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# Chloride Secretion by Canine Tracheal Epithelium: II. The Cellular Electrical Potential Profile

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**Summary.** We used intracellular microelectrode techniques to study the mechanisms responsible for Cl secretion by canine tracheal epithelium. Tissues were treated with indomethacin ($10^{-6}$ M, added to the mucosal solution) to reduce the baseline rate of Cl secretion and then stimulated by addition of epinephrine ($10^{-6}$ M) or prostaglandin $E_1$ ($10^{-6}$ M) to the submucosal solution.

Three conclusions emerged from our findings: First, secretagogues enhance the rate of transepithelial Cl transport primarily by increasing apical membrane Cl permeability, since: (i) stimulation of secretion produced parallel decreases in transepithelial resistance ($R_t$) and the membrane resistance ratio $R_a/R_b$, where $R_a$ and $R_b$ refer to the resistances of the apical and basolateral membranes; (ii) there was an inverse relation between the short-circuit current and $R_a/R_b$; (iii) secretagogues depolarized the electrical potential difference across the apical membrane ($\psi_a$) and produced an equivalent hyperpolarization of the transepithelial electrical potential difference ($\psi_t$) so that, in the steady-state, the basolateral membrane potential ($\psi_b$) was unchanged; and (iv) substitution of sulfate or gluconate for Cl in the bathing solutions prevented secretagogue-induced changes in $R_t$, $R_a/R_b$, $\psi_a$, and $\psi_t$.

Second, Cl entry into the cell across the basolateral membrane appears to be electrically-neutral since omission of Cl from the submucosal solution had no effect on $\psi_b$ and did not decrease $R_a/R_b$ as would be expected if Cl entered the cell by a conductive process.

Third, secretagogues decrease $R_b$. Approximately 20 sec after the onset of the secretory response $R_a/R_b$ underwent a secondary increase while $R_t$ continued to fall. The decrease in $R_b$ may reflect an increase in basolateral membrane K permeability.

**Key Words** tracheal epithelium · microelectrodes · membrane potentials · membrane resistances · chloride secretion · secretagogues

## Introduction

Active chloride transport from submucosa to mucosa underlies the capacity of canine tracheal epithelium for salt and water secretion (Olver, Davis, Marin & Nadel, 1975; Welsh, Widdicombe & Nadel, 1980). Chloride secretion by tracheal epithelium is an electrogenic transport process (Olver et al., 1975) that is inhibited by addition of ouabain (Al-Bazzaz & Al-Awqati, 1979; Widdicombe, Ueki, Bruderman & Nadel, 1979*b*) or furosemide (Davis et al., 1977) to the submucosal solution or removal of Na from the submucosal solution (Al-Bazzaz & Al-Awqati, 1979; Widdicombe et al., 1979*b*). The rate of active Cl secretion is regulated by intracellular cAMP and/or calcium levels (Smith, Welsh & Frizzell, 1982).

Our present understanding of the secretory process in canine tracheal epithelium is based on studies of transepithelial ion fluxes under short-circuit conditions. However, to further understand the mechanisms responsible for Cl secretion by this or other secretory epithelia, it is necessary to examine the transport properties of the individual cell membranes. In this study, we used intracellular microelectrode techniques to determine the electrical potential profile of the secretory cells and the relative resistances of their limiting membranes. We examined the effects of agents that inhibit or stimulate Cl secretion and performed ion-replacement studies to evaluate the ionic basis of their actions.

## Materials and Methods

Trachea were obtained from mongrel dogs (20–40 kg) that were anesthetized with Nembutal (25 mg/kg intravenously). After removing the posterior muscular layer, the posterior membraneous portion of the trachea was mounted horizontally, mucosal surface up, as a flat sheet between two halves of a small Lucite

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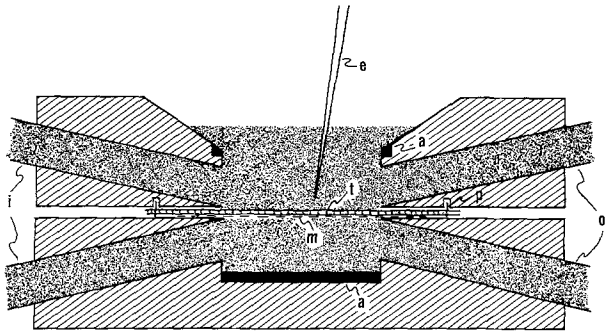


**Fig. 1.** Micropuncture chamber. *a*, Ag−AgCl electrodes implanted in chamber for passing current; *e*, glass microelectrode; *i*, inlets for perfusing solutions provided by gravity feed from reservoirs (not shown); *m*, nylon mesh; *o*, outlets for solutions; *p*, pins for holding tissue during mounting; *t*, tissue. Chamber was also waterjacketed (not shown)

chamber having an aperture of 0.125 $cm^2$ (Fig. 1). The submucosal surface of the tissue was supported by nylon mesh. The mucosal and submucosal compartments of the chamber contained approximately 0.03 ml of bathing media and were continuously perfused by gravity from reservoirs above the chamber at a rate of 1 to 1.5 $ml \cdot min^{-1}$. Both the chamber and the perfusion reservoirs were water-jacketed to assure that the perfusate was maintained at 37 °C.

The perfusing solution contained (in mM): Na, 143.0; K, 5.4; Ca, 1.2; Mg, 1.2; Cl, 123.7; $HCO_3$, 25; $HPO_4$, 2.4; $H_2PO_4$, 0.6; and glucose, 10. The pH was 7.4 during bubbling with 95% $O_2$–5% $CO_2$ at 37 °C. Chloride-free solutions were prepared by substituting $SO_4$ or gluconate for Cl. For the $SO_4$ substitution, osmolarity was maintained by addition of mannitol. The drugs used were prostaglandin $E_1$ (Sigma), indomethacin (Sigma), and epinephrine (Parke-Davis).

### *Electrical Measurements*

The transepithelial electrical potential difference ($\psi_t$) was monitored by an automatic voltage-current clamp apparatus (University of Iowa, Bioengineering) via two calomel half-cells that were connected to the bathing solutions by saturated KCl-agar bridges. The mucosal solution was used as reference for all electrical measurements. Transepithelial resistance ($R_t$) was calculated from the change in $\psi_t$ produced by bipolar, square-wave pulses of constant current (amplitude 40 $\mu A \cdot cm^{-2}$; duration 1 or 2 sec; period 10 sec) which were delivered by a pulse generator built into the voltage-current clamp. Experiments were performed with the tissues at their spontaneous $\psi_t$ but occasionally $\psi_t$ was clamped to zero to record the short-circuit current ($I_{sc}$). Current was passed across the tissue via Ag−AgCl rings implanted symmetrically in the chamber.

