

## Energetics of Chloride Secretion in Canine Tracheal Epithelium

### Comparison of the Metabolic Cost of Chloride Transport with the Metabolic Cost of Sodium Transport

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**A****bstract.** Canine tracheal epithelium secretes Cl and absorbs Na; the energy for both is derived from the activity of the basolateral membrane Na-K-ATPase. These properties allowed us to examine the energetics of Cl secretion by directly comparing the metabolic cost of Cl transport with the metabolic cost of Na transport. We measured the change in short-circuit current and O<sub>2</sub> consumption rate that was produced by a variety of maneuvers that alter Na and/or Cl transport rate. The experimental interventions included the use of the secretagogue epinephrine, the Cl transport inhibitor bumetanide, the Na transport inhibitor amiloride, the Na-K-ATPase inhibitor ouabain, and ion substitutions. The O<sub>2</sub> consumption rates required for Na and Cl transport were compared in each individual tissue. The results indicate that the oxygen cost of Cl transport is significantly less than the oxygen cost of Na transport: two to four Cl ions are transported for the same metabolic cost that is required to transport one Na ion. These findings suggest that the basolateral membrane Na-dependent Cl entry step couples the entry of more than one Cl ion to each Na ion.

### Introduction

Canine tracheal epithelium provides us with a unique opportunity to investigate the energetics of Cl transport because it has two active transport processes, Cl secretion and Na absorption. Thus, in a single tissue we can measure and directly

compare the metabolic cost of Cl transport with the metabolic cost of Na transport.

Several features of ion transport by tracheal epithelium allow this comparison. First, and most important, the energy source for both Na and Cl transport is Na-K-ATPase. In Na absorption, Na enters the cell passively through an amiloride-sensitive apical membrane Na conductance (1, 2). Na then exits against its electrochemical gradient via the activity of the basolateral Na-K-ATPase (3, 4). In Cl secretion, Cl enters the cell across the basolateral membrane against its electrochemical gradient by virtue of its coupling to Na (3–5). Cl then exits passively across a Cl-conductive apical membrane (2, 5, 6). The Na that enters with Cl is recycled back across the basolateral membrane by the Na-K-ATPase (7). Thus, the Na-K-ATPase provides the energy for Cl secretion by maintaining a low intracellular Na concentration.<sup>1</sup>

The second feature of tracheal epithelium important for this study is that both Na and Cl transport are electrogenic (8), so that ion transport rates can be measured as the short-circuit current, I<sub>sc</sub>,<sup>2</sup> simultaneously with O<sub>2</sub> consumption rate. Third, the rates of Na absorption and Cl secretion can be independently changed by a variety of transport inhibitors and secretagogues (1, 9).

The purpose of this study was to examine the energetics of Cl secretion in canine tracheal epithelium by comparing the oxygen consumption required for Cl transport with the oxygen consumption required for Na transport. I reasoned that: (a) the metabolic cost of Na and Cl transport would be equal if Cl entry at the basolateral membrane is tightly coupled to Na entry with a one- to-one stoichiometry, and there are no backleak pathways for Cl at the basolateral membrane; (b) the metabolic

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1. The Na-K-ATPase also indirectly supplies the energy for Cl secretion by maintaining a negative intracellular voltage. The negative electrical potential difference across both apical and basolateral membranes results from the K concentration gradient across the K conductive basolateral membrane (15, 27, 29).

2. Abbreviations used in this paper: I<sub>sc</sub>, short-circuit current; QO<sub>2</sub>, oxygen consumption rate;  $\mu\text{eq}/\mu\text{M}$  O<sub>2</sub>, ratio of microequivalents of ions transported per micromolar O<sub>2</sub> consumed.

cost of Cl transport would be greater than the cost of Na transport, if there are leak paths for Cl exit at the basolateral membrane or the stoichiometry of Cl entry involve more than one Na per Cl; or (c) the oxygen cost of Cl transport would be less than that for Na if the stoichiometry of the entry step involves more than one Cl per Na. The results suggest the third possibility.

## Methods

**Tissues and solutions.** Tracheal epithelium was prepared as previously described (10). Mongrel dogs (25–40 kg) of either sex were anesthetized with pentobarbital (25 mg/kg i.v.) and the trachea was removed. We used the posterior membranous portion after removing the muscular layer.

