J. Membrane Biol. 166, 15–35 (1998)

The Journal of
Membrane
Biology

Transcellular Chloride Pathways in *Ambystoma* Proximal Tubule

S. Abdulnour-Nakhoul*, E.L. Boulpaep
Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520-8026, USA

Received: 14 November 1997/Revised: 6 July 1998

Abstract. The transport mechanisms of *Ambystoma* proximal tubule that mediate transcellular Cl^- absorption linked to Na^+ were investigated in isolated perfused tubules using Cl^--selective and voltage-recording microelectrodes. In control solutions intracellular activity of Cl^- (a_i^{Cl}) is 11.3 ± 0.5 mM, the basolateral (V_1), apical (V_2), and transepithelial (V_3) potential differences are −68 ± 1.2 mV, +62 ± 1.2 mV and −6.4 ± 0.3 mV, respectively. When Na^+ absorption is decreased by removal of organic substrates from the lumen, a_i^{Cl} falls by 1.3 ± 0.3 mM and V_2 hyperpolarizes by +11.4 ± 1.7 mV. Subsequent removal of Na^+ from the lumen causes a_i^{Cl} to fall further by 2.3 ± 0.4 mM and V_2 to hyperpolarize further by +15.3 ± 2.4 mV. The contribution of transporters and channels to the observed changes of a_i^{Cl} was examined using ion substitutions and inhibitors. Apical Na/Cl or Na/K/2Cl symport is excluded because bumetanide, furosemide or hydrochlorothiazide have no effect on a_i^{Cl}. The effects of luminal HCO_3^- removal and/or of disulfonic stilbenes argue against the presence of apical Cl-base exchange such as Cl-HCO_3 or Cl-OH. The effects of basolateral HCO_3^- removal, of basolateral Na^+ removal and/or of disulfonic stilbenes are compatible with presence of basolateral Na-independent Cl-base exchange and Na-driven Cl-HCO_3 exchange. Several lines of evidence favor conductive Cl^- transport across both the apical and basolateral membrane. Addition of the chloride-channel blocker diphenylamine-2-carboxylate to the lumen or bath, increases the a_i^{Cl} by 2.4 ± 0.6 mM or 2.9 ± 1.0 mM respectively. Moreover, following inhibition by DIDS of all anion exchangers in HCO_3^--free Ringer, the equilibrium potential for Cl^- does not differ from the membrane potential V_2. Finally, the logarithmic changes in a_i^{Cl} in various experimental conditions correlate well with the simultaneous changes in either basolateral or apical membrane potential. These findings strongly support the presence of Cl^- channels at the apical and basolateral cell membranes of the proximal tubule.

Key words: Proximal tubule — Chloride channels — Chloride-base exchange — Na-driven Cl-HCO_3 exchange — Transcellular chloride transport — Disulfonic stilbenes

Introduction

The proximal tubule epithelium reabsorbs a major part of the filtered Cl^-. The paracellular pathway of this epithelium is highly permeable to Cl^- in both mammalian and amphibian kidneys (Boulpaep, 1971; Frömter & Gessner, 1974; Andreoli et al., 1979). Cl^- reabsorption occurs across both the paracellular and transcellular pathway, although the relative magnitude of Cl^- transport across each pathway remains controversial in the mammalian proximal tubule (Aronson & Giebisch, 1997). Tracer fluxes in *Necturus* proximal tubule indicate that about one-third of filtered Cl^- is reabsorbed via the transcellular pathway and two-thirds are reabsorbed via the paracellular pathway (Kimura & Spring, 1978).

Transcellular transport of Cl^- has been reported to be linked to Na^+, either directly or indirectly. A direct apical cotransport mechanism for Na^+ and Cl^- has been described in the proximal tubule of *Necturus* (Kimura & Spring, 1978). Other studies failed to demonstrate the presence of a NaCl symporter in the amphibian (Seifter & Aronson, 1984; Abdulnour-Nakhoul & Boulpaep, 1986), as well as in the mammalian proximal tubule (Seifter, Knickelbein & Aronson, 1984). Entry of chloride from the filtrate into the mammalian proximal tubule

* *Present Address:* Department of Medicine, Section of Gastroenterology SL 35, 1430 Tulane Avenue, New Orleans, LA 70112-2699, USA

Correspondence to: E.L. Boulpaep

cell is an uphill process probably mediated by exchange of Cl-formate (Karniski & Aronson, 1985; Schild et al., 1987; Wang, Giebisch & Aronson, 1992), Cl^- oxalate (Wang et al., 1992), or Cl-HCO_3 (Lucci & Warnock, 1979). Operation of apical Cl-base exchange in parallel with apical Na-H exchange would couple Na^+ and Cl^- transport indirectly. Moreover, apical Cl-base exchange would lead to intracellular accumulation of chloride above its predicted electrochemical equilibrium across the apical membrane. The nature of the exit step for chloride across the basolateral membrane of the mammalian proximal tubule remains controversial. Indirect studies have suggested that a Na-driven Cl-HCO_3 exchanger may be involved (Alpern & Chambers, 1987) or that chloride exits via an electroneutral KCl symporter (Eveloff & Warnock, 1987; Ishibashi, Rector & Berry, 1990). Patch-clamp studies have shown the presence of basolateral chloride channels in single isolated proximal tubule cells of *Ambystoma* (Segal & Boulpaep, 1990) and rabbit (Segal, Geibel & Boulpaep, 1993). Microperfusion studies in rabbit (Schild, Aronson & Giebisch, 1991) and rat (Wang et al., 1992, 1995) proximal tubule support the view that chloride may leave the cell through basolateral chloride channels.

The purpose of the present study was to provide more insight in the apical and basolateral transport mechanisms of Cl^- that participate in NaCl reabsorption by the proximal tubule. We used the isolated perfused proximal tubule preparation of the salamander, *Ambystoma tigrinum*, because the large cell size and shallow basal infoldings permit stable microelectrode impalements. To determine the linkage of Cl^- transport to Na^+ during transcellular NaCl reabsorption, we used luminal removal of organic metabolic substrates and Na^+, a maneuver known to abolish effectively and reversibly NaCl reabsorption (Forster, Steels & Boulpaep, 1980; Morgunov & Boulpaep, 1987). Using Cl^--ion selective microelectrodes we monitored changes in intracellular chloride activity (a_i^{Cl}) that accompany transcellular NaCl absorption. Changes in cell membrane potential differences were recorded simultaneously using conventional microelectrodes. We also examined the effect of diverse Cl^- transport inhibitors on steady-state a_i^{Cl} as well as on Na-linked Cl^- movement.

The present results indicate that both direct and indirect mechanisms couple Cl^- transport to the apical and basolateral transport of Na^+. At the apical membrane, neither direct Na/Cl cotransport nor a parallel arrangement of Na-H exchange with Cl-HCO_3 or other Cl-base exchange can explain the observed change in Cl^- transport. However, apical Cl^- channels in combination with the membrane potential changes induced by altered NaCl transport, can account for the modulation of a_i^{Cl}. At the basolateral membrane, Cl-HCO_3 or Cl-OH exchange is present and the transport of Cl^- may be linked to the opposite movement of basolateral Na^+ via Na-driven Cl-HCO_3 exchange, but neither transporter can explain the shifts of Cl^- activity observed during changes in transcellular Na^+ transport. In contrast, basolateral Cl^- channels in association with basolateral membrane potential changes account for the modulation of a_i^{Cl}, that accompanies altered NaCl transport. The present experiments also show that, when no anionic bases other than bicarbonate and lactate are present in the tubule lumen, the uphill accumulation of Cl^- into the cell results primarily from basolateral stilbene-sensitive Cl-base exchange and that under these conditions transepithelial Cl movement should proceed via a paracellular route.

Materials and Methods

Female tiger salamanders, *Ambystoma tigrinum*, in the neotenic phase were kept unfed in an aquarium at 4°C. The animals were anesthetized by immersion in 0.2% tricaine methane sulfonate solution. The kidneys were removed, placed in chilled pre-oxygenated PVP Ringer solution (solution 10, Table 1), and cut transversely into several pieces. Proximal tubules were isolated and perfused as described by Sackin and Boulpaep (1981*a*). Briefly, single tubules (100 μm O.D.) with glomeruli intact were isolated from the ventral surface of the kidney. The early part of the tubule, 700 to 1,000 μm in length, was dissected and the ends opened with very fine forceps.

Tubule segments were transferred in a small amount of medium to a perfusion chamber and connected to a pipette assembly as described by Burg et al. (1966) and by Sackin and Boulpaep (1981*a*). The apparatus consists of two sets of three concentric pipettes. The tubule was mounted at each end between an outer (holding) and a middle (perfusion) pipette by applying slight suction. Proper mechanical and electrical seal around the tubule was thus formed at a constriction in the holding pipette. Perfusion fluid was delivered to the inner (exchange) pipette of the right-hand side assembly, at a rate of 1 ml/min. Only a small amount of fluid entered the tip of the perfusion pipette and tubule lumen (50 nl/min), the rest leaving the pipette through a drain, where it contacted a flowing 3 M KCl electrode (tip diameter = 5 μm). The perfusate leaving the tubule entered the middle pipette of the left-hand side assembly, where continuous suction was applied to keep the liquid-air interface inside the middle pipette at a vertical height of 15 mm above bath level. The solution bathing the outside of the tubule was continuously exchanged at 3 ml/min. Experiments were conducted at 21–25°C. Tubule cells were visualized with an inverted microscope (Reichert, Vienna, Austria) and microelectrode impalements were made at a magnification of 400×.

Solutions

The composition of the solutions is given in Table 1. HEPES, N-methyl-D-glucamine (NMDG), furosemide, hydrochlorothiazide (HCTZ), and 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS) were purchased from SIGMA (St. Louis, MO). 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate (SITS) was obtained from International Chemical and Nuclear (Cleveland, OH). Whenever the isothiocyanate-containing disulfonic stilbenes SITS or DIDS were added to the solution, amino acids were omitted from the solution leaving only glucose and lactate as organic substrate (Minimal Substrates Solution 3 and 8 of Table 1). Diphenylamine-2-carboxylate (DPC), obtained from Fluka (Ronkonkoma, NY), was dissolved in a

small amount of dimethyl sulfoxide (DMSO) before addition to the solution. The concentration of DMSO in the final solution never exceeded 0.1%. Bumetanide, (obtained from Hoffman-LaRoche, Nutley, NJ) was dissolved in a small amount of NaOH before addition to the solution.

Electrodes

Ling-Gerard microelectrodes were pulled from 1 mm O.D. borosilicate fiber capillaries (FHC, Brunswich, ME) and filled with 3M KCl. Resistances ranged from 25–50 MΩ and tip potentials were <5 mV. Potential differences were recorded as defined earlier (Sackin & Boulpaep, 1981*a*). The basolateral membrane potential $V_1 = (\Psi_i - \Psi_{bath})$ was obtained from the intracellular voltage Ψ_i, recorded by a Ling-Gerard microelectrode with reference to Ψ_{bath}, recorded by a free-flowing 3M KCl electrode (tip ~5 μm) in the bath. The transepithelial potential $V_3 = (\Psi_{lumen} - \Psi_{bath})$ was measured as the voltage difference between a free flowing KCl electrode in the drain of the perfusion pipette connected to the lumen (Ψ_{lumen}), and the bath electrode (Ψ_{bath}). Apical membrane potential V_2 was defined as $(\Psi_{lumen} - \Psi_i)$, the potential in the lumen with reference to the cytosol, and calculated by subtracting V_1 from V_3.