The electrical potential difference across the apical cell membrane ($\psi_a$) was measured with a high input impedance electrometer (F23-B, W.P. Instruments, Inc., New Haven, CN) and referenced to the mucosal solution through a differential electrometer (750-B, W.P. Instruments, Inc.). Values of $\psi_a$, $\psi_t$, and the transepithelial current were recorded by a three-channel strip chart recorder (Gould Inc., Cleveland, OH). The electrical potential difference across the basolateral cell membrane ($\psi_b$) was calculated from $\psi_t - \psi_a$. The ratio of the changes in $\psi_a$ and $\psi_t$ that result from current pulsing ($\Delta\psi_a/\Delta\psi_t$) yields the fractional resistance of the apical membrane ($f_R$):

$$\frac{\Delta\psi_a}{\Delta\psi_t} = f_R = \frac{R_a}{R_a + R_b} \quad (1)$$

where $R_a$ and $R_b$ are the resistances of the apical and basolateral membranes, respectively. The membrane resistance ratio ($R_a/R_b$) was calculated from $f_R$ using the relation given above.

Microelectrodes were prepared from 1.2 mm OD fiber-filled borosilicate glass tubing (W.P. Instruments, Inc.), pulled on a horizontal micropipette puller (Model P-77, Sutter Instrument Co., San Francisco, CA). The micropipettes had shank lengths of 6.5–8 mm, and, by scanning electron microscopy, tip diameters of 0.05–0.09 μm. They were backfilled with 0.5 M KCl immediately before use and had resistances of 90–120 MΩ when the tips were immersed in 0.5 M KCl (Fromm & Schultz, 1981). Microelectrodes were mounted vertically above the tissue on a sliding micromanipulator (aus Jena, East Germany) and their tips were advanced into the epithelium, from the mucosal solution, with a hydraulic microdrive (Model MO-8, Narishige, Japan).

Our criteria for a successful impalement were: (i) an abrupt, negative deflection of the electrical potential sensed by the microelectrode tip, (ii) a stable plateau value of the electrical potential difference whose magnitude was at least as large as the deflection obtained on impalement of the tissue (rise time of the recorder greater than 10 mV/msec); (iii) a stable (±2 mV) plateau value of $\psi_a$ lasting at least 30 sec; (iv) an abrupt return to the baseline (pre-impalement) value (±2 mV) upon withdrawal of the electrode from the cell. For some of the studies reported here it was necessary to maintain cellular impalements for several minutes. There was no difference between the values of $\psi_a$ or $f_R$ obtained from impalements of long duration (>5 min) and those of 30–60 sec duration. When the microelectrode was advanced several μm beyond the epithelial cell layer, the recorded electrical potential difference was equipotential to the value of $\psi_t$ and the voltage deflections due to current pulsing were also equal to those obtained from the macroelectrodes present in the bathing media. This suggests that the electrical resistance of the submucosal collagen layer is negligible.

Data is presented as the mean and SEM. Statistical significance was evaluated using a paired or unpaired *t*-test, as indicated. A value of $p < 0.05$ was considered statistically significant.

## Results

### *Electrical Potential Profile*

Upon advancing the microelectrode to the surface of the tissue, we usually observed transient deflections in the recorded potential difference. These may result from contact of the microelectrode tip with the cell surface or with the cilia that protrude from the apical surface of these cells. A step advance of the microelectrode another 2–15 μm yielded cellular impalement. In general, these had one of the three forms, as illustrated by the representative recordings of Fig. 2. In the first type, shown in Fig. 2*a*, there was an abrupt negative deflection, followed by a transient decrease in potential difference that returned to a stable value similar in magnitude to the initial, rapid deflection. The secondary increase in potential was interpreted as a sealing of the apical membrane around the microelectrode tip following impalemant since it

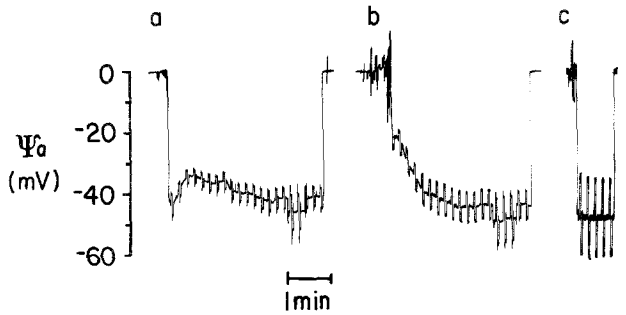


**Fig. 2.** Representative cellular impalements. *See* text for details

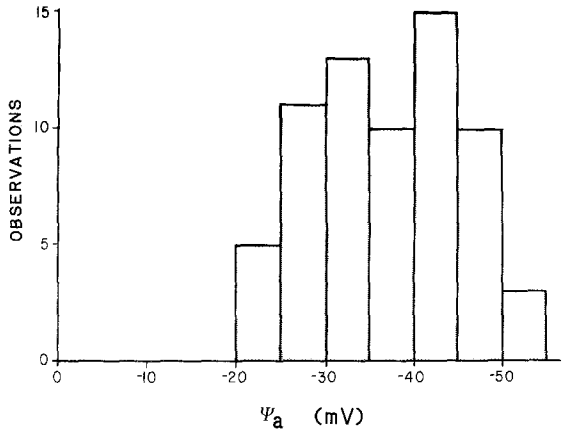


**Fig. 3.** Histogram of values of $\psi_a$. 67 cellular impalements were obtained in 12 tissues during baseline conditions (no agents added to the solutions)

was accompanied by an apparent increase in the relative resistance of the apical membrane, $f_R$. The second pattern, shown in Fig. 2*b*, is similar to the first; however, it lacks the transient decrease in voltage that followed the initial negative deflection. This type of impalement was also characterized by parallel increases in $\psi_a$ and $\Delta\psi_a/\Delta\psi_t$, which may represent a sealing phenomenon. Figure 2*c* shows a third pattern which was encountered less frequently than the others. In this pattern the potential difference and $f_R$ reached plateau values almost immediately. There was no systematic difference in the values of $\psi_a$ and $f_R$ obtained from these three different patterns of cellular impalement.

Figure 3 is a histogram of the values of $\psi_a$ obtained from 67 cellular impalements acquired from 12 tissues under control conditions. The values of $\psi_a$ appear to be distributed normally about a mean of $-38$ mV. The variance associated with repetitive sampling of $\psi_a$ values in individual tissues is less than that encountered between different tissues. This can be attributed to spontaneous variation in transport rate from tissue-to-tissue since, as will be shown below, $\psi_a$ varies with the rate of Cl secretion.

Table 1 provides values of the transmembrane electrical potential differences, transepithelial resistance, and the fractional resistance of the apical membrane under baseline conditions (no drugs added), during inhibition of Cl secretion with indomethacin ($10^{-6}$ M) and following stimulation of Cl secretion with epinephrine ($10^{-6}$ M) in six tissues.[1] All values were recorded during periods when $\psi_t$, $I_{sc}$ and $R_t$ were time-independent, i.e., under steady-state conditions. Addition of indomethacin to the mucosal solution decreased $\psi_t$ and $I_{sc}$ and increased $R_t$ as previously reported (Al-Bazzaz, Yadava & Westenfelder, 1981; Smith et al., 1982). These changes in transepithelial electrical properties were accompanied by a hyperpolarization of $\psi_a$ and an increase in $f_R$ so that the membrane resistance ratio ($R_a/R_b$) increased from $1.6\pm0.4$ to $3.0\pm0.7$. The finding that $R_t$ and $R_a/R_b$ increased in parallel strongly suggests that the resistance of the apical membrane increased following inhibition of Cl secretion by indomethacin.