The bathing solution contained (in millimolar): 118.9, NaCl; 20.4,  $\text{NaHCO}_3$ ; 2.4,  $\text{K}_2\text{HPO}_4$ ; 0.6,  $\text{KH}_2\text{PO}_4$ ; 1.2,  $\text{CaCl}_2$ ; 1.2,  $\text{MgCl}_2$ ; and 10, glucose. For Cl-free solutions, Na gluconate was substituted for NaCl,  $\text{CaSO}_4$  for  $\text{CaCl}_2$ , and  $\text{MgSO}_4$  for  $\text{MgCl}_2$ . For Na-free solutions, tetramethylammonium Cl was substituted for NaCl and choline  $\text{HCO}_3$  for  $\text{NaHCO}_3$ . Na concentrations were measured with a flame photometer (Instrumentation Laboratory, Inc., Boston, MA). Cl concentrations were measured with a chloridometer (Laboratory Glass and Instruments Corp., New York). Chemicals used were: epinephrine (Elkins-Sinn, Inc., Cherry Hill, NJ); bumetanide (a generous gift of Hoffman-LaRoche Inc., Nutley, NJ); amiloride (a generous gift of Merck Sharp & Dohme Research Laboratories, West Point, PA); ouabain and indomethacin (Sigma Chemical Co., St. Louis, MO). Gentamycin sulfate (50  $\mu\text{g}/\text{ml}$ ) was present in all solutions.

**Measurement of oxygen consumption.** The epithelium was mounted between two halves of a lucite chamber (Jim's Instruments Mfg. Inc., Coralville, IA) with a 1.5-cm<sup>2</sup> aperture. The volume of each bathing solution was 3.4 ml. The chamber was water-jacketed to maintain the solution at 37°C. Magnetic stir bars vigorously stirred both bathing solutions. Chemicals were injected through air-tight ports with a syringe (Hamilton Co., Reno, NV). When oxygen consumption was not being measured, both solutions were bubbled with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  (pH 7.4 at 37°C). Ringers' bridges were used to measure the transepithelial electrical potential difference and to pass current. Na-free and Cl-free agar bridges were prepared by substitution of tetramethylammonium for Na and gluconate for Cl. All studies were performed under short-circuit conditions.

To measure oxygen consumption ( $\text{QO}_2$ ), the bubbling was stopped and Clark-type oxygen electrodes (model 53, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) were inserted into each bathing solution. The percent  $\text{O}_2$  saturation of each bathing solution was continuously measured and recorded on a strip chart recorder. The oxygen electrodes were routinely calibrated in solutions equilibrated with room air and 95%  $\text{O}_2$ , and occasionally with 51 and 75.6%  $\text{O}_2$ . The rate of oxygen consumption was calculated from the change in percent saturation of each solution, the solubility of oxygen in Ringers' solution at 37°C, and the barometric pressure. In preliminary studies, we found that  $\text{QO}_2$  was stable for periods as long as 3½ h, if the rate of transport was stable. Furthermore,  $\text{QO}_2$  and  $I_{\text{sc}}$  were stable during repeated measurements (removing and reinserting the  $\text{O}_2$  electrode) and at  $\text{O}_2$  saturations from 95 to 30%. During the usual experiment the  $\text{O}_2$  saturation fell to ~50–60% by the end of the measurement period. We did not go <30%  $\text{O}_2$  saturation. The rate of  $\text{O}_2$  consumption in the mucosal solution was usually two to four times greater than that in the submucosal solution. The process of measuring  $\text{QO}_2$  did not change solution temperature (as

measured with a microthermistor, Bailey Instruments Co., Inc., Saddle Brook, NJ). pH (as measured with a miniature pH electrode, W-P Instruments, Inc., New Haven, CT) did not fall <7.2. Injection of Ringer's solution or drug vehicle did not change  $\text{QO}_2$ . Lactic acid was measured in the bathing solution by enzymatic analysis (Sigma Chemical Co.).

Results are presented as means  $\pm$  SEM. Statistical significance was evaluated by using a paired-*t* test; a *P* value < 0.05 was considered statistically significant.