Ion-selective microelectrodes were pulled from aluminosilicate glass (FHC, Brunswick, ME) on a Brown-Flaming micropipette puller (Sutter, San Raphael, CA) and dried in an oven at 200°C for 2 hr. Ten μl of tri-n-butyl-chlorosilane were then introduced in a closed vessel (300 ml) that contained the microelectrodes, left for 2 min, after which the silane fumes were vented and the electrodes left in the oven for an additional 30 min. A small amount of chloride liquid ion exchanger (Corning 477913) was introduced into the tip of the electrodes by means of very fine glass capillaries. The electrodes were further backfilled with 0.5 M KCl which made contact with a Ag-AgCl half cell. The slope of each electrode (S) was determined from the equation

$$S = \frac{V_{100\text{ mM NaCl}} - V_{10\text{mM NaCl}}}{0.94} \quad (1)$$

where $V_{100\text{ mM NaCl}}$ and $V_{10\text{ mM NaCl}}$ denote the electrode potential in the solutions in subscript, 0.94 equals the logarithm (base 10) of the Cl^- activity ratio of pure 100 mM over pure 10 mM NaCl. The average slope of the electrodes was 56.4 mV/10-fold change in activity. The selectivity for Cl^- over HCO_3^- was calculated from the equation:

$$k_{Cl-HCO_3} = 10^{[V_{100\ HCO_3^-} - V_{100\ Cl^-}]/S} \quad (2)$$

where $V_{100\ HCO_3^-}$ is the voltage of the Cl^- electrode in 100 mM $NaHCO_3$ and $V_{100\ Cl^-}$ the voltage in 100 mM NaCl. The k_{Cl-HCO_3} was 0.09, yielding a average selectivity ratio of Cl^- over HCO_3^- of 11:1 (11 ± 0.02) ($n = 61$).

The gross value of the ion-sensitive electrode potential (V_{Cl}) was measured as the voltage difference between the Cl^--selective electrode and a free flowing KCl electrode in the bath. The net Cl^- potential ($V_{Cl} - V_1$) was obtained by electronic subtraction of V_1 from V_{Cl}. All electrical potential differences were monitored and continuously recorded on a four-channel strip chart recorder (Brush 2300; Gould, Cleveland, OH).

The intracellular activity of Cl^- (a_i^{Cl}) was determined from:

$$a_i^{Cl} = a^{Cl}100\text{ mM NaCl} \cdot 10^{[(V_{Cl} - V_1)_i - V_{Cl} - V_1)100\text{ mM NaCl}]/S} - k_{Cl-HCO_3}(a_i^{HCO_3}) \quad (3)$$

where the subscripts ''*i*'' or ''$_{100\text{ mM NaCl}}$'' refer to recordings of both electrodes when they are located inside the cell or in a 100 mM NaCl solution respectively, and $a^{Cl}{}_{100\text{ mM NaCl}}$ is 0.778 at 25°C.

The correction term $k_{Cl\text{-}HCO_3}(a_i^{HCO_3})$ requires independent knowledge of the intracellular $a_i^{HCO_3}$. Assuming a control value of 4.7 mM for $a_i^{HCO_3}$ as determined in our laboratory in *Ambystoma* proximal tubule cells (Boron & Boulpaep 1983*b*), the correction would be 0.4 mM. Because the $a_i^{HCO_3}$ could not be monitored independently and the correction is smaller than the standard error of the determination of the uncorrected chloride activity (*see* Results), the second term of Eq. 3 was omitted from the calculation of a_i^{Cl}. It is unlikely that unknown interfering anions, other than HCO_3^-, would have a higher selectivity or be present in the cell at a substantially higher activity than HCO_3^-. Moreover, it is impossible to estimate the changes in intracellular concentration of such unknown interfering anions during the experimental maneuvers in this study that affect anion transport. Therefore, no fixed correction for interfering anions was used (Spring & Kimura, 1978).

Because the Cl^- ion-selective electrode is sensitive to anion transport inhibitors, such as SITS or DIDS (Chao & Armstrong, 1987), we took special care not to expose the electrode to the inhibitor in the bathing solution. Only those Cl^--selective electrode readings were included where cell impalement had preceded the exposure of the tubule to the inhibitor. Since cell membrane permeability to disulfonic stilbenes is negligible (Grinstein, McCulloch & Rothstein, 1979), any effect of the inhibitor on the intracellular recording could be neglected.

The Nernst equilibrium potential for Cl^- across the basolateral membrane was calculated from intracellular and bath activities of Cl^-

$$E_{Cl} = -\frac{RT}{zF} ln \frac{a_i^{Cl}}{a_{bath}^{Cl}} \quad (4)$$

The Nernst equilibrium potential for Cl^- across the apical membrane was calculated from lumen and intracellular activities of Cl^-

$$E_{Cl} = -\frac{RT}{zF} ln \frac{a_{lumen}^{Cl}}{a_i^{Cl}} \quad (5)$$

Statistical Analysis

The results are presented as mean ± SEM. The number of observations is shown as *n*. Data were analyzed using the two-tailed paired Student's *t* test. When two different sets of experiments were compared, unpaired *t*-test was used for analysis.

Results

In 49 proximal tubules perfused on both lumen and bath side with control HCO_3^- solution (Solution 1 of Table 1) the basolateral membrane potential difference, V_1, was −68 ± 1.2 mV, the apical membrane potential difference, V_2 was +62 ± 1.2 mV, the transepithelial membrane potential difference, V_3, was −6.4 ± 0.3 mV and the intracellular activity of chloride a_i^{Cl} was 11.3 ± 0.5 mM ($n = 66$). The measured a_i^{Cl} yields an electrochemical equilibrium potential for Cl^- (E_{Cl}) across the basolateral membrane of −48.0 mV (Eq. 4) and across the apical membrane of +48.0 mV (Eq. 5). Since E_{Cl} for each membrane is smaller than the electrical potential difference ($V_1 - E_{Cl} = -20$ mV and $V_2 - E_{Cl} = +14$ mV), chloride is at a higher intracellular activity then predicted

Table 1. Composition of solutions

Component	Control HCO_3^-	0 Substrate HCO_3^-	Minimal Substrates HCO_3^-	0 Na 0 Substrate HCO_3^-	0 Na HCO_3^-	Standard HCO_3^--free	0 Substrate HCO_3^--free	Minimal Substrates HCO_3^--free	0 Na 0 Substrate HCO_3^--free	PVP HCO_3^-
Na^+	100.95	100.9	100.9	0	0.05	97.6	97.6	97.6	0	100.95
$NMDG^+$	0	0	0	100.4	100.4	0	0	0	97.1	0
K^+	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mg^{++}	1	1	1	1	1	1	1	1	1	1
Ca^{++}	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Lys^+	0.2	0	0	0	0.2	0.2	0	0	0	0.2
Cl^-	94.7	98.1	94.5	97.6	94.2	94.7	98.1	94.5	97.6	94.7
HCO_3^-	10	10	10	10	10	0	0	0	0	10
$H_2PO_4^-$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPO_4^-	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
$Lactate^-$	3.6	0	3.6	0	3.6	3.6	0	3.6	0	3.6
Glu^-	0.05	0	0	0	0.05	0.05	0	0	0	0.05
$HEPES^-$	0	0	0	0	0	6.7	6.7	6.7	6.7	0
Glucose	2.2	0	2.2	0	2.2	2.2	0	2.2	0	2.2
Gln	0.5	0	0	0	0.5	0.5	0	0	0	0.5
Ala	0.5	0	0	0	0.5	0.5	0	0	0	0.5
HEPES	0	0	0	0	0	6.7	6.7	6.7	6.7	0
Mannitol	0	4	0	4	0	0	4	0	4	0
PVP g/l	0	0	0	0	0	0	0	0	0	15
CO_2 (%)	1.5	1.5	1.5	1.5	1.5	0	0	0	0	1.5
O_2 (%)	98.5	98.5	98.5	98.5	98.5	100	100	100	100	98.5

Composition of solutions given in mM unless otherwise indicated.
PVP is polyvinylpyrrolidone (average molecular weight 40,000).

by equilibrium across either the basolateral or the apical membrane of the cell. This is in agreement with previous findings in *Ambystoma* proximal tubule (Sackin & Boulpaep, 1981*b*), in *Necturus* proximal tubule (Khuri et al., 1975; Guggino, Boulpaep & Giebisch, 1982; Guggino et al., 1983), and in mammalian proximal tubule (Cassola, Mollenhauer & Frömter, 1983; Ishibashi, Sasaki & Yoshiyama, 1988).

Organic Substrates and/or Sodium in the Lumen Modulate Intracellular Chloride

To examine the effect of luminal Na^+ on Cl transport, the initial step was to remove substrates from the lumen. Removal of organic substrates reduces fluid and salt absorption in the amphibian proximal tubule (Forster, Steels & Boulpaep, 1980; Morgunov & Boulpaep, 1987). Deletion of organic substrates, glucose, amino-acids and lactate from the lumen eliminates the major pathways for apical Na^+ entry into the cell. The contribution of other Na-linked transport mechanisms to Cl^- transport was examined by monitoring a_i^{Cl} during subsequent removal of luminal Na^+. Deletion of organic substrates from the luminal solution (Solution 2) hyperpolarized V_1 by -10.2 ± 1.4 mV ($n = 23$), V_2 by 11.4 ± 1.7 mV ($n = 15$), depolarized V_3 by $+3.2 \pm 0.4$ mV ($n = 16$) and decreased the a_i^{Cl} by 1.3 ± 0.3 mM ($n = 22$) (data of Table 2-I). The electrogenic response of V_1, V_2, and V_3 to the removal of substrates is similar to that observed by Morgunov and Boulpaep (1987) in the same preparation. The observed changes upon removal of luminal substrates required the presence of Na^+ in the lumen (*data not shown*).

Figure 1 shows a tracing of a representative experiment in which Na^+ was removed from the lumen (Solution 4), following the removal of substrates. In addition to the electrical effect due to substrate removal, the absence of Na^+ caused V_1 and V_2 to hyperpolarize further by -10.8 ± 1.6 mV ($n = 22$) and $+15.3 \pm 2.4$ mV ($n = 16$), respectively, V_3 to depolarize by $+3.0 \pm 0.5$ mV ($n = 16$), and a_i^{Cl} to fall further by 2.3 ± 0.4 mM ($n = 22$). The data are summarized in Table 2-II. Since the effects on a_i^{Cl} were reversible, it can be assumed that substrate-linked or Na-linked fluid absorption is associated with an elevated a_i^{Cl}. A fall in a_i^{Cl} was also observed in Necturus proximal tubule cells after removal of Na^+ from the lumen (Spring & Kimura, 1978; Guggino et al., 1983).