The use of concomitant changes in $R_t$ and $R_a/R_b$ to localize resistance changes to the apical and/or basolateral membranes requires that secretagogue-induced changes in paracellular pathway resistance, $R_p$, are minimal. This proposal is supported by two observations: first, agents that stimulate or inhibit Cl secretion by canine tracheal epithelium have no effect on unidirectional mannitol fluxes across this tissue (Al-Bazzaz et al., 1981; Welsh & Widdicombe, 1980). Since transepithelial movement of mannitol appears to be restricted to the paracellular pathway, these findings suggest that $R_p$ is not markedly altered by agents affecting Cl secretion rate. Second, if the observed decrease in $R_t$ were produced largely by a decrease in $R_p$, secretagogues would decrease $\psi_t$; the opposite is observed.

Table 1 also gives the values of $\psi_a$, $\psi_b$, and $f_R$ during the steady-state following stimulation of Cl secretion with epinephrine ($10^{-6}$ M), added to the submucosal solution of indomethacin-treated tissues. As has been observed previously (Al-Bazzaz & Cheng, 1979), epinephrine increased $\psi_t$ and $I_{sc}$ and decreased $R_t$. These changes were accompanied by depolarization of $\psi_a$ and decrease in $f_R$ so that $R_a/R_b$ decreased from $3.3\pm0.7$ to $0.5\pm0.1$. The parallel decreases in $R_t$ and $R_a/R_b$ indicate that stimulation of secretion decreased the resistance of the apical membrane.

[1] Table 1 provides not only the mean values for each group of tissues, but also the mean values obtained in individual tissues. The data is presented in detail to illustrate the consistency of measurements made within individual tissues and the variation of electrical properties with Cl secretion rate among tissues.

**Table 1.** Electrical profile of the epithelium during baseline, indomethacin treated, and stimulated conditions

| Tissue | (*n*) | $\psi_t$ (mV) | $I_{sc}$ ($\mu A \cdot cm^{-2}$) | $R_t$ ($\Omega \cdot cm^2$) | $\psi_a$ (mV) | $\psi_b$ (mV) | $f_R$ |
|---|---|---|---|---|---|---|---|
| | | | | Baseline | | | |
| A | (6) | 15.3±0.7 | 110±6.9 | 126±3 | −27.8±1.9 | 43±1.9 | 0.46±0.05 |
| B | (7) | 12.5±0.5 | 109±2.4 | 104±2 | −42.7±1.2 | 55.2±1.4 | 0.61±0.17 |
| C | (7) | 11.1±0.5 | 63±6 | 167±1 | −36.4±3.2 | 47.4±2.9 | 0.58±0.05 |
| D | (6) | 21.0±0.7 | 207±2 | 95±2 | −28.8±2.9 | 49.8±3.4 | 0.32±0.07 |
| E | (6) | 14.2±0.1 | 105±1 | 110±2 | −46.7±0.9 | 60.8±1.0 | 0.71±0.03 |
| F | (6) | 14.9±0.7 | 59±6 | 227±7 | −30.0±3.3 | 44.9±3.3 | 0.73±0.06 |
| $\bar{X}$±SEM | | 14.8±1.4 | 109±22 | 138±21 | −35.4±3.2 | 50.2±2.7 | 0.57±0.06 |
| | | | | Indomethacin ($10^{-6}$ M) | | | |
| A | (6) | 7.3±0.4 | 46±3 | 149±1.5 | −41.9±1.4 | 49.2±1.4 | 0.64±0.04 |
| B | (6) | 10.7±0.3 | 70±6 | 150±6 | −38.8±2.7 | 49.6±2.9 | 0.72±0.06 |
| C | (10) | 7.5±0.4 | 39±2.7 | 183±2.5 | −48.0±3.5 | 55.6±3.4 | 0.85±0.03 |
| D | (6) | 13.5±0.8 | 112±8 | 115±1 | −36.2±2.5 | 49.6±2.6 | 0.44±0.06 |
| E | (7) | 10.0±0.7 | 67±3 | 128±1 | −49.9±1.6 | 59.8±1.9 | 0.82±0.02 |
| F | (7) | 8.7±0.6 | 30±1.4 | 254±5 | −38.1±2.7 | 46.0±2.1 | 0.71±0.05 |
| $\bar{X}$±SEM | | 9.6±1.0 | 61±12 | 163±20 | −42.2±2.3 | 51.6±2.1 | 0.70±0.06 |
| | | | | Epinephrine ($10^{-6}$ M) | | | |
| A | (7) | 28.9±0.3 | 253±6 | 86±3 | −29.2±2.3 | 58.1±2.6 | 0.15±0.01 |
| B | (3) | 19.1±0.3 | 130±2 | 121±4 | −34.3±2.5 | 53.4±1.7 | 0.44±0.03 |
| C | (3) | 17.0±0.5 | 102±1 | 141±7 | −26.3±1.9 | 43.3±2.1 | 0.33±0.04 |
| D | (5) | 29.1±0.4 | 261±10 | 96±2 | −27.2±1.5 | 56.3±1.6 | 0.28±0.07 |
| E | (8) | 16.4±0.6 | 124±2 | 104±2 | −39.2±1.9 | 55.6±1.9 | 0.62±0.04 |
| F | (5) | 24.8±0.2 | 132±1 | 143±2 | −21.9±2.9 | 46.7±2.7 | 0.46±0.09 |
| $\bar{X}$±SEM | | 22.6±2.4 | 167±29 | 115±10 | −29.7±2.5 | 52.2±2.4 | 0.38±0.07 |

*n* represents the number of impalements in each tissue. Values represent the mean±SEM. All measurements were made during steady-state conditions.

**Table 2.** Effect of prostaglandin $E_1$ on the electrical profile

| Condition | $\psi_t$ (mV) | $I_{sc}$ ($\mu A \cdot cm^{-2}$) | $R_t$ ($\Omega \cdot cm^2$) | $\psi_a$ (mV) | $\psi_b$ (mV) | $f_R$ |
|---|---|---|---|---|---|---|
| Control | 16.4±2.2 | 82±14 | 207±24 | −47.9±3.3 | 64.3±1.5 | 0.78±0.08 |
| $PGE_1$ ($10^{-6}$ M) | 28.2±4.3 | 171±34 | 146±15 | −33.5±3.6 | 61.6±3.2 | 0.61±0.08 |

Indomethacin ($10^{-6}$ M) was present during both periods. All values represent the mean and SEM for five tissues. All measurements were made during steady-state conditions.

Table 2 shows the steady-state values of the electrical properties of tissues during the control period (indomethacin-treated) and following stimulation with $PGE_1$. The changes in $\psi_t$, $R_t$ and $I_{sc}$ elicited by $PGE_1$ are quantitatively similar to those observed with epinephrine (Table 1); the depolarization of $\psi_a$ and the decrease in $f_R$ are also similar to those shown in Table 1. Despite these similarities in the responses to epinephrine and prostaglandin, they differ in the extent to which their effects are reversible. The stimulation produced by $PGE_1$ was reversed entirely 10 to 20 min following its removal whereas that elicited by epinephrine was usually slowly (1–2 hr) and incompletely reversible.