## Results

Fig. 1 shows the time course of the simultaneous measurement of  $I_{\text{sc}}$  and  $\text{QO}_2$  in one representative tissue. This figure also illustrates the protocol that we used for the first series of experiments. Indomethacin ( $10^{-6}$  M) was added to the mucosal bathing solution for at least 60 min before the start of the experiment. Indomethacin decreases endogenous prostaglandin production and decreases intracellular cyclic AMP levels, thereby minimizing the rate of Cl secretion without interfering with the subsequent response to secretagogues (6, 9, 11). Thus, the  $I_{\text{sc}}$  in the first part of the figure primarily results from Na absorption. After measuring a steady state  $I_{\text{sc}}$  and  $\text{QO}_2$ , we added amiloride ( $10^{-4}$  M) to the mucosal solution to inhibit Na transport (1, 2). Amiloride decreased both the  $I_{\text{sc}}$  and the  $\text{QO}_2$ . The decrease in  $\text{QO}_2$  was attributed to a decrease in the  $\text{O}_2$  required for Na transport. After  $I_{\text{sc}}$  and  $\text{QO}_2$  stabilized, epinephrine ( $10^{-6}$  M, mucosal solution) was added to stimulate Cl secretion (6, 12). The magnitude of the change in  $I_{\text{sc}}$   $\text{QO}_2$  was used to calculate the rate of Cl transport and the rate of Cl transport-related  $\text{O}_2$  consumption.

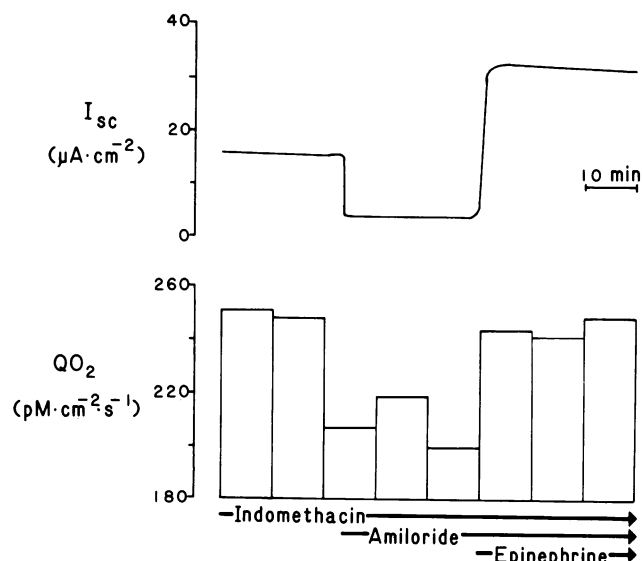


Figure 1. Effect of amiloride ( $10^{-4}$  M, mucosal solution) and epinephrine ( $10^{-6}$  M, submucosal solution) on short-circuit current and oxygen consumption. Values are from one representative tissue. Indomethacin ( $10^{-6}$  M, mucosal solution) was present throughout.

The magnitude of the changes in  $I_{sc}$  and  $QO_2$  resulting from the sequential inhibition of Na transport and then stimulation of Cl secretion, suggest that the oxygen consumption required for Cl transport may be less than that for Na transport. That is, for a similar change in  $QO_2$ , there is a greater change in the rate of Cl transport than Na transport. This point is made more clearly by the data shown in Table I, which gives the values for eight epithelia treated in the same manner as the tissue in Fig. 1. For each individual tissue we calculated a value for the microequivalents of Na transported per micromolar  $O_2$  consumed, from the amiloride-induced changes, and a value for the microequivalents of Cl transported per micromolar  $O_2$  consumed, from the epinephrine-induced changes. Furthermore, we could directly compare these two values for each individual tissue as shown in Fig. 2: For a given  $O_2$  consumption, a greater quantity of Cl than Na was transported. The mean ratio of Cl to Na transported was  $2.0 \pm 0.3 \mu\text{eq Cl}/\mu\text{eq Na}$ , a ratio significantly different from one.

In the analysis of this data, I assume that the experimental interventions did not alter the rate of  $O_2$  consumption unrelated to ion transport; i.e., the change in  $QO_2$  was entirely attributed to the change in transport. One way of indirectly examining this assumption was to use other agents to change the rate of ion transport. Table II shows the results obtained when amiloride was used to inhibit Na transport and submucosal bumetanide was used to inhibit Cl transport. Bumetanide and other loop

Table I. Oxygen Cost of Na and Cl Transport Determined from the Effect of Amiloride and Epinephrine

	$I_{sc}$	$QO_2$	$\mu\text{eq}/\mu\text{M } O_2$
	$\mu\text{A} \cdot \text{cm}^{-2}$	$\text{pmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	
Na transport			
Indomethacin ( $10^{-6}$ M)	$34 \pm 4$	$256 \pm 19$	
+ Amiloride ( $10^{-4}$ M)	$13 \pm 3$	$220 \pm 19$	
$\Delta$	$-21 \pm 3$	$-37 \pm 5$	$6.7 \pm 1.3$
Cl transport			
Indomethacin ( $10^{-6}$ M) and			
Amiloride ( $10^{-4}$ M)	$13 \pm 2$	$226 \pm 20$	
+ Epinephrine ( $10^{-6}$ M)	$104 \pm 17$	$313 \pm 29$	
$\Delta$	$+92 \pm 16$	$+87 \pm 15$	$11.5 \pm 1.5$
$\left( \frac{\mu\text{eq Cl}}{\mu\text{M } O_2} \right) \div \left( \frac{\mu\text{eq Na}}{\mu\text{M } O_2} \right) = 2.0 \pm 0.3^* \frac{\mu\text{eq Cl}}{\mu\text{eq Na}}$			