The hyperpolarization of V_1 and V_2 observed upon removal of luminal Na^+ in the absence of substrates suggests the presence of a conductive pathway for Na^+ at the apical cell membrane (Abdulnour-Nakhoul & Boulpaep,

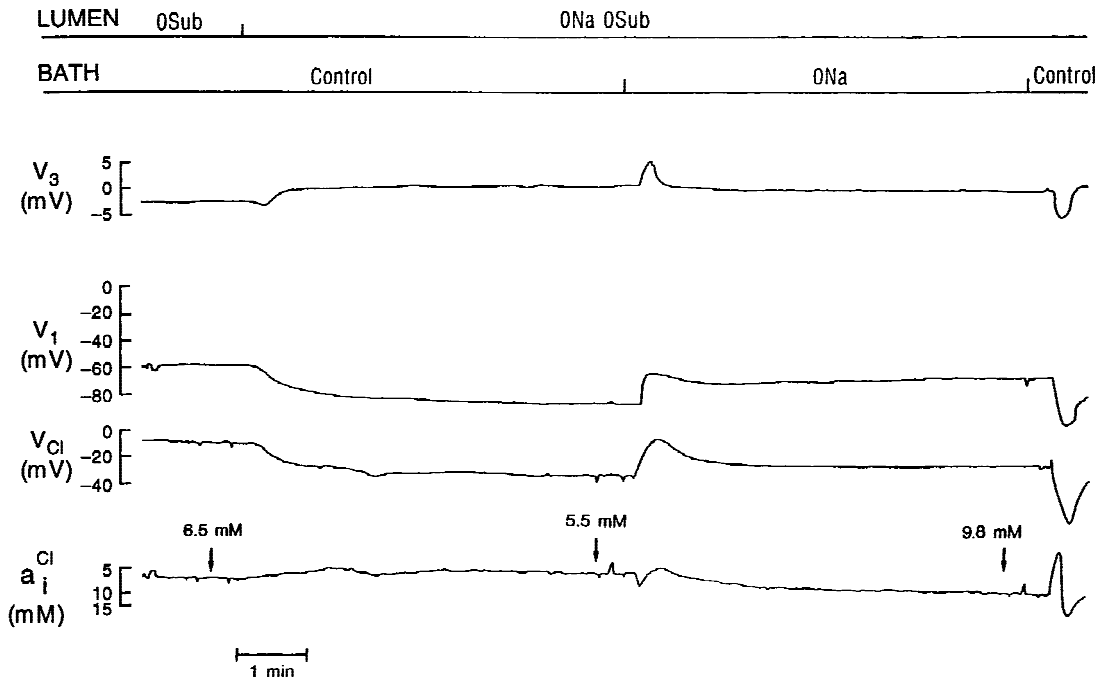

Fig. 1. Effects of removal of luminal Na^+ subsequent to the removal of luminal substrates and the effect of basolateral Na^+ removal on four electrical recordings. Top tracing: transepithelial potential (V_3 = lumen − bath = $V_1 + V_2$). Second tracing from top: basolateral membrane potential (V_1 = cytosol − bath); Third tracing from top: gross potential of the chloride electrode (V_{Cl}). Bottom tracing: ($V_{Cl} - V_1$), expressed as a logarithmic scale of intracellular activity of chloride (a_i^{Cl}). Data from this and similar experiments are summarized in Table 2-II and 2-III.

Table 2. Effect of removal of luminal substrates and Na^+, and removal of basolateral Na^+, on the basolateral (V_1), luminal (V_2), and the transepithelial membrane (V_3) potential differences and on intracellular activity of Cl^-, (a_i^{Cl})

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)	V_1-E_{Cl} (mV)	V_2-E_{Cl} (mV)
I	Control (L and B)	−56 ± 2.5	51 ± 2.9	−4.7 ± 0.4	12.8 ± 1.0	−8.6 ± 1.8	5.5 ± 1.8
	0 Sub (L), Control (B)	−66 ± 2.4	63 ± 3.2	−1.5 ± 0.4	11.5 ± 0.8	−16.5 ± 1.5	15.1 ± 2.0
	n	(23)	(16)	(16)	(22)	(22)	(15)
	P	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001
II	0 Sub (L), Control (B)	−66 ± 2.3	62 ± 2.9	−1.2 ± 0.4	10.6 ± 0.7	−15.1 ± 1.2	13.1 ± 2.2
	0 Na 0 Sub (L), Control (B)	−77 ± 2.0	78 ± 2.6	1.8 ± 0.4	8.3 ± 0.6	−19.4 ± 1.4	20.4 ± 1.8
	n	(22)	(16)	(16)	(22)	(22)	(16)
	P	<0.001	<0.001	<0.001	<0.001	<0.005	<0.005
III	0 Na 0 Sub (L), Control (B)	−77 ± 4.5	76 ± 5.7	2.1 ± 0.8	8.2 ± 0.1	−19.3 ± 3.8	18.8 ± 4.6
	0 Na 0 Sub (L), 0 Na (B)	−57 ± 4.8	165 ± 4.7	1.9 ± 0.8		−5.8 ± 3.7	5.3 ± 3.7
	n	(8)	(6)	(6)	(8)	(8)	(6)
	P	<0.001	<0.001	NS	<0.02	<0.001	<0.001

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 11 different tubules. E_{Cl} is the Nernst equilibrium for Cl^- calculated as outlined in Materials and Methods Eq. (4) or (5).

1986). The hyperpolarization caused by luminal Na^+ removal increases the passive driving forces $V_1 - E_{Cl}$ and $V_2 - E_{Cl}$, for Cl^- exit across both the basolateral membrane and apical membrane and could explain in part the fall in a_i^{Cl}. However, the a_i^{Cl} remains markedly above equilibrium ($V_1 - E_{Cl}$ = −19.4 ± 1.4 mV and $V_2 - E_{Cl}$ = +20.4 ± 1.8 mV) after the combined removal of luminal substrates and Na^+. These findings indicate the existence of an active transport mechanism for Cl^- entry into the cell which is independent of luminal Na^+.

Following the removal of substrates and Na^+ from the lumen, Na^+ was also omitted from the bath (Solution 5) as shown in Fig. 1. V_1 and V_2 depolarized on average by +19.6 ± 1.1 mV (n = 8) and by −20.7 ± 1.6 mV (n

Table 3. Effect of 10^{-4} M bumetanide in the lumen and the effect of removal of luminal substrates and Na^+, in the presence of bumetanide

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)
I	Control (L and B)	−70 ± 2.6	62 ± 2.7	−7.6 ± 0.5	11.1 ± 1.6
	Bumetanide (L), Control (B)	−72 ± 2.1	66 ± 2.1	−5.6 ± 0.3	11.0 ± 1.6
	n	(13)	(13)	(14)	(7)
	P	<0.001	<0.001	<0.001	NS
II	Bumetanide (L), Control (B)	−68 ± 3.3	63 ± 3.3	−5.0 ± 0.3	9.8 ± 0.9
	0 Sub + Bumetanide (L), Control (B)	−81 ± 3.3	78 ± 3.4	−2.2 ± 0.3	8.5 ± 0.9
	n	(14)	(14)	(14)	(10)
	P	<0.001	<0.001	<0.001	<0.001
III	0 Sub + Bumetanide (L), Control (B)	−79 ± 4.5	78 ± 4.3	−1.4 ± 0.3	9.5 ± 0.9
	0 Na 0 Sub + Bumetanide (L), Control (B)	−86 ± 3.1	88 ± 3.1	2.2 ± 0.3	8.1 ± 0.8
	n	(11)	(11)	(11)	(9)
	P	<0.005	<0.001	<0.001	<0.001

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 4 different tubules.

= 6), respectively, while the a_i^{Cl} increased by 2.1 ± 0.6 mM (n = 8). The data are summarized in Table 2-III. The depolarizations of V_1 and V_2 agree with an overriding effect of Na^+ removal on the membrane potential via the electrogenic Na/HCO_3 cotransporter on the basolateral membrane (Boron & Boulpaep, 1983*b*). The depolarizations caused by Na^+ removal from the bath decrease also the passive driving forces ($V_1 - E_{Cl}$) and ($V_2 - E_{Cl}$) for Cl^- exit across both the basolateral and apical membrane and could explain in part the rise in a_i^{Cl}.

Several mechanisms may contribute directly or indirectly to the observed change in a_i^{Cl} that is associated with Na-substrate-linked or Na-linked absorption. These include: (i) at the apical membrane of the cell: Na/Cl or Na/K/2Cl cotransport, (ii) at the apical membrane: Na-independent Cl-base exchange such as Cl-OH, Cl-HCO_3, Cl-formate or Cl-oxalate exchange, (iii) at the basolateral membrane: Na-independent Cl-HCO_3 exchange or Na-driven Cl-HCO_3 exchange (with stoichiometry of Na/HCO_3^- Cl/H or Na/2HCO_3-Cl), and (iv) at apical and/or basolateral membrane: Cl^- channels. The following sections describe experimental maneuvers that test for the presence of the transporters and channels listed and examine their role in chloride transport.

Effect of Bumetanide, Furosemide, and Hydrochlorothiazide

The presence of a Na/Cl symporter as described for *Necturus* proximal tubule (Spring & Kimura, 1978), or a Na/K/2Cl cotransporter similar to that present in the mammalian thick ascending limb of Henle's loop (Greger & Schlatter, 1981), would explain the fall in a_i^{Cl} upon removal of Na^+ from the lumen. Bumetanide and furosemide are two potent loop diuretics known to inhibit the Na/K/2Cl transporter (Oberleithner et al., 1982; Schlatter, Greger & Weidtke, 1983). Bumetanide has also been reported to block NaCl transport in *Necturus* gallbladder (Larton & Spring, 1983).

Table 3 shows the effect of the addition of 10^{-4} M bumetamide to the luminal perfusion fluid, as well as the effect of removal of luminal substrates and sodium in the presence of bumetamide. Perfusing the lumen with a control solution containing 10^{-4} M bumetamide caused slight but significant hyperpolarizations of V_1 and V_2 of −2.1 ± 0.6 mV and +4.2 ± 0.8 mV (n = 13), respectively, and a depolarization of V_3 of +2.0 ± 0.2 mV (n = 14). However, the a_i^{Cl} remained unchanged (Table 3-I). When substrates were removed from the lumen in the presence of bumetanide, V_1 and V_2 hyperpolarized by −12.6 ± 0.8 mV and +15.3 ± 0.8 mV (n = 14), respectively, V_3 depolarized by +2.8 ± 0.2 mV (n = 14), and the a_i^{Cl} fell by 1.3 ± 0.3 mM (n = 10) (data of Table 3-II). These values are not different from those obtained when substrates are removed in the absence of bumetanide. Further removal of Na^+ from the lumen in the presence of bumetanide (Table 3-III) hyperpolarized V_1 and V_2 by −7 ± 1.9 mV and +10.6 ± 1.7 mV (n = 11) respectively, depolarized V_3 by +3.6 ± 0.5 mV (n = 11), and reduced a_i^{Cl} by 1.3 ± 0.2 mM (n = 9, $P < 0.001$). Thus, the loss of 1.3 mM a_i^{Cl} upon removal of luminal Na^+ in the presence of bumetanide, results likely from mechanism(s) other than Na/Cl or Na/K/2Cl symport. Moreover, we found in a previous study using Na^+ ion-selective microelectrodes (Abdulnour-Nakhoul & Boulpaep, 1986), that the activity of intracellular Na^+ (a_i^{Na}) did not change upon the removal of luminal Cl^- in the presence or absence of bumetanide, which provides additional evidence against a sizable Na/Cl or Na/K/2Cl cotransport at the apical membrane. The addition of furosemide (10^{-4} M) to the luminal solution showed no effect on a_i^{Cl}. The substrates and Na^+ removal effects are not altered by the presence of furosemide in the lumen (*data not shown*).

Table 4. Effect of 5×10^{-4} M SITS in the lumen and the effect of removal of luminal substrates and Na^+ in the presence of luminal SITS

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)
I	Minimal Sub (L), Control (B)	−68 ± 4.2	66 ± 4.4	−2.0 ± 0.3	11.1 ± 0.9
	SITS (L), Control (B)	−63 ± 4.0	61 ± 4.9	−2.5 ± 0.6	14.0 ± 2.8
	n	(3)	(3)	(3)	(3)
	P	<0.025	<0.02	NS	NS
II	SITS (L), Control (B)	−53 ± 3.4	50 ± 3.7	−2.8 ± 0.4	14.2 ± 1.0
	0 Sub + SITS (L), Control (B)	−60 ± 4.4	59 ± 4.8	−1.1 ± 0.5	13.5 ± 1.1
	n	(10)	(10)	(10)	(10)
	P	<0.001	<0.001	<0.001	NS
III	0 Sub + SITS (L), Control (B)	−60 ± 4.4	59 ± 4.9	−1.3 ± 0.5	13.6 ± 1.3
	0 Na 0 Sub + SITS (L), Control (B)	−76 ± 2.1	78 ± 1.8	1.9 ± 0.3	9.5 ± 0.5
	n	(9)	(9)	(9)	(9)
	P	<0.001	<0.001	<0.005	<0.025

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 3 different tubules.