Results for the individual tissues shown in Table 1, as well as the mean values in Tables 1 and 2, indicate that inhibition or stimulation of Cl transport does not alter $\psi_b$ in the steady-state. This suggested an inverse relationship between $\psi_a$ and $\psi_t$, and thus an inverse relationship between $\psi_a$ and the Cl secretion rate. In Fig. 4 the average values of $\psi_t$ and $\psi_a$ under control and stimulated conditions are plotted for the 11 tissues that provided the data shown in Tables 1 and 2. For each

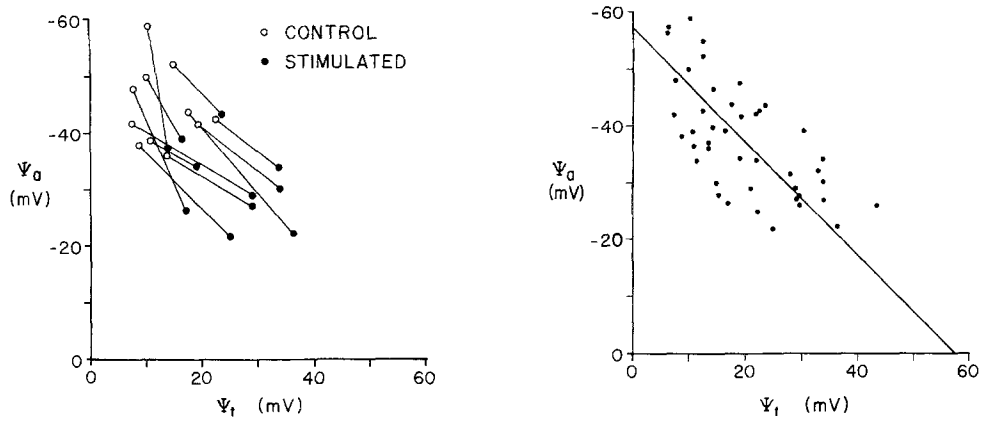


**Fig. 4** (*left*). Relationship between $\psi_a$ and $\psi_t$ in 11 tissues. Open circles represent steady-state, control conditions (indomethacin $10^{-6}$ M present in the mucosal solution); closed circles represent steady-state, stimulated conditions with epinephrine ($10^{-6}$ M) or prostaglandin $E_1$ ($10^{-6}$ M) added to submucosal solution

**Fig. 5** (*right*). Relationship between $\psi_a$ and $\psi_t$. Circles represent steady-state values during all experimental conditions (baseline, indomethacin treatment, and stimulation). $r=0.75$. Line was arbitrarily drawn to have slope of one and indentical $x$ and $y$ intercepts. The regression analysis yielded a relation that does not differ significantly from that drawn here

tissue, we found an inverse relationship between $\psi_t$ and $\psi_a$; that is, as $\psi_a$ depolarizes, $\psi_t$ hyperpolarizes. In Fig. 5, average values of $\psi_a$ and $\psi_t$ are given for all tissues studied under baseline, indomethacin-treated, or stimulated (epinephrine or $PGE_1$) conditions. Although there is some scatter, the data is adequately described by a straight line with slope of $-1$ and identical $x$ and $y$ axis intercepts. This relationship confirms the stability of $\psi_b$ despite changes in the steady-state rate of Cl secretion.

Figure 6 shows the relationship between the average steady-state values of $f_R$ and $I_{sc}$ for all tissues under all experimental conditions examined (baseline, indomethacin-treated, and stimulated). There is an inverse correlation between the rate of Cl secretion (reflected by the $I_{sc}$) and $f_R$. Thus, an increase in Cl secretion rate is closely correlated with a decrease in apical membrane resistance. In addition, this finding suggests that spontaneous variations in the rate of Cl secretion and $f_R$, which are most readily observed under baseline conditions (filled circles of Fig. 6 and Table 1), are causally related and are due to variations in the secretory state of the tissue rather than variable damage to the apical cell membrane produced by microelectrode impalement.

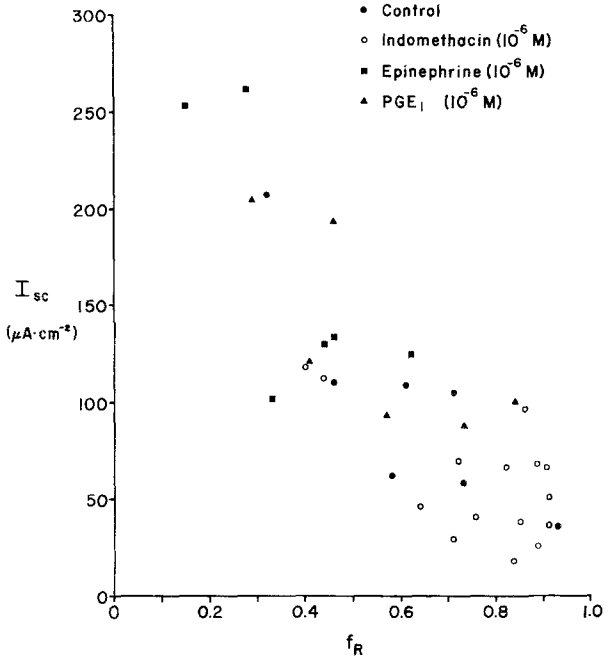


**Fig. 6.** Relationship between fractional resistance of the apical membrane ($f_R$) and $I_{sc}$. $r=0.84$

### *Cl Replacement Studies*

To determine the contribution of Cl to the changes in $\psi_a$ and $f_R$ that accompany stimulation of secretion, we examined the effects of replacing Cl in one or both bathing media with sulfate (three tissues) or gluconate (one tissue). The results obtained with gluconate and sulfate replacement were indistinguishable and were therefore combined. Replacement of Cl in the mucosal, submucosal, or both solutions was performed in random order and five cellular impalements were obtained for each experimental condition. No correction has been made for liquid junction potentials during unilateral Cl replacement. These are small, due to use of saturated KCl electrodes for determination of $\psi_t$, and would not affect measured values of $R_t$ or $f_R$.

**Table 3.** The effect of Cl substitutions on the electrical profile

| Condition | [Cl] (mM) $m-s$ | $\psi_t$ (mV) | $I_{sc}$ ($\mu A \cdot cm^{-2}$) | $R_t$ ($\Omega \cdot cm^2$) | $\psi_a$ (mV) | $\psi_b$ (mV) | $f_R$ |
|---|---|---|---|---|---|---|---|
| Control | 124–124 | 20.1±6.6 | 69±23 | 288±27 | −37.2±6.7 | 57.3±4.6 | 0.71±0.11 |
| $PGE_1$ | 124–124 | 31.3±5.5 | 157±29 | 159±12 | −27.6±2.2 | 58.9±4.7 | 0.43±0.06 |
| Control | 0–0 | 17.5±5.0 | 41±19 | 403±50 | −42.3±3.4 | 59.8±1.7 | 0.88±0.04 |
| $PGE_1$ | 0–0 | 23.7±5.2 | 65±11 | 347±43 | −38.7±4.2 | 62.4±2.9 | 0.84±0.05 |
| $PGE_1$ | 124–0 | 7.9±3.6 | – | 228±24 | −51.9±4.8 | 64.8±5.4 | 0.60±0.06 |
| $PGE_1$ | 0–124 | 52.0±11.5 | – | 231±13 | − 7.7±7.3 | 59.7±5.9 | 0.48±0.09 |

Indomethacin ($10^{-6}$ M) was present in the mucosal solution during all conditions. [Cl] represents the choride concentration in the perfusing solutions; $m$ and $s$ represent the mucosal and submucosal solutions, respectively. $PGE_1$ indicates that prostaglandin $E_1$ ($10^{-6}$ M) is present in the submucosal solution. All measurements were made during steady-state conditions. Values represent the mean ±SEM for four tissues. Measurements were obtained under each of these conditions in each tissue.