Values are mean  $\pm$  SEM from eight tissues. Mean ratio of microequivalents of ions transported per micromolar  $O_2$  consumed ( $\mu\text{eq}/\mu\text{M } O_2$ ) and ratio of microequivalents of Cl transported to microequivalents of Na transported per micromolar  $O_2$  consumed, were obtained from values calculated for each individual tissue.  $\Delta$  represents the difference between the two measurement periods.

\* Value significantly different from 1,  $P < 0.05$ .

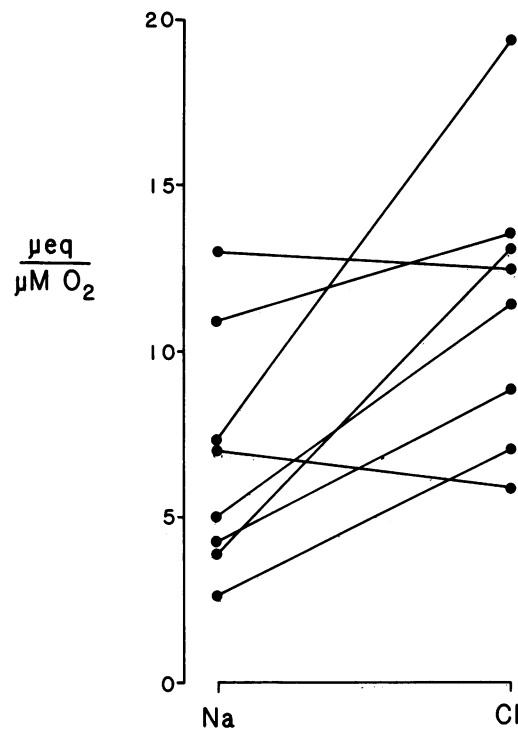


Figure 2. Comparison of the oxygen cost of Na and Cl transport ( $\mu\text{eq}/\mu\text{M } O_2$ ). The  $O_2$  cost of Na transport was determined from the change in  $I_{sc}$  and  $QO_2$  induced by amiloride ( $10^{-4}$  M). The  $O_2$  cost of Cl transport was determined from the change in  $I_{sc}$  and  $QO_2$  induced by epinephrine. Indomethacin ( $10^{-6}$  M) was present throughout.

diuretics inhibit Cl secretion by inhibiting the electrically neutral, Na-dependent Cl entry step at the basolateral membrane (5, 13, 14). For these studies the tissues were first incubated with indomethacin, to minimize Cl secretion, and the change in  $I_{sc}$  and  $QO_2$  produced by amiloride was used to evaluate Na transport. After this, submucosal epinephrine was added to stimulate Cl secretion. Then we measured the change in  $I_{sc}$  and  $QO_2$  produced by submucosal bumetanide ( $10^{-4}$  M), to evaluate Cl transport. Fig. 3 shows that in all six tissues the  $O_2$  cost for Cl transport was less than that for Na transport. The ratio of Cl to Na transport for a given  $QO_2$  was significantly greater than one, again suggesting that Cl transport is energetically more efficient.

The energy for both Na and Cl transport is derived from the Na-K-ATPase in tracheal epithelium. Therefore, we also examined the energetics of Na and Cl transport by directly inhibiting the Na pump with ouabain ( $10^{-4}$  M, submucosal solution) (3, 4). For each experiment, three tissues from the same dog were studied. One tissue was used to evaluate Na transport; indomethacin ( $10^{-6}$  M, mucosal solution) and bumetanide ( $10^{-4}$  M, submucosal solution) inhibited Cl transport. One tissue was used to evaluate Cl transport; amiloride ( $10^{-4}$

Table II. Oxygen Cost of Na and Cl Transport Determined from the Effect of Amiloride and Bumetanide