Hydrochlorothiazide (HCTZ) is a known inhibitor of Na/Cl symport in the distal tubule (Costanzo & Windhager, 1978) and in the urinary bladder of the winder flounder (Stokes, 1984). In a series of experiments on *Ambystoma* proximal tubules, addition of HCTZ (10^{-4} M) to the luminal solution had no effect on the cell membrane potential differences nor on the a_i^{Cl}. The effects of luminal removal of substrates in the presence of HCTZ were not different from the effects of substrate removal under control conditions. When Na^+ was removed from the lumen in the presence of HCTZ, V_1 hyperpolarized from −81 ± 3.9 mV to −84 ± 3.4 mV ($n = 5$; $P < 0.01$), V_2 also hyperpolarized from +81 ± 3.9 mV to +85 ± 3.5 mV ($n = 5$; $P < 0.005$) and a_i^{Cl} decreased from 8.2 ± 1.2 mM to 6.9 ± 1.1 mM ($n = 4$; $P < 0.01$). The reduced hyperpolarization of V_1 and V_2 following removal of luminal Na^+ in the presence of HCTZ, cannot be attributed to the inhibition of the electroneutral Na/Cl cotransport and is probably due to secondary actions of the drug on the cells (Fernandez & Puschett, 1973). The present results indicating the absence of Na/Cl or Na/K/2Cl cotransport in the apical membrane of the amphibian proximal tubule, confirm the report on rabbit renal microvillus membrane vesicles (Seifter et al., 1984).

Effect of SITS

Apical Cl-Base Exchangers

Several Cl-base exchangers have been shown to exist in mammalian brush-border membrane preparations, such as Cl-OH, Cl-HCO_3, Cl-formate or Cl-oxalate exchange (Warnock & Yee, 1981; Seifter et al., 1984; Karniski & Aronson, 1985; Karniski & Aronson, 1987). The operation of Cl-base exchange in parallel with Na-H exchange can lead to net NaCl uptake at the apical border of the proximal tubule cell (Aronson, 1989). In the present experiments, removal of Na^+ from the lumen would reverse the operation of the apical Na-H exchanger (Boron & Boulpaep, 1983*a*) and cause a drop in pH_i. Removal of lactate from the lumen of *Ambystoma* proximal tubule would also decrease intracellular pH (pH_i) in Hepes-buffered media (Siebens & Boron, 1987). Therefore, both the removal of substrates and that of Na^+ from the lumen are likely to reduce pH_i and buffer anions in the cell. In both maneuvers apical Cl-base exchangers (Cl-OH, Cl-HCO_3, Cl-formate or Cl-oxalate exchange), carrying Cl^- into the cell and buffer anions out, would slow down and cause a fall of a_i^{Cl}.

The disulfonic stilbene SITS blocks Cl-HCO_3 exchange (Cabantchik & Rothstein, 1972) and Cl^- oxalate exchange (McConnell & Aronson, 1994), but is less effective on Cl-formate exchange (McConnell & Aronson, 1994). Thus, SITS was applied to the lumen to examine the contribution of Cl-base exchange to Cl^- entry from lumen to cell. To avoid covalent binding of SITS to amino groups of amino acids, particularly of lysine, amino acids were omitted (Solution 3) in the following experiments, prior to the addition of SITS. The addition of $5 \cdot 10^{-4}$ M SITS to the luminal solution gradually depolarized V_1 and V_2 by +4.7 ± 0.7 mV and −5.3 ± 0.7 mV ($n = 3$), respectively, and there was a small but not significant increase in the a_i^{Cl}. The data are summarized in Table 4-I.

The removal of glucose and lactate from the lumen in the presence of $5 \cdot 10^{-4}$ M SITS (Table 4-II) caused V_1 and V_2 to hyperpolarize by −7.8 ± 1.3 mV and +9.4 ± 1.4 mV ($n = 10$) respectively, V_3 to depolarize by +1.7 ± 0.2 mV ($n = 10$) and the a_i^{Cl} decreased slightly by 0.7 ± 0.4 mM ($n = 10$), but not significantly. This change in a_i^{Cl} upon removal of luminal substrates in SITS was not different ($P < 0.3$) from the decrease in a_i^{Cl} of 1.3 ± 0.3 mM ($n = 22$, Table 2-II), observed in the absence of SITS. The electrical changes were also not significantly differ-

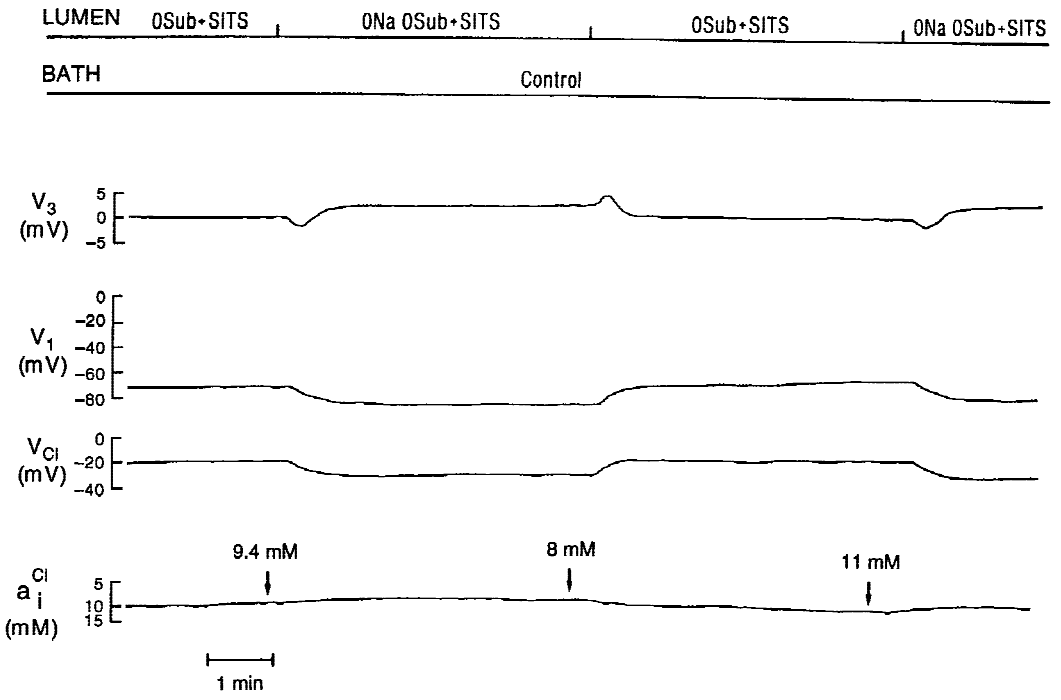

Fig. 2. Effects of removal of luminal Na^+ in the absence of luminal substrates and in the presence of SITS ($5 \cdot 10^{-4}$ M) in the lumen. The changes observed are not different from the changes in the absence of luminal SITS. V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Table 4-III.

ent from those observed in the absence of SITS. Thus, SITS-sensitive apical Cl-base exchange does not appear to play a role in the observed fall in a_i^{Cl} after substrate removal.

Subsequent removal of Na^+ from the lumen further decreased the a_i^{Cl} by 4.1 ± 1.5 mM ($n = 9$, $P < 0.025$) alongside a hyperpolarization of V_1 by −16.1 ± 2.8 mV, of V_2 by +19 ± 3.4 mV ($n = 9$, $P < 0.001$) and a depolarization of V_3 by +3.3 ± 0.7 mV (Fig. 2 and Table 4-III). Those values are not significantly different from the values obtained upon removal of luminal Na^+ under control conditions. The persistent change in a_i^{Cl} upon removal of luminal Na^+ cannot be caused by a SITS-inhibitable Cl-base exchanger operating in the apical membrane in parallel with Na-H exchange.

Basolateral Cl-Base Exchangers

Na-independent Cl-HCO_3 or Cl-OH exchange has been described in the basolateral membrane of *Necturus* kidney (Edelman, Bouthier & Anagnostopoulos, 1981), of rat and rabbit proximal tubule (Alpern & Chambers, 1987; Grassl, Holohan & Ross, 1987; Nakhoul, Chen & Boron, 1990). Guggino et al. (1983) provided evidence for a basolateral Na-driven Cl-HCO_3 exchange mechanism (Na/HCO_3-Cl/H) in *Necturus* proximal tubule, that translocates Cl^- out of the cell and Na^+ and 2 HCO_3^- into the cell. A Na-driven Cl-HCO_3 exchange has also been described in the rat and rabbit proximal convoluted tubules (Alpern & Chambers, 1987; Sasaki & Yoshiyama, 1988). SITS blocks both Na-independent Cl-HCO_3 (Edelman et al., 1981; Alpern & Chambers, 1987) and Na-driven Cl-HCO_3 (Guggino et al., 1983) transporters, and in addition inhibits the electrogenic Na/HCO_3 cotransporter (Boron & Boulpaep, 1983*b*). Both Na-independent and Na-driven Cl-HCO_3 exchange could account for the decrease in a_i^{Cl} following the removal of luminal substrates and Na^+. Indeed, removal of luminal substrates and Na^+ decreases not only a_i^{Na} (Morgunov & Boulpaep, 1987) but also pH_i (Boron & Boulpaep, 1983*a*). As a result of both a fall in a_i^{Na} and pH_i, the driving force would be increased for the Na/HCO_3-Cl/H exchanger to carry more Na^+ and HCO_3^- into the cell and more Cl^- out. Similarly, as a result of the fall in pH_i Cl-HCO_3(OH) exchange would be reduced causing less Cl^- to enter.

To examine whether the effect of luminal Na^+ removal on a_i^{Cl} is mediated indirectly by an effect on basolateral Na/HCO_3-Cl/H or Cl-HCO_3(OH) exchangers, the inhibition by SITS in the bath was tested in four experimental protocols. The data are summarized in Table 5 and a typical experiment is shown in Fig. 3. In the first protocol, SITS ($5 \cdot 10^{-4}$ M) alone was added to the basolateral solution (Table 5-I). As a result, V_1, V_2, and V_3 gradually hyperpolarized by −7.3 ± 1.2 mV, +5.6 ± 1.2 mV, and −1.6 ± 0.3 mV, respectively ($n = 14$). The a_i^{Cl} fell by 1.8 ± 0.6 mM ($n = 10$). This hy-

Table 5. Effect of 5×10^{-4} M SITS in the bath and the effect of removal of luminal substrates and Na^+ as well as the removal of basolateral Na^+ in the presence of SITS in the bath

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)	V_1-E_{Cl} (mV)	V_2-E_{Cl} (mV)
I	Control (L), Minimal Sub (B)	−71 ± 2.3	65 ± 2.3	−6.4 ± 0.7	10.3 ± 0.8	−21.3 ± 1.9	14.2 ± 1.8
	Control (L), SITS (B)	−78 ± 1.7	70 ± 1.9	−8.0 ± 1.0	8.6 ± 0.7	−23.2 ± 1.4	14.4 ± 1.6
	n	(14)	(14)	(14)	(10)	(10)	(10)
	P	<0.001	<0.001	<0.001	<0.02	NS	NS
II	Control (L), SITS (B)	−75 ± 2.8	66 ± 3.0	−8.8 ± 0.9	10.5 ± 1.1	−21.2 ± 1.2	11.8 ± 1.1
	0 Sub (L), SITS (B)	−82 ± 3.0	80 ± 3.3	−1.2 ± 0.4	9.0 ± 1.3	−23.5 ± 1.6	22.1 ± 1.6
	n	(13)	(13)	(13)	(11)	(11)	(11)
	P	<0.001	<0.001	<0.001	<0.005	NS	<0.001
III	0 Sub (L), SITS (B)	−77 ± 3.4	75 ± 3.5	−1.5 ± 0.3	8.5 ± 1.0	−18.9 ± 3.2	17.2 ± 3.2
	0 Na 0 Sub (L), SITS (B)	−80 ± 3.2	82 ± 3.1	1.8 ± 0.4	7.3 ± 0.8	−18.7 ± 3.3	20.6 ± 3.2
	n	(15)	(15)	(16)	(14)	(14)	(14)
	P	<0.005	<0.001	<0.001	<0.001	NS	<0.02
IV	0 Na 0 Sub (L), SITS (B)	−74 ± 3.9	76 ± 3.9	2.8 ± 0.2	6.2 ± 0.8	−7.9 ± 2.4	11.2 ± 2.5
	0 Na 0 Sub (L), 0 Na+ SITS (B)	−71 ± 2.8	71 ± 2.7	0.7 ± 0.2	6.2 ± 0.8	−5.1 ± 1.9	5.7 ± 1.9
	n	(14)	(14)	(14)	(14)	(14)	(14)
	P	NS	<0.05	<0.001	NS	<0.02	<0.005

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 16 different tubules.

