$</td>
<td>14.0 mM</td>
<td>13.6 $\pm$ 0.4 mM [58]</td>
</tr>
<tr>
<td>$[K^+]_i$</td>
<td>123.5 mM</td>
<td></td>
</tr>
<tr>
<td>$[Cl^-]_i$</td>
<td>19.7 mM</td>
<td>23 $\pm$ 1.9 mM [26]</td>
</tr>
<tr>
<td>$[HCO_3^-]_i$</td>
<td>20.7 mM</td>
<td></td>
</tr>
</tbody>
</table>

Potential difference at the end of duct

$V_A = -15.2 \text{ mV}$
$V_B = -57.1 \text{ mV}$
Lumenal concentration profile

Electrogenic AE

Electroneutral AE

B

Flow Rate (1=\nu_0 for 0.3 \mu M CCh + 5 \mu M IPR)