	Isc	QO <sub>2</sub>	$\mu\text{eq}/\mu\text{M O}_2$
	$\mu\text{A} \cdot \text{cm}^{-2}$	$\text{pmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	
Na transport			
Indomethacin ( $10^{-6}$ M)	33±4	271±22	
+ Amiloride ( $10^{-4}$ M)	14±4	231±24	
Δ	-19±2	-40±5	5.6±1.2
Cl transport			
Epinephrine ( $10^{-6}$ M)	94±18	300±26	
+ Bumetanide ( $10^{-4}$ M)	37±8	262±28	
Δ	-57±10	-38±6	14.9±2.1
$\left(\frac{\mu\text{eq Cl}}{\mu\text{M O}_2}\right) \div \left(\frac{\mu\text{eq Na}}{\mu\text{M O}_2}\right) = 3.1 \pm 0.5^* \quad \frac{\mu\text{eq Cl}}{\mu\text{eq Na}}$			

Values are mean±SEM from six tissues. During measurement of O<sub>2</sub> cost of Cl transport, indomethacin and amiloride were present in the mucosal solution. See legend of Table I.

\* Value significantly different from one,  $P < 0.05$ .

M, mucosal solution) inhibited Na transport and epinephrine ( $10^{-6}$  M, submucosal solution) stimulated Cl secretion. A third tissue was used to evaluate the effect of ouabain on Isc and

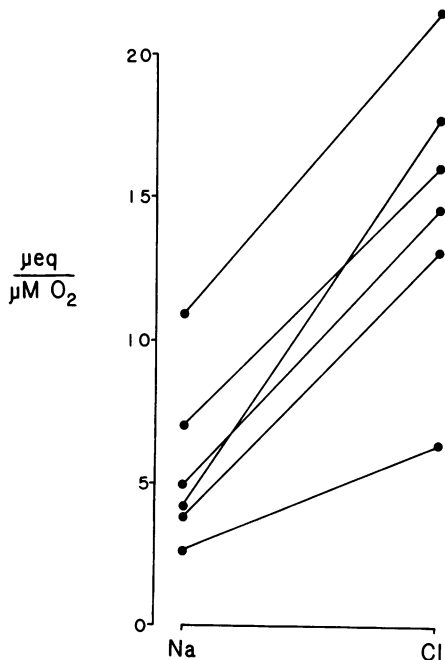


Figure 3. Comparison of the oxygen cost of Na and Cl transport. The O<sub>2</sub> cost of Na transport was determined from the change in Isc and QO<sub>2</sub> induced by amiloride. The O<sub>2</sub> cost of Cl transport was determined from the change produced by bumetanide ( $10^{-4}$  M, submucosal solution). Indomethacin was present throughout.

QO<sub>2</sub> when transport rates were minimal; amiloride, indomethacin, and bumetanide inhibited both Na and Cl transport. The changes in this third tissue will be referred to as "residual." Isc and QO<sub>2</sub> were measured during a base-line period and then 20 min after addition of ouabain. The order of studying the three tissues was randomized.

The results are shown in Table III. Ouabain decreased Isc and QO<sub>2</sub> in both the Cl-transporting and the Na-transporting tissues and the ratio of Cl to Na transport per micromolar QO<sub>2</sub> was significantly greater than one. However, despite the presence of both Na and Cl transport inhibitors, there was a residual Isc in the third group of tissues. This residual Isc probably represents incomplete inhibition of both Na and Cl transport (1, 2, 11). Ouabain decreased the residual Isc to zero and decreased QO<sub>2</sub>. Because of this effect, we subtracted the residual change in Isc and QO<sub>2</sub> from the other two tissues in each experiment and recalculated the ratio of the oxygen cost of Cl vs. Na transport. The corrected values showed more variability (larger standard errors) but the mean ratio was still significantly different from one.

One final way of comparing the O<sub>2</sub> cost of Na and Cl transport was to replace Na and Cl in the bathing solutions. For this study, epinephrine was present throughout to stimulate Cl secretion. Isc and QO<sub>2</sub> were measured during five separate periods: a "base-line" period (NaCl Ringers); a period with a Na-free mucosal solution; a base-line period; a period with both solutions

Table III. Oxygen Cost of Na and Cl Transport Determined from the Effect of Ouabain