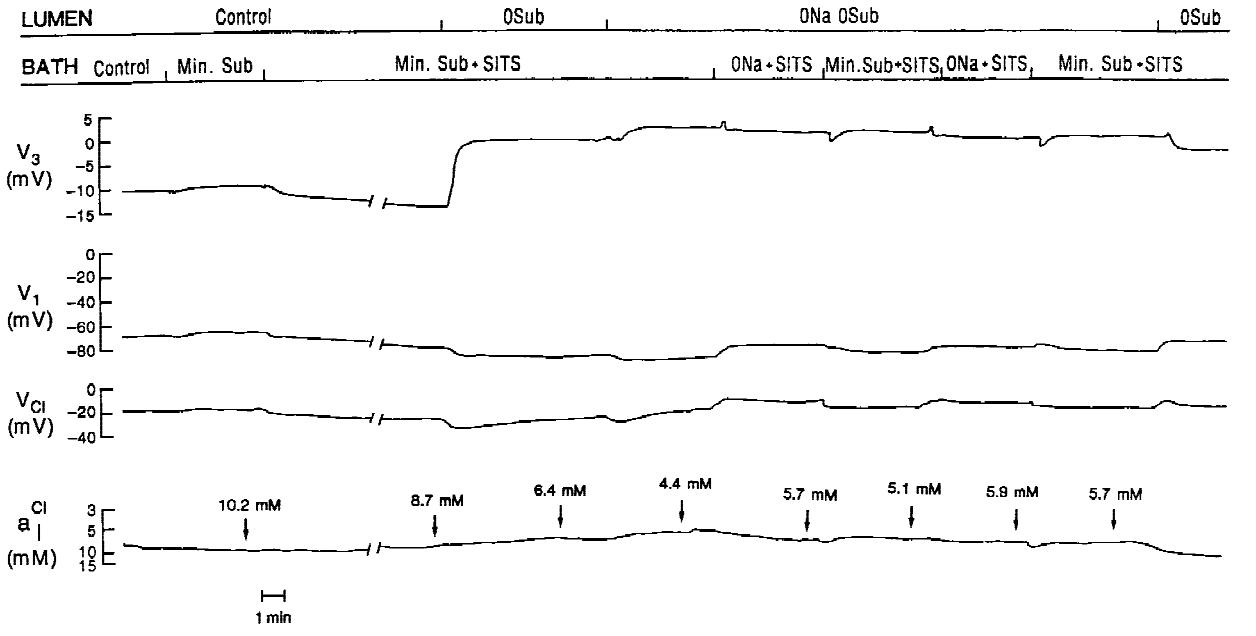

Fig. 3. Effect of addition of SITS ($5 \cdot 10^{-4}$ M) to a bath that contained only the minimal substrates glucose and lactate (Min. Sub), followed by the removal of luminal substrates, the removal of luminal Na^+, and finally the removal of basolateral Na^+ in the presence of SITS in the bath. The gap in the record represents an interval of 6 min. V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Table 5-I, 5-II, 5-III, and 5-IV.

perpolarization of V_1 is largely due to inhibition by SITS of the electrogenic Na/HCO_3 cotransporter (Boron & Boulpaep, 1983*b*), that carries negative charge out of the cell. The decrease in a_i^{Cl} upon addition of basolateral SITS could be the result of the inhibition of a Cl^- entry step at the basolateral membrane such as $Cl\text{-}HCO_3(OH)$ exchange.

In the second protocol substrates were removed from the lumen, while the bath contained $5 \cdot 10^{-4}$ M SITS (Table 5-II). The a_i^{Cl} still fell by 1.2 ± 0.3 mM (n = 11), V_1 hyperpolarized by −6.5 ± 0.9 mV, V_2 by +14.1 ± 1.1 mV, and V_3 depolarized by +7.6 ± 0.9 mV (n = 13). The various isoforms of the H^+/monocarboxylate cotransporter (MCT) differ in sensitivity to stilbenes

(Wang, Levi & Halestrap, 1996). Renal basolateral lactate transport via the basolateral H^+/monocarboxylate cotransporter (MCT) is not blocked by low concentrations of DIDS (Rosenberg, Fadil & Schuster, 1993) and only weakly inhibited by SITS in a nominally HCO_3-free bath (Siebens & Boron, 1987). Therefore, any fall in pH_i, potentially caused by the apical removal of lactate, should not have been altered by the presence of SITS. In contrast, inhibition of the basolateral rheogenic Na/HCO_3 transporter by SITS could have increased intracellular HCO_3^- and pH (Boron & Boulpaep, 1983*b*). There are two interpretations for the persistent change in a_i^{Cl}: either lactate removal does not lead to a drop in pH_i in HCO_3-containing solutions, or basolateral Cl^- base exchange is not responsible for the observed changes in a_i^{Cl}.

In the third protocol luminal Na^+ was removed in the presence of basolateral $5 \cdot 10^{-4}$ M SITS (Table 5-III). The sodium removal hyperpolarized V_1 by −3.3 ± 0.8 mV ($n = 15$) and V_2 by +6.7 ± 1.1 mV ($n = 15$), depolarized V_3 by +3.4 ± 0.5 mV ($n = 16$), and decreased the a_i^{Cl} by 1.2 ± 0.3 mM ($n = 14$). The hyperpolarization of V_1 and V_2 upon removal of luminal Na^+ was significantly less in the presence of SITS ($P < 0.005$, $n = 37$ unpaired *t*-test and $P < 0.005$, $n = 31$ unpaired *t*-test, respectively) as compared to that observed upon removal of luminal Na^+ in the absence of SITS. The reduced hyperpolarization of V_1 and V_2 is most likely due to the simultaneous inhibition of the rheogenic Na/HCO_3 cotransporter by SITS. The change in a_i^{Cl} is reduced but not abolished by the presence of SITS in the bath ($P < 0.05$, $n = 36$ unpaired *t*-test). Thus, the reduced response of a_i^{Cl} to the assumed fall in a_i^{Na} and pH_i may be due to SITS-inhibitable basolateral Na/HCO_3-Cl/H or Cl-HCO_3 (OH) exchange. The remaining portion of a_i^{Cl} change upon removal of luminal Na^+ can indicate the operation of SITS-insensitive Cl^- transport mechanisms.

In the fourth protocol we verified the ability of $5 \cdot 10^{-4}$ M SITS to effectively block basolateral Na-driven Cl-HCO_3 exchange. When basolateral Na^+ was removed, in the presence of SITS in the bath, no significant change in a_i^{Cl} resulted (Table 5-IV). This is in contrast to the increase in a_i^{Cl} observed upon removal of basolateral Na^+ in the absence of SITS in the bath (Table 2). Thus, the rise in a_i^{Cl} observed upon removal of basolateral Na^+ appears to be mediated by a SITS-inhibitable mechanism, most likely Na/HCO_3-Cl/H exchange. It may be noted that basolateral Na^+ removal, which normally depolarizes V_1 by +19.6 ± 1.1 mV (Table 2-III), did not affect V_1 in the presence of SITS. This confirms the concomitant inhibition of a basolateral rheogenic Na/HCO_3 transporter by SITS.

Role of Bicarbonate

To examine whether the effect of luminal Na^+ removal on a_i^{Cl} is mediated indirectly by altered driving forces on basolateral Na/HCO_3-Cl/H or Cl-HCO_3 exchange, the role of bicarbonate removal was investigated. A typical experiment is shown in Fig. 4 and the results are summarized in Table 6. When the lumen was perfused with a HCO_3^--free solution (Solution 6), V_1 and V_2 hyperpolarized by −5.3 ± 0.8 mV and +6.2 ± 0.7 mV ($n = 12$), respectively, V_3 depolarized by +0.7 ± 0.2 mV ($n = 12$), whereas a_i^{Cl} decreased by 1.1 ± 0.3 mM ($n = 12$) (Table 6-I). If Cl^- uptake occurred in exchange for cellular HCO_3^-, removal of HCO_3^- from the lumen would be expected to drive more Cl^- into the cell. In contrast, the fall of a_i^{Cl} upon removal of luminal bicarbonate argues against apical CL-HCO_3 exchange contributing to Cl^- uptake. When bicarbonate was subsequently removed from the bath, V_1, V_2, and V_3 depolarized by +5.7 ± 1.1 mV, −5.1 ± 0.8, and +0.8 ± .03 mV ($n = 11$), respectively whereas a_i^{Cl} increased by 1.5 ± 0.5 mM ($n = 11$) (Table 6-II). The effect of basolateral bicarbonate removal on a_i^{Cl} is compatible with the operation of either a basolateral Cl-HCO_3 exchanger or a Na/HCO_3-Cl/H exchanger driving bicarbonate out and Cl^- into the cell. The changes in cell potential differences must be due to the electrogenic exit of Na/HCO_3 (Boron & Boulpaep, 1983*b*).

Long-term removal of HCO_3 from lumen and bath did not achieve electrochemical equilibrium for Cl^- across either basolateral or apical membrane (Table 7-I). Several explanations exist why a_i^{Cl} remains above equilibrium in nominally HCO_3-free solutions: (i) the experimental solutions were not totally CO_2- and HCO_3-free, (ii) an apical Cl-base exchanger that is not inhibited by luminal SITS, (iii) basolateral Cl-OH exchange, also is not fully inhibited by SITS.

Effect of Organic Substrate Removal and Na^+ Removal in HCO_3^- Free Solutions

In view of the preceding results, the response to substrate and/or luminal Na^+ removal was further examined in nominally HCO_3-free solutions in both lumen and bath (Fig. 4 and Table 7). In the absence of bicarbonate, the removal of substrates (Table 7-I) had no effect on a_i^{Cl} and the hyperpolarization of V_1 by −4.5 ± 1.7 mV ($n = 10$) was significantly smaller than that observed in Table 2-I upon removal of substrates in the presence of bicarbonate ($P < 0.02$, $n = 33$, unpaired *t*-test). V_2 hyperpolarized by +6.4 ± 1.8 mV ($n = 10$) also less than in HCO_3^- solutions ($P < 0.05$, $n = 26$, unpaired *t*-test). The depolarization of V_3 by +1.9 ± 0.4 mV ($n = 10$) was not different from that seen in bicarbonate. When substrates are removed from the lumen, the inward current of apical Na-glucose and Na-aminoacid transporters is abolished and V_1 and V_2 will hyperpolarize to values determined by the diffusional e.m.f.s at the apical and basolateral membrane, in particular the K conductance of the basolateral membrane (Morgunov & Boulpaep, 1987). However,