Table 3 shows the results of these studies, in which $PGE_1$ served as the secretory stimulus. Values obtained in the presence of Cl are in good agreement with those given in Tables 1 and 2. In unstimulated (indomethacin-treated) tissues bilateral Cl replacement increased $R_t$, but had only minimal effects on $I_{sc}$, $\psi_t$, $\psi_a$ and $f_R$.[2] Bilateral exposure to Cl-free media abolished the effects of $PGE_1$ on $\psi_t$, $\psi_a$ and $f_R$. The small changes in $I_{sc}$ and $R_t$ which followed addition of $PGE_1$ to Cl-free media may result from the presence of a small amount of residual Cl in the submucosal connective tissue layer; however, the response to $PGE_1$ is, for all intents and purposes, abolished in the absence of Cl. Of particular interest is the failure of $PGE_1$ to reduce $f_R$ in the absence of Cl. This indicates that the decrease in apical membrane resistance that normally accompanies stimulation of secretion is specific for Cl and results from the development of an electrically-conductive pathway for Cl movement across the apical cell membrane. This pathway is impermeable to sulfate and gluconate.

The last two rows of Table 3 provide results obtained during replacement of Cl in either the mucosal or sumucosal solution. During unilateral Cl replacement, $R_t$ is less than the value observed in the complete absence of Cl but is greater than the value obtained when Cl Ringer bathes both surfaces of the tissue. While this undoubtedly reflects the contribution of Cl to the conductance of the paracellular pathway, the presence of a transcellular Cl conductance in $PGE_1$-stimulated tissues is apparent from the corresponding values of $f_R$ which also lie midway between those observed in the presence and absence of Cl. The parallel changes in $f_R$ and $R_t$ during Cl replacement in one or both bathing media suggest that part of the change in $R_t$ is due to a change in the conductance of the apical membrane. The presence of Cl on the mucosal surface alone would decrease $R_a$ by increasing the ionic content of a Cl-selective pathway traversing the apical membrane, and thus decrease $f_R$. Similarly, the presence of Cl in the submucosal solution alone would also decrease $R_a$ and $f_R$ below the value observed during bilateral Cl replacement since submucosal solution Cl has access to the cell interior via the process(es) responsible for net Cl transport across the basolateral membrane.

The changes in $\psi_a$ of $PGE_1$-treated tissues during unilateral Cl replacement are consistent with the generation of diffusion potentials across a Cl-selective apical membrane. However, these changes in $\psi_a$ are complicated by corresponding changes in $\psi_t$ that arise from diffusion potentials across the paracellular pathway, which has been shown to possess a relatively high Cl permeability (Welsh & Widdicombe, 1980).

Finally, the values of $\psi_b$ were not significantly affected by the presence or absence of $PGE_1$ or by unilateral or bilateral Cl replacement. The stability of $\psi_b$ in the face of dramatic changes in Cl secretion rate and despite marked alterations in bathing solution Cl concentrations will be discussed below.

### *The Acute Electrical Response to Stimulation*

In five tissues we were able to maintain individual cellular impalements for at least 3 min before and 3 min following the onset of the response to epi-

[2] The slight increase in $f_R$ of unstimulated tissues upon replacement of Cl with nontransported anions suggests that a small Cl-dependent conductance may be present in the apical membrane, despite pretreatment with indomethacin. This finding is consistent with the observations of Al-Bazzaz et al. (1981), that a low rate of Cl secretion persists even in the presence of indomethacin. However, the magnitude of this residual Cl secretion appears to depend on the duration of exposure to indomethacin (Smith et al., 1982).

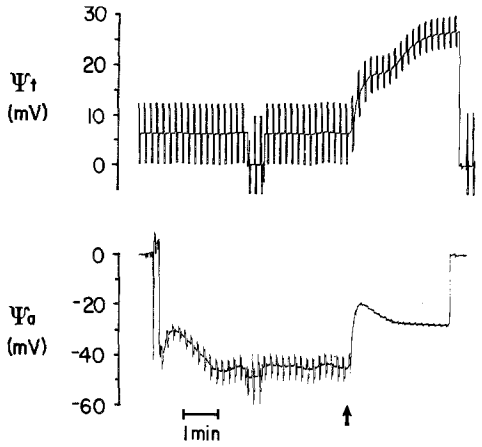


**Fig. 7.** Representative recording of the acute electrical response to epinephrine. The onset of the electrical response to the addition of epinephrine ($10^{-6}$ M) to the submucosal solution is indicated by the arrow. The period when $\psi_t = 0$ represents short circuiting of the tissue

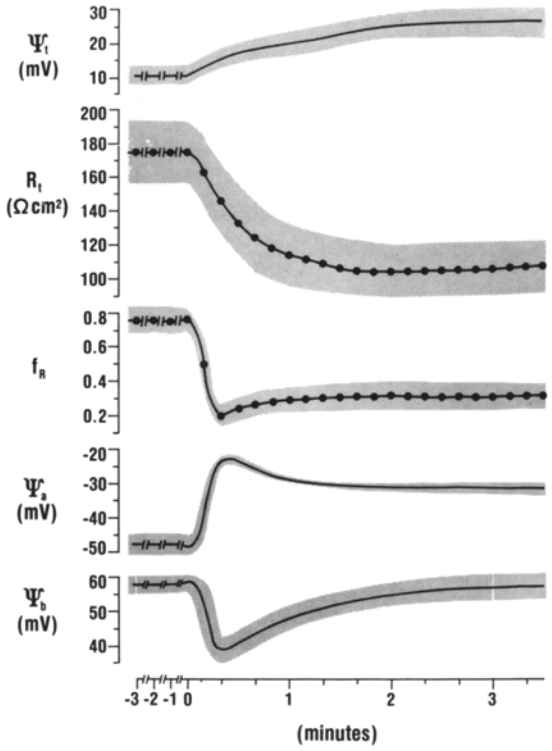


**Fig. 8.** Acute electrical response to stimulation with epinephrine. Data represents mean ± SEM (shaded area). The onset of the response to the addition of epinephrine ($10^{-6}$ M ) to the submucosal solution is indicated by time zero. $R_t$ and $f_R$ were measured at times indicated by dots. $\psi_t$, $\psi_a$, and $\psi_b$ were measured continuously

nephrine. Figure 7 is an experimental record illustrating the time courses of $\psi_t$ and $\psi_a$ in one tissue (tissue *A* of Table 1). All tissues were pretreated with indomethacin, so that during the initial phase of this record, $\psi_t$ is low (7 mV). Epinephrine ($10^{-6}$ M) was added to the submucosal solution at the time indicated by the arrow and caused $\psi_t$ to increase to a new steady-state value of 28 mV. $\psi_a$ was initially −42 mV, and the addition of epinephrine depolarized $\psi_a$ to a new steady-state value of −30 mV. However, the acute depolarization of $\psi_a$ is greater than the value reached during steady-state stimulation, and the deflections in $\psi_a$ induced by current pulsing ($\Delta\psi_a$) are also smaller during this transient than they are 2 min after the onset of the secretory response.