	Na transport	Cl transport	Residual
ΔIsc ( $\mu\text{A} \cdot \text{cm}^{-2}$ )	-34±6	-81±14	-8±1
ΔQO <sub>2</sub> ( $\text{pmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ )	-70±16	-69±12	-19±10
$\mu\text{eq}/\mu\text{M O}_2$	6.0±1.1	15.2±4.4	7.9±7.1
$\mu\text{eq}/\mu\text{M O}_2$ (corrected)	6.8±2.1	20.3±4.2	
$\left(\frac{\mu\text{eq Cl}}{\mu\text{M O}_2}\right) \div \left(\frac{\mu\text{eq Na}}{\mu\text{M O}_2}\right) = 2.6 \pm 0.5^* \quad \frac{\mu\text{eq Cl}}{\mu\text{eq Na}}$			
$\left(\frac{\mu\text{eq Cl}}{\mu\text{M O}_2}\right) \div \left(\frac{\mu\text{eq Na}}{\mu\text{M O}_2}\right) (\text{corrected}) = 4.1 \pm 1.1^* \quad \frac{\mu\text{eq Cl}}{\mu\text{eq Na}}$			

ΔIsc is the change in short-circuit current and ΔQO<sub>2</sub> is the change in O<sub>2</sub> consumption rate produced by addition of ouabain ( $10^{-4}$  M, submucosal solution). Six groups of three tissues were studied. In each group one tissue was treated with indomethacin ( $10^{-6}$  M) and bumetanide ( $10^{-4}$  M), "Na transport;" one tissue received epinephrine ( $10^{-6}$  M) and amiloride ( $10^{-4}$  M), "Cl transport;" one tissue received indomethacin, bumetanide, and amiloride, "Residual." The QO<sub>2</sub> after addition of ouabain was 320±45, 304±32, and 345±59 pmol·cm<sup>-2</sup>·s<sup>-1</sup> for the three groups, respectively. Because ouabain inhibited a small Isc and produced a small change in QO<sub>2</sub> in the "Residual" tissue, the O<sub>2</sub> cost of ion transport ( $\mu\text{eq}/\mu\text{M O}_2$ ) and the ratio of Cl to Na transported per  $\mu\text{M O}_2$  ( $\mu\text{eq Cl}/\mu\text{eq Na}$ ) were also "corrected" for the residual changes.

\* Value significantly different from one,  $P < 0.05$ .

Cl-free; and a final base-line period. Between periods, the O<sub>2</sub> probes were removed from the chamber; the solutions were changed; and then the O<sub>2</sub> probes were reinserted. The order of the two ion substitutions was randomized. At the end of the measurement period the Na concentration in the Na-free solution was 2.95±0.40 mM and the Cl concentration in the Cl-free solution was 0.44±0.07 mM. Unilateral mucosal Na substitution will not only inhibit Na absorption, but may also generate a small diffusion voltage across the epithelium (10, 15). However, a diffusion voltage will be offset to some extent by a junction voltage of the opposite polarity between the tetramethylammonium-Cl bridges and the NaCl submucosal solution. Thus, the absolute value of I<sub>sc</sub> with a Na-free mucosal solution may be subject to a small error.

Table IV shows the results of this study. Due to variability in the individual values, the mean value of microequivalents of Cl/microequivalents Na was not statistically different from one (*P* = 0.08, by paired analysis). However, in each of the five experiments the ratio of microequivalents of Cl/micromolar O<sub>2</sub> was greater than the microequivalents of Na/micromolar O<sub>2</sub>. Thus, these results support the conclusion that the metabolic cost of Cl transport is less than that for Na.

To further test the assumption that the maneuvers used to change Cl transport rate do not alter cellular energy metabolism other than that responsible for ion transport, we examined the effect of epinephrine and bumetanide in tissues treated with ouabain. Fig. 4 shows that QO<sub>2</sub> was not altered by these agents when active transport was inhibited.

To determine if there was any difference in lactate production between Na- and Cl-transporting tissues, we measured the lactate

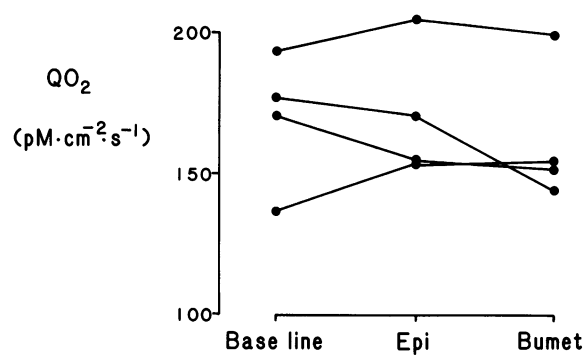


Figure 4. Effect of epinephrine (Epi) and bumetanide (Bumet) on oxygen consumption in ouabain-treated tissues. Ouabain (10<sup>-4</sup> M, submucosal solution) was present throughout. Oxygen consumption was measured during a base-line period, then after addition of epinephrine, and finally after addition of bumetanide.

in the bathing solution at the beginning and end of a 1-h period. The lactate produced by Na-transporting tissues (indomethacin treated) was 44±30 μM/h (*n* = 6). Addition of amiloride and epinephrine, to inhibit Na transport and stimulate Cl transport, did not significantly change the rate of lactate production (73±42 μM/h) (*P* = 0.23 by paired analysis).