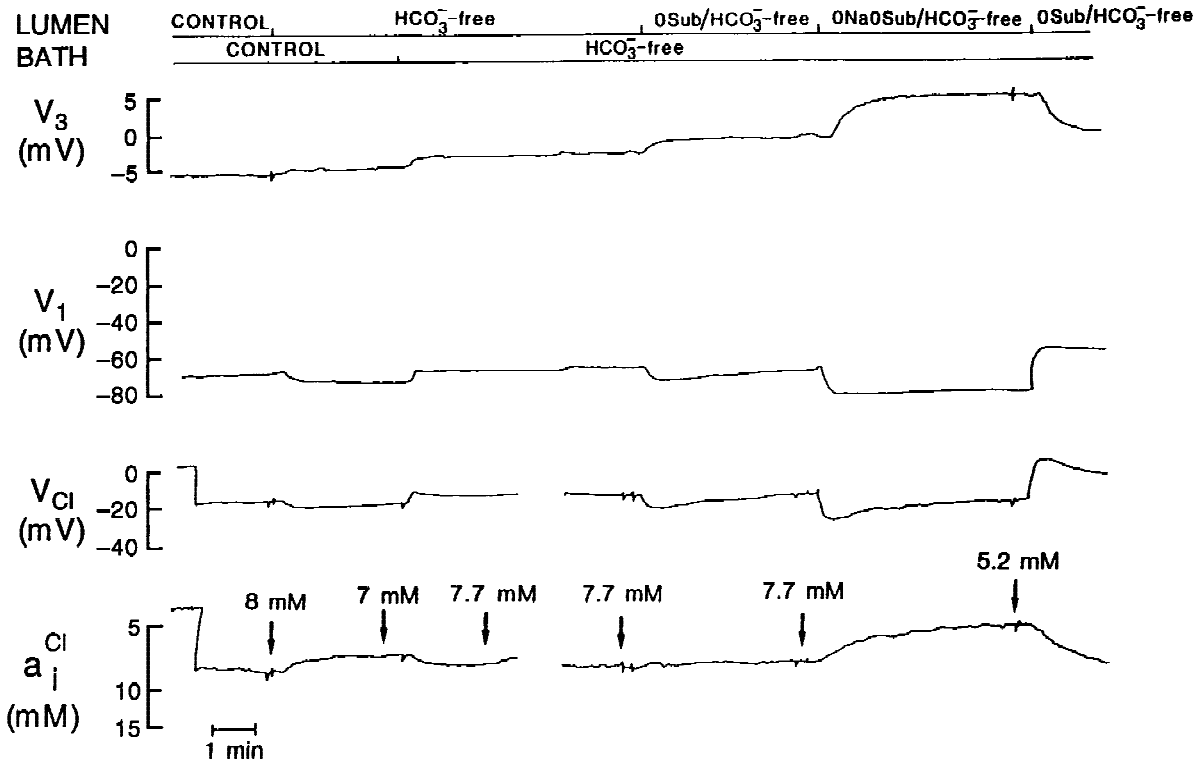

Fig. 4. Effects of removal of HCO_3^- and the effects of removal of luminal substrates and Na^+ in the absence of HCO_3^-. V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Tables 6-I, 6-II, 7-I and 7-II.

Table 6. Effect of removal of bicarbonate from the lumen first, then from the bath on V_1, V_2, and V_3 and on a_i^{Cl}

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)
I	Control (L and B)	−62 ± 3.0	56 ± 2.9	−6.8 ± 0.4	13.0 ± 1.1
	HCO_3^--free (L), Control (B)	−68 ± 2.9	62 ± 2.9	−6.1 ± 0.4	11.9 ± 1.1
	n	(12)	(12)	(12)	(12)
	P	<0.001	<0.001	<0.005	<0.005
II	HCO_3^--free (L), Control (B)	−68 ± 3.1	62 ± 3.2	−6.4 ± 0.5	12.0 ± 1.1
	HCO_3^--free (L and B)	−62 ± 3.2	57 ± 3.0	−5.6 ± 0.6	13.5 ± 1.4
	n	(11)	(11)	(11)	(11)
	P	<0.001	<0.001	<0.02	<0.02

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 10 different tubules.

the lactate removal in bicarbonate-free solutions induces a marked fall of pH_i of up to 0.4 pH units (Siebens & Boron, 1987). Low pH_i is known to depolarize V_1 (Boron & Boulpaep, 1983*a*) and to reduce the basolateral K conductance (Steels & Boulpaep, 1987). Thus, the important fall of pH_i due to the bicarbonate-free solutions used in this experiment explains the reduced hyperpolarizations of V_1 and V_2 as well as the depression of the a_i^{Cl} response that accompanies such hyperpolarization.

In contrast to the effect of substrate removal, the removal of Na^+ from the lumen (Solution 9) had very sizable effects (Table 7-II). a_i^{Cl} decreased by 3.6 ± 1.1 mM (n = 10), V_1 hyperpolarized by −13.7 ± 1.5 mV (n = 12), V_2 hyperpolarized by +25.8 ± 1.3 mV and V_3 depolarized by 11.9 ± 1.0 mV (n = 12). The potential changes of V_2 and V_3 are significantly larger than the values observed upon removal of luminal Na^+ in bicarbonate ($P < 0.005$, n = 28, unpaired t-test). It should be noted that V_3 in the presence of a bath to lumen Na^+ gradient was +9.4 mV. The enhanced electrical response of V_2 and V_3 upon removal of luminal Na^+ may result from an increased paracellular diffusional e.m.f. for Na^+, perhaps due to a higher paracellular Na^+ permeability in bicarbonate-free solutions.

It should be noted that the a_i^{Cl} of 8.7 mM, measured in the absence of bilateral bicarbonate and luminal Na^+

Table 7. Effect of removal of luminal substrates and Na^+, in the absence of HCO_3^-

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)	V_1-E_{Cl} (mV)	V_2-E_{Cl} (mV)
I	HCO_3^--free (L and B)	−54 ± 3.4	50 ± 3.3	−4.4 ± 0.5	14.0 ± 1.2	−10.9 ± 1.9	6.5 ± 1.7
	0 Sub (L), HCO_3^--free (L and B)	−59 ± 3.5	56 ± 3.7	−2.5 ± 0.4	13.7 ± 1.2	−15.1 ± 2.6	12.7 ± 2.6
	n	(10)	(10)	(10)	(10)	(10)	(10)
	P	<0.025	<0.01	<0.005	NS	<0.025	<0.005
II	0 Sub (L), HCO_3^--free (L and B)	−58 ± 2.7	55 ± 2.8	−2.5 ± 0.3	12.3 ± 1.3	−11.0 ± 1.3	8.3 ± 1.3
	0 Na 0 Sub (L), HCO_3^--free (L and B)	−72 ± 2.3	81 ± 1.9	9.4 ± 0.8	8.7 ± 0.9	−15.8 ± 1.5	26.0 ± 2.3
	n	(12)	(12)	(12)	(12)	(12)	(12)
	P	<0.001	<0.001	<0.001	<0.01	<0.01	<0.001

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 4 different tubules.

(Table 7-II), still exceeds that predicted by passive distribution across either the basolateral or the apical membrane. Indeed, Table 7-II shows that $V_1 - E_{Cl} = 15.8 \pm 1.5$ mV, and $V_2 - E_{Cl} = +26 \pm 2.3$ mV ($n = 12$). Thus, the secondary active transport mechanism which raises a_i^{Cl} above equilibrium is not dependent on HCO_3^- and does not require the presence of luminal Na^+.

Effect of Organic Substrate Removal and Na^+ Removal in HCO_3^- Free Solutions Containing DIDS

The stilbene derivative DIDS is a powerful inhibitor of Cl-base exchange including Cl-formate and Cl-oxalate exchangers (Aronson, 1989), and of some Cl^- channels (Pusch & Jentsch, 1994). DIDS also inhibits the electrogenic Na/HCO_3 cotransporter, but DIDS was applied in the present experiments always in combination with HCO_3^- free solutions containing minimal substrate (Solution 8). Thus, DIDS was used to exclude the contribution of all anion exchangers to a_i^{Cl} homeostasis or to transcellular Cl^- transport. When DIDS (10^{-4} M) and HCO_3^- free solutions were present in both lumen and bath, the proximal tubule continued transepithelial NaCl absorption as evidenced by a very negative V_3 (Table 8-I). The larger V_3 can result from a decrease in paracellular anion-selective conductance, if DIDS also inhibited paracellular Cl^- conductive pathways. Lucci & Warnock (1979) suggested a possible effect of SITS on the paracellular permeability and Schild et al. (1987) attributed some effects of luminal DIDS to an action on the paracellular pathway permeability. Although a_i^{Cl} did not differ from control conditions, the distribution of Cl^- after DIDS inhibition approached equilibrium (Table 8-I). Indeed, the passive driving force for Cl^- exit across the apical membrane ($V_2 - E_{Cl}$) was not different from zero. This suggests complete inhibition of all apical Cl-base exchangers that may hold a_i^{Cl} above equilibrium across that membrane. In addition, the passive driving force for Cl^- exit across the basolateral membrane ($V_1 - E_{Cl}$) became also very small, thus suggesting that any basolateral Cl-OH exchange persisting in nominally HCO_3^- free solutions was further inhibited by DIDS. Finally, it should be noted that, despite a complete inhibition of all Cl^- transporters, it is physically impossible to achieve simultaneously electrochemical equilibrium across the Cl^- channels of both cell membranes, because the two membrane potentials are different whereas the external luminal and basolateral solutions are solute-clamped at identical Cl^- concentrations.

Figure 5 and Table 8-I show the effect of glucose and lactate removal from the lumen by substituting solution 7 for solution 8, in the bilateral presence of DIDS. V_1 shows a minimal transient hyperpolarization followed by a depolarization (Fig. 5). Similarly, V_2 transiently hyperpolarized followed by the depolarization, that is reported in Table 8-I. This contrasts with the immediate and sustained hyperpolarization observed when all substrates (including amino acids) are removed in HCO_3^- containing solutions in the absence of DIDS. Since only glucose and lactate were removed in this experiment, abolishing the inward current of Na-glucose cotransport may cause the modest transient hyperpolarization whereas lactate removal probably causes the subsequent depolarization over 3 to 4 minutes. Indeed, luminal lactate removal by itself in HCO_3^- free solutions induces a marked fall of pH_i over a similar time course as that of the depolarization (*see* Fig. 1 of Siebens & Boron, 1987) and intracellular acidification causes V_1 to depolarize (Steels & Boulpaep, 1987). The basolateral H^+/monocarboxylate cotransporter in *Ambystoma* normally mediates the basolateral exit of lactate with protons (Siebens & Boron, 1987). To the extent that the basolateral H^+/monocarboxylate cotransporter is inhibited by DIDS (Wang et al. 1996, Rosenberg et al., 1993), the resulting decrease in H^+/monocarboxylate efflux from the cell, or even flux reversal, would predict a further fall in pH_i. As a result V_1 would depolarize along the same time course. The important difference between this experiment and that reported in Table 5-II is the absence of HCO_3^-. In the presence of HCO_3^-, stilbenes in the bath may exert an overriding alkalinization of pH_i via the inhibition of the basolateral rheogenic Na/HCO_3 cotrans-

Table 8. Effect of removal of luminal glucose and lactate (minimal substrates) and of Na^+, in HCO_3^--free solutions in the presence of DIDS (10^{-4} M) in the lumen and bath

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)	V_1-E_{Cl} (mV)	V_2-E_{Cl} (mV)
I	Minimal Sub HCO_3^--free + DIDS (L and B)	−60 ± 7.2	52 ± 7.5	−8.7 ± 1.1	12.7 ± 2.7	−12.5 ± 5.1	3.8 ± 4.8
	0 Sub (L), HCO_3^--free + DIDS (L and B)	−53 ± 7.4	46 ± 7.9	−7.0 ± 1.2	14.7 ± 2.7	−9.5 ± 4.5	2.7 ± 4.4
	n	(10)	(10)	(10)	(10)	(10)	(10)
	P	<0.05	NS	<0.02	NS	<0.05	NS
II	0 Sub (L), HCO_3^--free + DIDS (L and B)	−53 ± 5.1	48 ± 5.2	−5.4 ± 0.7	13.3 ± 1.5	−8.1 ± 2.9	2.0 ± 3.1
	0 Na 0 Sub (L), HCO_3^--free + DIDS (L and B)	−65 ± 3.1	81 ± 3.4	16.0 ± 1.0	5.5 ± 1.1	5.5 ± 2.6	10.5 ± 2.3
	n	(14)	(14)	(14)	(14)	(14)	(14)
	P	<0.005	<0.001	<0.001	<0.001	<0.001	<0.005

L = lumen; B = bath; Sub = substrates. The observations are paired and the experiments were performed on 7 different tubules.