This pattern was characteristic of all five tissues studied in this manner, and the time courses of $\psi_t$, $R_t$, $f_R$, $\psi_a$ and $\psi_b$ are presented in Fig. 8 as the mean ± SEM (shaded areas). Zero time represents the onset of the epinephrine response. The major points to be noted from this figure are: (i) all electrical properties were stable for 3 min before the addition of epinephrine, i.e., the indomethacin-treated tissues were in a steady-state; (ii) following addition of epinephrine, $\psi_t$ increased and $R_t$ decreased to new steady-state values by 2.5 min. This was accompanied by an increase in $I_{sc}$ (determined by briefly clamping the $\psi_t$ to zero) from $69 \pm 18$ $\mu A \cdot cm^{-2}$ to $238 \pm 53$ $\mu A \cdot cm^{-2}$; (iii) $f_R$ was decreased in a biphasic manner so that the control value of $R_a/R_b$ (3.31) decreased to its lowest value (0.25) twenty seconds following the onset of the epinephrine response and subsequently increased to a steady-state value of 0.58. At 20 sec, when $f_R$ reached its lowest value, $R_t$ had decreased by only 42% of the entire decrease observed in the new steady-state; (iv) both $\psi_a$ and $\psi_b$ were depolarized in a biphasic manner and the maximal depolarization of $\psi_a$ (24 mV) and $\psi_b$ (19 mV) coincided with the minimum in $f_R$; finally, (v) during the period from 20 to 120 sec, $\psi_b$ repolarized completely and $\psi_a$ repolarized by 9 mV. The steady-state values of $\psi_a$, $\psi_b$ and $f_R$ determined before and after stimulation by epinephrine, and illustrated in Fig. 8, are not different from the average values obtained from multiple cellular impalements before and after stimulation, given in Table 1. That is, there is no reason to suspect that the acute, transient alterations in $f_R$, $\psi_a$, and $\psi_b$ displayed in Fig. 9 represent properties unique to those cellular impalements that lasted 6 min or more.

The acute response to epinephrine provides additional insight into the changes in individual apical and basolateral membrane properties that are associated with stimulation of secretion. As discussed above, stimulation of Cl secretion produces a decrease in $R_t$ that appears to be restricted to the cellular pathway. The time courses of $R_t$ and $f_R$ indicate that the initial decrease in $R_t$ seen during

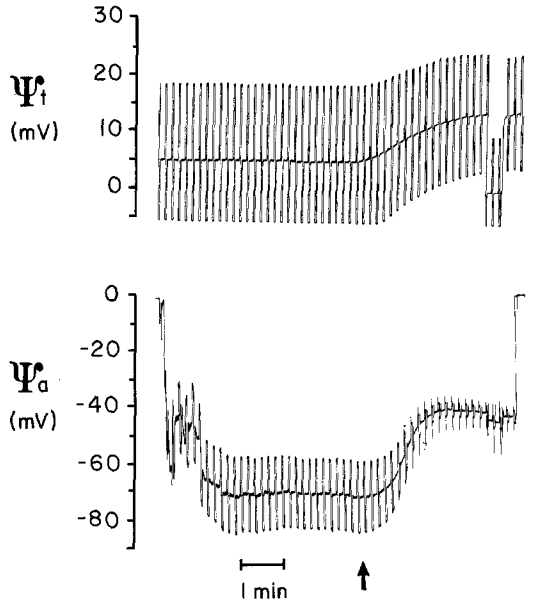


**Fig. 9.** Representative recording of the acute electrical response to prostaglandin $E_1$. Prostaglandin $E_1$ ($10^6$ M) was added to the submucosal solution at the time indicated by the arrow

the first 20 sec is predominantly, if not entirely, due to a rapid decrease in apical membrane resistance, as was evident from examination of the steady-state values (Tables 1 and 2). However, after 20 sec, $R_t$ continues to decrease as $f_R$ increases, so that much of the reduction in $R_t$ during the period from 20–120 sec is due to a secondary reduction in basolateral membrane resistance, $R_b$. In addition, the steady-state data in Tables 1 and 2 demonstrate that depolarization of $\psi_a$ accounted entirely for the hyperpolarization of $\psi_t$, so that $\psi_b$ was unaffected. However, the data illustrated in Fig. 8 indicate that the initial depolarization of $\psi_a$ is accompanied by a concomitant depolarization of $\psi_b$. This is most likely the result of passive electrical coupling of $\psi_b$ to the primary change in $\psi_a$ since the depolarization of $\psi_a$ exceeds that of $\psi_b$ and these events occur during the period (first 20 sec) when apical membrane resistance is decreasing. However, after 20 sec, $\psi_b$ repolarizes to its prestimulation value. During this time, $\psi_a$ partially repolarizes, and this is probably also due to passive electrical coupling; in this case the primary event appears to be localized to the basolateral membrane since the change in $\psi_b$ is greater than the change in $\psi_a$ and since $R_b$ is decreasing during this period.

The steady-state response to stimulation with $PGE_1$ is similar to that observed with epinephrine (Tables 1 and 2); however, the acute electrical response to $PGE_1$ is quantitatively different. The time courses of $\psi_t$ and $\psi_a$ are illustrated in Fig. 9 before and during stimulation with prostaglandin $E_1$ ($10^{-6}$ M). The changes in $\psi_t$ and $\psi_a$ are less rapid than with epinephrine, and the biphasic response of $\psi_a$ and $\Delta\psi_a$ is not observed during stimulation by $PGE_1$. However, analysis of this data indicates that the decreases in $f_R$ and $R_t$ accompanying stimulation are still dissociated in time. That is, $f_R$ approaches its steady-state value at a time when $R_t$ is still declining. Similarly, the depolarization of $\psi_a$ is more rapid than the hyperpolarization of $\psi_t$ so that $\psi_b$ is transiently depolarized as with epinephrine. Thus, the acute response to $PGE_1$ is qualitatively similar to that observed with epinephrine but shows a slower onset. This is presumably due to differences in the rapidity with which these agents raise cellular cAMP levels rather than differences in the rate at which they penetrate the submucosal connective tissue layer. Prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) also reversibly stimulates electrogenic Cl secretion (Al-Bazzaz et al., 1981). Addition of $PGF_{2\alpha}$ to the submucosal bathing solution resulted in steady-state changes in the electrical potential differences and membrane resistance ratio similar to those obtained with epinephrine and $PGE_1$ and an acute, biphasic response to stimulation similar to that seen with addition of epinephrine.

## Discussion

In this section, we will review a model for Cl secretion which appears to be generally applicable to Cl-secreting epithelia and discuss the results of prior studies suggesting that this model is appropriate for canine tracheal epithelium. Our electrophysiologic findings will then be discussed within the framework of this model.