## Discussion

The results of this study show that in canine tracheal epithelium, under short-circuit conditions, Cl secretion is energetically more efficient than Na absorption. We simultaneously measured the rate of oxygen consumption and the rate of ion transport under a variety of experimental interventions that were designed to alter either Na or Cl transport. The data indicate that for one mole of oxygen consumption, the epithelium transports more Cl ions than Na ions.

There are only a few previous studies of the energetics of Cl transport. In isolated perfused shark rectal gland, Silva et al. (16) measured the O<sub>2</sub> consumption rate and the rate of NaCl secretion. Since Cl secretion is the active transport process (17), they used the rate of Cl efflux from the gland duct and the total rate of O<sub>2</sub> consumption (determined from the arterial-venous O<sub>2</sub> difference) to calculate the microequivalents of Cl/micromolar O<sub>2</sub> ratio. On the basis of the predicted number of ATP moles produced per O<sub>2</sub> used, and the assumed number of Na transported per ATP hydrolyzed by the Na pump, they concluded that the gland transported 70% more Cl than predicted if 1 mol of Cl were transported for every mole of Na transported by the Na pump. Oxygen consumption has also been measured in frog cornea, another Cl-secreting epithelium. Reinach et al. (18) measured O<sub>2</sub> consumption and Zadunaisky et al. (19) measured the rate of CO<sub>2</sub> production from radiolabeled glucose. However, the tissues were not mounted as epithelial sheets, so that the rate of metabolism could not be directly correlated

Table IV. Oxygen Cost of Na and Cl Transport Determined from the Effect of Ion Substitutions

	I <sub>sc</sub>	QO <sub>2</sub>	
	μA · cm <sup>-2</sup>	pmol · cm <sup>-2</sup> · s <sup>-1</sup>	μeq/μM O <sub>2</sub>
Na			
Base line	84±10	322±26	
Na-free	70±10	296±32	
Δ	-14±2	-26±8	8.6±3.1
Cl			
Base line	89±14	279±22	
Cl-free	21±3	248±24	
Δ	-68±12	-31±9	26.3±4.7
$\left(\frac{\mu\text{eq Cl}}{\mu\text{M O}_2}\right) \div \left(\frac{\mu\text{eq Na}}{\mu\text{M O}_2}\right) = 5.4 \pm 1.9 \quad \frac{\mu\text{eq Cl}}{\mu\text{eq Na}}$			

Values are mean±SEM from five tissues. In "Na-free" condition, tetramethylammonium was substituted for Na in the mucosal solution. In "Cl-free" condition, gluconate was substituted for Cl in both bathing solutions. Epinephrine (10<sup>-6</sup> M) was present throughout. See legend of Table I.

with the rate of ion transport. Martin and Diamond (20) studied the energetics of Cl absorption in rabbit gallbladder and found a good correlation between  $\text{QO}_2$  and isotonic fluid absorption. On the basis of the observed stoichiometry of Na and Cl transported per  $\text{O}_2$  consumed, they concluded that the total number of ions transported (Na plus Cl), was twice as great as in epithelia that only transport Na actively, while the stoichiometry of Cl transport or Na transport per  $\text{O}_2$  was similar to that found in Na-transporting epithelia.

It is appropriate to consider the assumptions involved in the present study. First, I assume that the experimental interventions do not change the ratio of  $\text{O}_2$  consumption to ATP production. The lack of any change in lactate production rate at least suggests that there are not large changes in anaerobic ATP generation. Second, I assume that the various interventions do not change the magnitude of nontransport-related metabolism or ATP utilization. In support of this assumption, neither epinephrine nor bumetanide altered  $\text{QO}_2$  in tissues in which all ion transport was inhibited with ouabain. Third, I assume that the various interventions do not change the number of Na ions transported per mole of ATP consumed by the Na-K-ATPase. In support of this assumption, previous studies suggest a constant ratio of Na transported per  $\text{O}_2$  consumed within an individual tissue (21, 22). Furthermore, in support of all three assumptions, we used four different experimental maneuvers to change Cl transport and three different experimental maneuvers to alter Na transport. In all the protocols, the results consistently show that the  $\text{O}_2$  consumption used for Cl transport is less than for Na transport, thereby providing an indirect test of these assumptions.