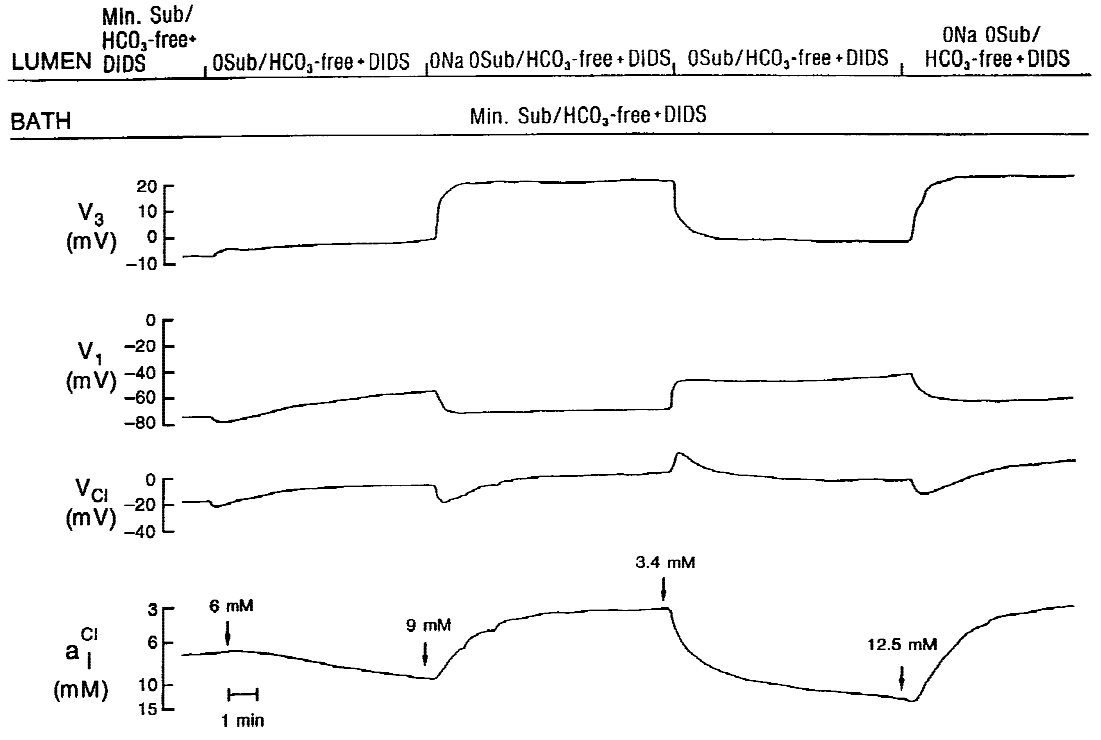

Fig. 5. Effects of removal of minimal substrates (glucose and lactate) and the effect of subsequent removal of luminal Na^+ in the presence of bilateral DIDS (10^{-4} M). V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Table 8-I and 8-II.

porter. In turn, opposite effects on pH_i between the experiments of Table 5-II and Table 8-I may explain the opposite responses of the membrane potential.

Of importance is the finding that upon glucose and lactate removal, a_i^{Cl} slightly increased, although not significantly (Table 8-I). This effect on a_i^{Cl} is opposite to that seen in all previous substrate removal experiments, but agrees with electrodiffusive shifts of Cl^- driven by the membrane potential changes, that are also opposite in sign to those seen in previous substrate removal experiments. It is noteworthy that neither ($V_1 - E_{Cl}$) nor ($V_2 - E_{Cl}$) differ from zero, indicating a passive distribution of a_i^{Cl}. An electrodiffusive Cl^- pathway, that is not inhibited by DIDS, was also found in rat renal basolateral vesicles (Grassl et al., 1987).

Figure 5 and Table 8-II show the effects of Na^+ removal from the lumen, in the presence of DIDS and HCO_3^--free solutions. V_1 hyperpolarized by −11.9 ± 2.9 mV (n = 14) alongside a hyperpolarization of V_2 by +33.2 ± 3.1 mV. V_3 depolarized by +21.4 ± 1.1 mV, a value significantly larger than the depolarization obtained in the absence of DIDS in either bicarbonate-free

Table 9. Effect of addition of DPC (10^{-4} M) to the lumen and to the bath

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)
I	Control (L and B)	−71 ± 2.6	62 ± 2.6	−8.2 ± 0.7	9.9 ± 0.8
	DPC (L), Control (B)	−69 ± 2.8	63 ± 2.7	−5.4 ± 0.5	12.3 ± 1.2
	n	(22)	(22)	(22)	(22)
	P	<0.005	NS	<0.001	<0.001
II	Control (L and B)	−62 ± 4.1	53 ± 4.2	−8.6 ± 0.7	12.7 ± 2.3
	Control (L), DPC (B)	−65 ± 4.5	54 ± 4.7	−9.4 ± 0.6	15.6 ± 2.4
	n	(6)	(6)	(6)	(6)
	P	NS	NS	<0.025	<0.05

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 7 different tubules.

solutions (Table 7-II) ($P < 0.001$, $n = 26$, unpaired *t*-test), and also larger than in bicarbonate containing solutions (Table 2-II). The electrical response of V_3 upon removal of luminal Na^+ is possibly augmented by two mechanisms: an enhanced paracellular diffusional e.m.f. for Na^+, resulting from a higher paracellular Na^+ permeability in bicarbonate-free solutions and an increased paracellular resistance resulting from partial inhibition of the paracellular conductance for Cl^-. The change in a_i^{Cl} upon removal of luminal Na^+ was also enhanced by the presence of DIDS; a_i^{Cl} fell from 13.3 ± 1.5 mM to a very low value of 5.5 ± 1.1 mM ($n = 14$, Table 8-II). Since ($V_1 - E_{Cl}$) is 5.5 ± 2.6 mV and does not differ significantly from 0, the low a_i^{Cl} in the absence of transcellular NaCl transport and in the presence of DIDS does not differ from a passive distribution across the basolateral membrane. Large changes in a_i^{Cl} occurred in parallel with large changes in membrane potential. Thus, DIDS appears to block effectively all Cl-base exchangers responsible for keeping a_i^{Cl} normally above equilibrium, but DIDS fails to block those Cl^- channels that underlie the large electrodiffusive shifts driven by membrane potential changes. This agrees with the finding that DIDS fails to block a Cl-conductive pathway in rat basolateral membrane vesicles (Grassl et al. 1987).

Effect of the Cl^- Channel Blocker DPC

The basolateral membrane conductance to Cl^- in the intact amphibian proximal tubule was reported to be low (Guggino et al., 1982). However, in the present experiments changes in a_i^{Cl} occurred alongside changes in membrane potential difference and the changes in a_i^{Cl} were in the direction as predicted by the altered membrane potential difference. These changes in a_i^{Cl} were maximized by inhibition with DIDS. To further investigate the presence of Cl^- channels in the proximal tubule, we used the Cl^- channel blocker diphenylamine-2-carboxylate (DPC). DPC inhibits Cl^- channels in Cl-transporting epithelia (Di Stefano et al., 1985; Gögelein & Greger, 1986) and the Cl^- channels in rat proximal tubule that are stimulated by cAMP (Wang et al., 1995). In our control conditions, the electrochemical driving forces at the basolateral membrane ($V_1 - E_{Cl}$) and apical membrane ($V_2 - E_{Cl}$), favor Cl^- exit from the cell through Cl^- channels. Blocking these Cl^- channels with DPC would cause a_i^{Cl} to rise. The effect of DPC (10^{-4} M) in the lumen and in the bath is summarized in Table 9. A typical tracing is shown in Fig. 6. When DPC was added to the luminal solution (Table 9-I), V_1 depolarized by +2 ± 0.6 mV; there was no significant effect on V_2; V_3 depolarized by +2.7 ± 0.3 mV, and the a_i^{Cl} increased significantly by 2.4 ± 0.6 mM ($n = 22$). In contrast, when DPC was added to the bath only (Table 9-II), there was no significant effect on V_1 or V_2; V_3 hyperpolarized slightly by −0.8 ± 0.2 mV, but a_i^{Cl} increased by 2.9 ± 1.0 mM ($n = 6$). When DPC was added to lumen and bath consecutively, the effects were additive as shown in Fig. 6. With DPC in both lumen and bath, a_i^{Cl} rose to 17.6 ± 1.6 mM, whereas V_1, V_2, and V_3 were −54 ± 3 mV, +50 ± 3.2 mV and −4.6 ± 0.6 mV ($n = 11$), respectively. These changes in a_i^{Cl} upon addition of DPC are consistent with the presence of Cl^- channels at both the apical and the basolateral membranes of the cell. The effects of DPC were completely reversible. Whereas DPC affected transepithelial potential difference, especially when applied from the lumen, it did not have a major effect on either the apical or the basolateral membrane potential difference. This could be explained if Cl^- channels contribute only a small fraction of the overall conductance of either plasma membrane.

Effect of Organic Substrate Removal and Na^+ Removal in the Presence of a Cl^- Channel Blocker

The hyperpolarizations of the basolateral and apical membrane potential that accompany the removal of organic substrates and Na^+ (Table 2-I and 2-II) are in the

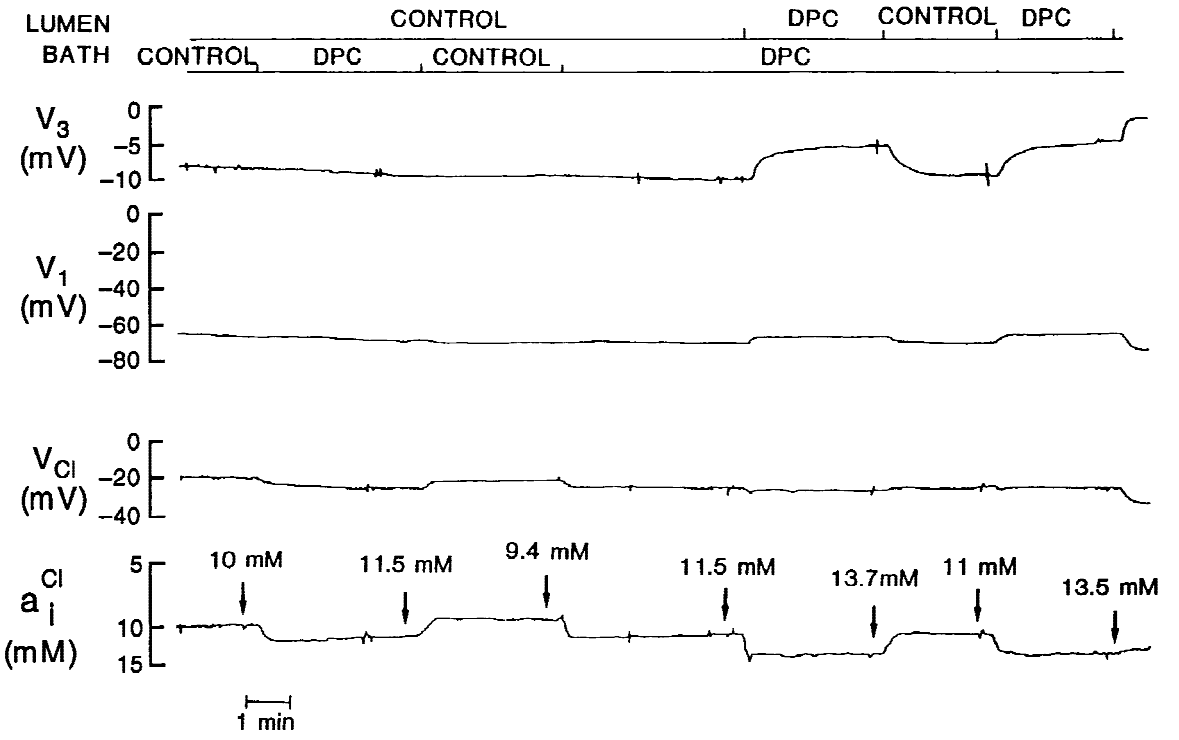

Fig. 6. Effects of the addition of DPC (10^{-4} M) to the bath and to the lumen. V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Table 9-I and 9-II.

right direction to predict a fall in a_i^{Cl} caused by increased efflux through Cl^- channels. Similarly, the depolarizations of the basolateral and apical membrane potential that accompany the removal of Na^+ from the bath (Table 2-III) are in the right direction to predict a rise in a_i^{Cl} caused by decreased efflux through Cl^- channels.