### *A Model of Cl Secretion*

A model for electrolyte transport by canine tracheal epithelium, which is similar to the model reviewed by Frizzell, Field and Schultz (1979*a*) for Cl-secreting epithelia, is illustrated in Fig. 10. According to this model, Cl enters the cell via an electrically neutral, NaCl co-transport process at the basolateral membrane. The driving force for Cl entry is the sum of chemical potential differences for Na and Cl across the basolateral membrane. This coupled entry step results in thermodynamic accumulation of Cl within the cell at an activity greater than that predicted for electrochemical equilibrium. Accordingly, Cl exit from the cell across the apical membrane occurs in the direction of a favorable electrochemical potential difference. The Na that enters the cell coupled to Cl is recycled across the basolateral membrane via

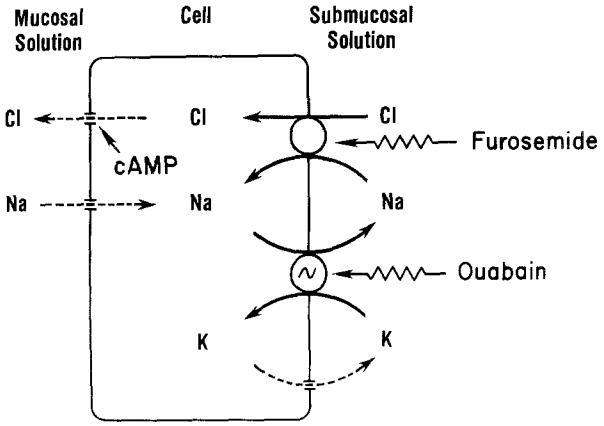


**Fig. 10.** A model for chloride secretion by the canine tracheal epithelium. (*See* text for details)

the Na-K-pump. All of the K entering in exchange for Na exits across the basolateral membrane since there is no net K secretion across the epithelium (Gatzy & Boucher, 1979). Finally, a pathway for Na entry across the apical membrane is included in this model since short-circuited tracheal epithelium displays a small rate of active Na absorption. According to this model, net current flow across the epithelial cells during secretion will be carried by Cl exiting and Na entering across the apical membrane and by K and Na exiting across the basolateral membrane.

Prior observations which support the applicability of this model to canine tracheal epithelium are: (i) the epithelial cells contain a Na-K-ATPase (Westenfelder, Earnest & Al-Bazzaz, 1980) that has been localized to the basolateral membrane by autoradiographic localization of $^3$H-ouabain binding (Widdicombe, Basbaum & Yee, 1979*a*); (ii) addition of ouabain ($10^{-4}$ M) to the submucosal bathing solution abolishes the $I_{sc}$, Cl secretion and Na absorption (Al-Bazzaz & Al-Awqati, 1979; Widdicombe et al., 1979*b*); (iii) replacement of Na with choline abolishes Cl secretion (Al-Bazzaz & Al-Awqati, 1979). This effect is due to removal of Na from the submucosal solution alone (Widdicombe et al., 1979*b*), (iv) stimulation of Cl secretion decreases tissue resistance (Al-Bazzaz & Al-Awqati, 1979) with no apparent change in the permeability of the paracellular pathway (Welsh & Widdicombe, 1980); this decrease in $R_t$ could result from an increase in Cl conductance of the apical membrane; and (v) addition of furosemide ($10^{-3}$ M) to the submucosal solution inhibits Cl secretion (Davis et al., 1977). It appears that loop diuretics interfere with NaCl co-transport in other epithelia (Frizzell, Smith, Vosburgh & Field 1979*b*).

*Cl Transport across the Apical Membrane*

In the companion paper (Smith et al., 1982) we show that epinephrine and $PGE_1$ increase intracellular cAMP concentration and stimulate Cl secretion. Indomethacin decreases intracellular cAMP, $PGE_2$ production, and Cl secretion rate. The results of the present study indicate that changes in cellular cAMP alter Cl secretion rate by influencing the permeability of the apical membrane to Cl. This conclusion is supported by three observations: First, there is an inverse relation between the rate of Cl secretion and the fractional resistance of the apical membrane, $f_R$. Second, replacement of Cl with $SO_4$ or gluconate abolishes the decrease in $R_t$ produced by secretagogues. Third, unilateral replacement of Cl with nontransported anions alters $R_a/R_b$ and $\psi_a$ of secretagogue-treated tissues in the manner excepted for a conductive Cl transport pathway across the apical membrane.

Our findings are similar to those of Klyce and Wong (1977) who used microelectrode techniques to study Cl secretion by rabbit corneal epithelium. They found that brief exposure to epinephrine produced a transient decrease in the resistance of the outer membrane of the squamous cell layer and that substitution of Cl with $SO_4$ blocked this response. They also noted that the resistance decrease produced by epinephrine was present, but reduced in magnitude, when Cl was omitted from one bathing solution alone. Nagle and Reinach (1980) made similar observations using intracellular microelectrodes in frog corneal epithelium.

If the electrochemical potential difference for Cl across the apical membrane is oriented from cell to mucosal solution, a secretagogue-induced increase in apical membrane Cl conductance would permit Cl to leave the cell in the secretory direction. Studies conducted with Cl-selective microelectrode techniques indicate that intracellular Cl activity exceeds the value predicted for electrochemical equilibrium in two other Cl-secreting epithelia: In the isolated, perfused, shark rectal gland (Welsh, Smith & Frizzell, 1981), we demonstrated that cell Cl is accumulated at an activity six times greater than that predicted for a passive distribution across the basolateral membrane and approximately twice the value for equilibrium across the apical membrane. Zadunaisky, Spring and Shindo (1979) found that in frog corneal epithelium the intracellular Cl activity is twice the value expected for a passive distribution at the apical membrane. Determinations of intracellular Cl concentration in isolated cells from the canine tracheal epithelium by chemical assay techniques yields a value of

50 mM (Widdicombe, Basbaum & Highland, 1981). Since isolated cells are short circuited, the value of $\psi_a$ under short-circuit conditions with no drugs added ($\psi_a = -45$ mV, M.J. Welsh, P.L. Smith and R.A. Frizzell, *unpublished observation*) can be used to calculate a corresponding equilibrium cell Cl concentration of 23 mM, or half the value obtained by Widdicombe et al.

These considerations are also consistent with the observation that $\psi_a$ is depolarized by secretagogues. If cell Cl concentration is approximately 50 mM, the equilibrium potential for Cl across the apical membrane is roughly $-24$ mV. A marked increase in apical membrane Cl permeability would therefore shift $\psi_a$ toward this value, as we have observed during stimulation of secretion (Tables 1 and 2, Figs. 7, 8 and 9).

### *Cl Transport across the Basolateral Membrane*

Our results suggest that Cl enters the cell across the basolateral membrane via an electrically-neutral transport process. This conclusion is based on two observations. First, replacement of Cl in the submucosal bathing solution increased $f_R$. The presence of an electrically-conductive Cl transport pathway across the basolateral membrane would result in a decrease in $f_R$ (due to an increase in $R_b$) when submucosal solution Cl is replaced by an impermeant, nontransported anion. Second, the Cl replacement studies also indicate that the chemical potential difference for Cl across the basolateral membrane plays little or no part in determining $\psi_b$. Therefore, no significant conductive pathway for Cl transport across the basolateral membrane can be detected.