One of the advantages of this study, in comparison to previous studies, is that our conclusions do not depend upon assumptions about the actual stoichiometry of the metabolic steps between  $\text{O}_2$  consumption and Na-pump activity; we only assume that the stoichiometry does not change.<sup>3</sup> We directly compared the rate of  $\text{O}_2$  consumption required for Cl transport to the  $\text{QO}_2$  required for Na transport. We were able to do this because the metabolic energy for both transport processes is derived from the Na-K-ATPase. Another advantage of this study is that the ratio of microequivalents of Cl/microequivalents of Na transported per micromolar  $\text{O}_2$  consumed was calculated for each individual tissue. The ability to analyze the data in this way avoids the problems and uncertainties inherent in analysis of averages obtained from a population of epithelia (21, 23).

The most straightforward interpretation of this data is that the Na-dependent Cl entry step at the basolateral membrane

couples the entry of more than one Cl to each Na. This interpretation explains the observation that two to three (significantly more than one) Cl are transported for the same metabolic cost as one Na.<sup>4</sup> The mechanism that mediates Cl entry may represent the cotransport of one Na-one K-two Cl as has been described in a number of cells including Ehrlich ascites tumor cells (24) and the cortical thick ascending limb of the loop of Henle (25). Several observations would support such a mechanism. First, the Cl entry step is electrically neutral (6), as would be predicted by this mechanism. Second, loop diuretics inhibit Cl entry in trachea with a half-maximal dose similar to that required to inhibit the one Na-one K-two Cl cotransport system (5, 13, 14). Third, neither removal of  $\text{HCO}_3^-$  from the bathing solutions, application of substituted stilbenes (4-acetamido-4'-isothiocyanato-2,2'-stilbene disulfonate, SITS, or 4,4'-diisothiocyanostilbene-2,2'-disulfonate, DIDS), nor addition of acetazolamide was able to inhibit Cl secretion (unpublished observations). These maneuvers would be expected to inhibit neutral Cl transport via parallel Cl- $\text{HCO}_3^-$  and Na-H cotransport processes. Finally, in shark rectal gland, another Cl-secreting epithelium, studies of the Cl entry step using basolateral membrane vesicles are consistent with coupled entry of Na, K, and 2 Cl (26).

Certainly the energy in the Na gradient is sufficient to drive the entry of more than one Cl for each Na transported. Under secreting short-circuit conditions, the electrochemical gradient for Cl is  $-22$  mV (5) and the electrochemical gradient for K is  $-24$  mV (27). Thus, if the entry step involved the cotransport of one Na-one K-two Cl, 68 mV would be required from the Na electrochemical potential difference. An estimate of the Na electrochemical gradient from the basolateral membrane voltage (55–60 mV) and the intracellular Na concentration of 20 mM measured in isolated canine tracheal epithelial cells (28) yields a value of  $+107$  mV, more than sufficient to energize the entry of two Cl and one K.

The electrochemical potential difference for Na is used to energize "secondary-active" Cl transport in a wide variety of both secretory and absorptive epithelia. Thus, these results may be of significance for epithelia other than trachea by suggesting that Cl transport is coupled to Na transport in a way that maximizes the use of the metabolic energy used to generate the Na gradient.

3. In some Na-transporting epithelia the mean ratio of microequivalents of Na transported per micromolar  $\text{O}_2$  consumed was  $\sim 18$ , although substantial variability in the ratio has also been observed. The ratio of 18 is larger than the ratio found in this study; I do not know the reason for this difference. However, as indicated above, the conclusions and validity of this study do not depend upon the absolute value of the stoichiometry between ion transport and oxygen consumption.

4. We observed some variability in the mean ratio of microequivalents of Cl/microequivalents of Na transported per micromolar  $\text{O}_2$  consumed with the different experimental protocols (Tables I–IV). This variability may have resulted from small discrepancies between the measured changes in  $I_{sc}$  and the changes in the Na and Cl transport rates. Another possible explanation for the variability is that some of the maneuvers may have had a small and variable effect on nontransport metabolism. Despite this variability, the important point is that the ratio of microequivalents of Cl per microequivalents of Na was significantly greater than a ratio of one with all the different maneuvers. In addition, the mean values of microequivalents of Cl per microequivalents of Na obtained with the different protocols were not significantly different from one another.

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