The possibility that electrically-neutral Cl entry across the basolateral membrane results from Cl-$HCO_3$ exchange is unlikely since neither removal of $HCO_3$ from the bathing solutions (Al-Bazzaz & Al-Awqati, 1979) nor addition of the substituted stilbene, SITS, to the submucosal solution (M.J. Welsh and J.H. Widdicombe, *personal observation*) inhibits Cl secretion. SITS has been observed to inhibit Cl-$HCO_3$ exchange in both human erythrocytes (Cabantchik & Rothstein, 1974) and turtle urinary bladder (Cohen, Mueller & Steinmetz, 1978). It seems most likely that Cl enters the cell coupled to the entry of Na, since removal of Na from the submucosal solution abolishes Cl secretion (Al-Bazzaz & Al-Awqati, 1979; Widdicombe et al., 1979*b*). In addition, using isolated, perfused, shark rectal gland (Welsh et al., 1981), we recently demonstrated that removal of Na from the perfusing solution decreased intracellular Cl activity toward the value predicted for electrochemical equilibrium. Taken together, these findings strongly suggest that Cl enters the cell across the basolateral membranes of this and other Cl-secreting epithelia via a NaCl co-transport process.

### *Conductive Ion Flow across the Basolateral Membrane*

Our findings indicate that the primary decrease in apical membrane resistance that accompanies stimulation of secretion is followed by a secondary decrease in basolateral membrane resistance. This conclusion emerges from examination of the acute electrical response of the tissue to epinephrine, shown in Figs. 7 and 8, where $f_R$ undergoes a secondary rise as $R_t$ continues to decline. Together with the decrease in basolateral membrane resistance, the electrical potential difference across this barrier, $\psi_b$, repolarizes toward the prestimulation level (Fig. 8). In the steady-state, $\psi_b$ remains fairly constant despite wide variations in the rate of chloride secretion (Tables 1 and 2, Figs. 4 and 5).

The most likely explanation for the decrease in $R_b$ and repolarization of $\psi_b$ that reflect increased conductive ion flow across the basolateral membrane is an increase in conductance specific for one ion. For example, an increase in basolateral membrane K permeability would account for the decrease in $R_b$ and cause $\psi_b$ to approach the equilibrium potential for K across this barrier. Inasmuch as $\psi_b$ repolarizes toward a value of 60 mV, cell interior negative, the ion whose specific conductance is increased must have an equilibrium potential in excess of this value. It seems likely that the only ion that satisfies this criterion is K. Indeed, the intracellular K concentration of 150 mM, recently reported by Widdicombe et al. (1981), would yield an equilibrium potential ($E_K$) of 90 mV, cell interior negative. If the basolateral membrane is largely permeable to K under nonstimulated conditions, an increase in apical membrane Cl permeability will cause $\psi_b$ to shift away from the equilibrium potential for K through electrical coupling between the limiting membranes. However, a subsequent increase in basolateral membrane K permeability will shift $\psi_b$ back toward the K equilibrium potential. This situation is analogous to the repolarization of membrane potential observed in excitable cells, where the membrane is initially depolarized by an increase in Na permeability, but a secondary increase in potassium permeability is, in part, responsible for the repolarization of membrane potential. A sus-

tained elevation of K permeability would, in fact, repolarize the membrane even if Na permeability (in our case chloride permeability) remained elevated.

Several lines of evidence suggest that the electrical conductance of the basolateral membrane is determined predominantly by a high permeability to K: First, we have shown that there is no apparent Cl-conductance pathway across this barrier (Table 3). Second, a sizeable basolateral membrane Na conductance seems unlikely since this would permit energetically inefficient recycling of Na across the basolateral membrane. Third, a high basolateral membrane K conductance is consistent with the observations of Stutts, Boucher and Gatzy (1980), who found that increasing the K concentration of the submucosal bathing solution to 100 mM decreased the rate of Cl secretion to zero. Presumably, this inhibition of Cl secretion results from K-induced depolarization of the basolateral membrane and, via electrical coupling, the apical membrane so that the driving force for Cl exit from cell to mucosal solution is abolished.

Nagel and Reinach (1980) observed that stimulation of chloride secretion across isolated frog cornea with epinephrine was associated with a decrease in $R_b$ which they attributed to an increase in basolateral membrane chloride permeability. However, this conclusion was based on the permeabilities of the epithelium to Na and K derived from studies of transepithelial Na and K fluxes. The notion that the decrease in basolateral membrane resistance results from an increase in basolateral membrane K permeability is more appealing for the following reasons: a threefold increase in Na-K pump activity, which is likely to be associated with the transition from indomethacin to epinephrine-treated tissues, as shown in Table 1, would result in a threefold increase in the rate at which K is pumped from the submucosal solution into the cell. Since there is no evidence for active K secretion by the canine tracheal epithelium (Gatzy & Boucher, 1979), this increase in pump rate must be balanced, in the steady state, by an increase in the rate at which K diffuses from cell to submucosal solution. In the absence of a change in basolateral membrane K permeability, this requires that the driving force for K exit from cell to submucosal solution also increase by a factor of three. Calculations based on reasonable estimates for cell K activity, together with the known electrical potential difference across the basolateral membrane, indicate that this would involve an enormous increase in cell K activity (e.g., an increase of 200 mM above the normal value). It seems more reasonable that the K permeability of the basolateral membrane is regulated in parallel with changes in Na-K-pump activity, so that the requirement for increased diffusional outflow of K from the cell is met by a change in the resistance of the basolateral membrane to K flow rather than a marked change in cellular composition. As indicated above, this would account for the decrease in $R_b$ and repolarization of $\psi_b$ seen in Fig. 9.

### *Relation of these Findings to Na-Absorbing Epithelia*

Finally, we would like to note two similarities in the cellular mechanisms of electrogenic ion transport by Cl-secreting and Na-absorbing epithelia. First, regulation of the rate of transepithelial ion transport in both types of epithelia appears to be mediated by an ion-specific apical membrane conductance pathway. In Na-absorbing epithelia, such as rabbit urinary bladder (Lewis, Eaton & Diamond, 1976), rabbit descending colon (Schultz, Frizzell & Nellans, 1977), and *Necturus* urinary bladder (Fromter & Gebler, 1977), there is a direct relationship between apical membrane Na-conductance and the rate of Na absorption. Likewise, in the tracheal epithelium and corneal epithelium (Klyce & Wong, 1977; Nagel & Reinach, 1980) stimulation of Cl secretion elicits a decrease in apical membrane resistance that is specific for Cl.

Second, our results indicate that an increase in Cl secretion rate is accompanied by a decrease in basolateral membrane resistance. A similar inverse relation between the rate of transepithelial transport and basolateral membrane resistance has been observed in *Necturus* urinary bladder (Fromter & Gebler, 1977), toad urinary bladder (Davis & Finn, 1980), and *Necturus* small intestine (Gunter-Smith, Grasset & Schultz, 1982). Although the ionic basis of this change in $R_b$ has not been established with certainty, it seems likely that changes in Na-K-pump activity in both Na-absorbing and Cl-secreting epithelia are associated with parallel changes in basolateral membrane K permeability, so that several-fold alterations in the rate of transepithelial transport do not lead to marked alterations in cell composition.

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