Table 10 shows the effect of the Cl^- channel blocker DPC on the responses to substrate and Na^+ removal from the lumen. First, in the presence of luminal DPC, the response to substrate removal (Table 10-I) was quite similar to that seen earlier without DPC: a hyperpolarization of V_1 and V_2 by −8.1 ± 1.5 mV and +11.0 ± 1.6 mV, a depolarization of V_3 by +2.8 ± 0.3 mV, and a fall of a_i^{Cl} by 1.7 ± 0.4 mM ($n = 15$). Second, in the presence of luminal DPC, the response to luminal Na^+ removal (Table 10-II) was also quite similar to that seen earlier without DPC: a hyperpolarization of V_1 and V_2 by −8.9 ± 1.6 mV and +14.4 ± 2.1 mV respectively, a depolarization of V_3 by +5.9 ± 0.8 mV, and a fall of a_i^{Cl} by 2.1 ± 0.6 mM ($n = 17$). Third, in the bilateral presence of DPC, the response to substrate removal (Table 10-III) was also similar to that seen earlier without DPC: a hyperpolarization of V_1 and V_2 by −7.5 ± 1.2 mV and +9.0 ± 1.2 mV, respectively, a depolarization V_3 by +1.6 ± 0.4 mV, and a fall of a_i^{Cl} by 1.7 ± 0.4 mM ($n = 6$). Fourth, in the bilateral presence of DPC, the response to Na^+ removal (Fig. 7 and Table 10-IV) was not diminished: V_1 and V_2 hyperpolarized by −15.6 ± 2.3 mV and +21.6 ± 2.7 mV respectively, V_3 depolarized by +6.0 ± 0.9 mV, and a_i^{Cl} fell by 5.9 ± 1.3 mM ($n = 12$).

The changes in a_i^{Cl} observed upon the addition of DPC of Table 9 strongly suggest the presence of some DPC-sensitive Cl^- channels on both the apical and the basolateral membrane of the cell. However, the Cl^- channels, that mediate the responses of a_i^{Cl} elicited by removal of substrates or Na^+ (Table 10), do not appear to be completely blocked by DPC.

Discussion

The present experiments confirm that intracellular chloride activity in the *Ambystoma* proximal tubule is higher than predicted by electrochemical equilibrium of chloride across either the apical or the basolateral membrane of the cell (Boron & Boulpaep, 1983*a*). This finding requires the presence of an active entry step for chloride at the apical and/or basolateral membrane.

Whereas it has been generally accepted that Cl^- absorption in the proximal tubule is linked to Na^+ transport, the mechanism of the linkage is still poorly understood. Apical Na/organic-substrate cotransport is the major pathway for Na^+ uptake into the proximal tubule cell and a primary determinant of transepithelial potential differ-

Table 10. Effect of removal of luminal substrates and Na^+, in the presence of 10^{-4} M DPC in the lumen and in both lumen and bath

		V_1 (mV)	V_2 (mV)	V_3 (mV)	a_i^{Cl} (mM)
I	DPC (L), Control (B)	−60 ± 3.3	55 ± 3.4	−5.4 ± 0.6	14.6 ± 1.6
	0 Sub + DPC (L), Control (B)	−68 ± 3.5	66 ± 3.9	−2.6 ± 0.7	12.8 ± 1.6
	n	(15)	(15)	(15)	(15)
	P	<0.001	<0.001	<0.001	<0.005
II	0 Sub + DPC (L), Control (B)	−67 ± 3.1	65 ± 3.4	−2.4 ± 0.6	12.6 ± 1.4
	0 Na 0 Sub + DPC (L), Control (B)	−76 ± 1.7	79 ± 1.7	3.4 ± 0.6	10.6 ± 1.1
	n	(17)	(17)	(17)	(17)
	P	<0.001	<0.001	<0.001	<0.005
III	DPC (L and B)	−52 ± 4.0	48 ± 4.4	−4.8 ± 1.0	17.5 ± 2.7
	0 Sub (L), DPC (L and B)	−60 ± 4.8	57 ± 5.4	−3.2 ± 1.1	15.8 ± 2.5
	n	(6)	(6)	(6)	(6)
	P	<0.001	<0.001	<0.005	<0.005
IV	0 Sub (L), DPC (L and B)	−51 ± 3.9	48 ± 4.1	−2.1 ± 0.7	18.5 ± 1.8
	0 Na 0 Sub (L), DPC (L and B)	−66 ± 2.4	70 ± 2.7	3.9 ± 0.5	12.5 ± 1.1
	n	(12)	(12)	(12)	(12)
	P	<0.001	<0.001	<0.001	<0.001

L = lumen; B = bath; Sub = substrates. The observations (*n*) are paired and the experiments were performed on 11 different tubules.

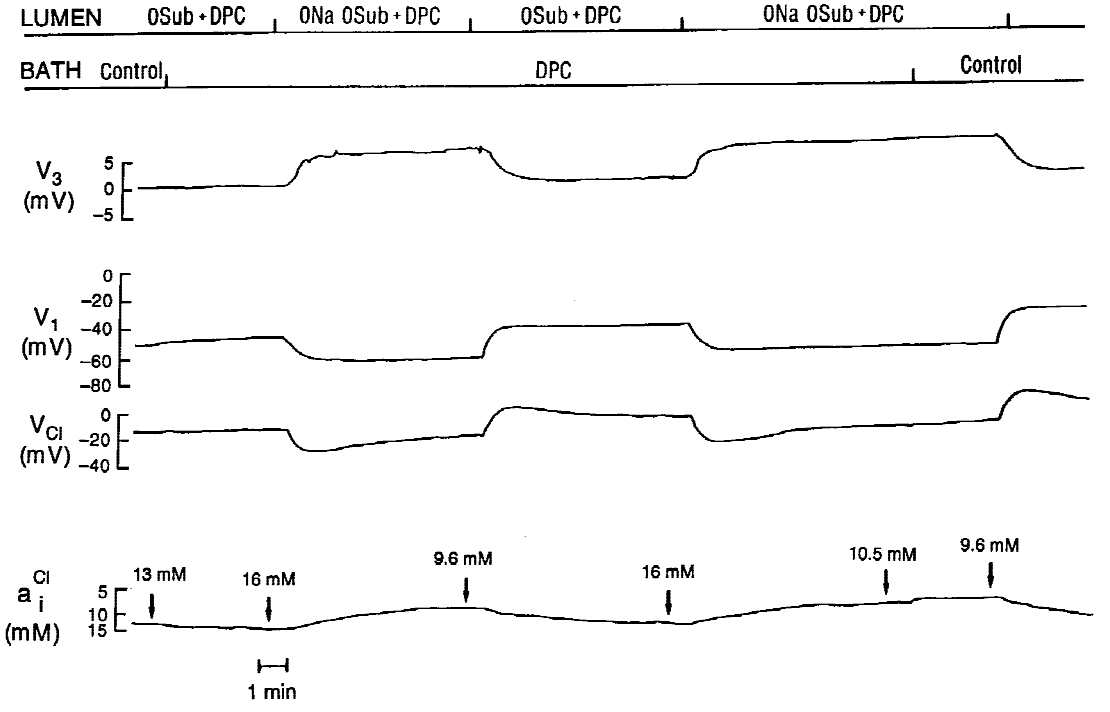

Fig. 7. Effects of removal of luminal Na^+ in the absence of luminal substrates and the bilateral presence of DPC (10^{-4} M). V_3, V_1, V_{Cl}, and a_i^{Cl} have the same meaning as in Fig. 1. Data from this and similar experiments are summarized in Table 10-IV.

ence and of transepithelial fluid absorption (Forster, Steels & Boulpaep, 1980; Morgunov & Boulpaep, 1987). Chloride is the major anion accompanying Na^+ during isotonic fluid reabsorption in the amphibian proximal tubule, because of the lower bicarbonate concentration in the glomerular filtrate as compared to the mammalian tubule, and also because the amphibian proximal tubule secretes less acid (O'Regan, Malnic & Giebisch 1982). Cl^- reabsorption can follow either the paracellular or transcellular pathway. Because the paracellular compo-

nent of Cl^- absorption is directly proportional to the paracellular Cl^- conductance and the transepithelial electrochemical driving force for Cl^-, coupling of paracellular Cl^- absorption to Na^+ absorption could be mediated by altered paracellular driving forces. In contrast, the transcellular component of Cl^- absorption involves transport steps at both cell membranes that may affect intracellular Cl^- activity. Therefore, we examined Na-linked Cl-transport by monitoring a_i^{Cl} when Na^+ uptake was modified by means of two standard maneuvers: the removal of substrates from the lumen, and the removal of Na^+ from the lumen.

Our data indicate that in control HCO_3^- solutions, the removal of substrates from the lumen reduces the a_i^{Cl}. Subsequent removal of Na^+ from the lumen reduces the a_i^{Cl} further. Both changes occur alongside a hyperpolarization of V_1, V_2 and a depolarization of V_3. The present results indicate that several transport mechanisms, both apical and basolateral, contribute to the alterations of a_i^{Cl} linked to transcellular NaCl absorption.

Apical Na/Cl and/or Na/K/2Cl Cotransport

An apical transporter for Na^+ and Cl^- has been described in the amphibian proximal tubule and would account for the uphill accumulation of Cl^- (Kimura & Spring, 1978). The present experiments argue against the presence of a Na/Cl or Na/K/2Cl cotransporter at the apical membrane. First, in the absence of luminal Na^+, the a_i^{Cl} is still at a higher value than predicted by equilibrium ($V_1 - E_{Cl} = -19.4$ mV and $V_2 - E_{Cl} = 20.4$ mV, Table 2-II), which strongly suggests that Na/Cl or Na/K/2Cl is not the mechanism for uphill Cl^- entry into the cell. Second, the steady state a_i^{Cl} was not affected by the addition to the lumen of bumetanide, hydrochlorothiazide, or furosemide, all of which are expected to inhibit Na/Cl or Na/K/2Cl cotransport. In the presence of bumetanide, furosemide, or hydrochlorothiazide the fall in a_i^{Cl} upon removal of luminal Na^+ is still highly significant, which implies that Na/Cl or Na/K/2Cl symport is not responsible for the observed fall in a_i^{Cl}. Finally, previous data using ion-selective Na^+ microelectrodes have shown no dependence of a_i^{Na} on luminal Cl^- (Abdoulnour-Nakhoul & Boulpaep, 1986).

Apical Cl-Base Exchange

The transport of Cl^- across the apical membrane has been suggested to occur by Na-independent Cl-base exchange such as Cl-OH, Cl-HCO_3, Cl-formate or Cl-oxalate exchange (Warnock & Yee, 1981; Seifter et al., 1984; Seifter & Aronson, 1984; Karniski & Aronson, 1985; Karniski & Aronson, 1987). Cl-base exchange working in parallel with Na-H exchange at the apical membrane would result in net NaCl uptake leading to active electroneutral NaCl reabsorption in the rabbit proximal tubule (Schild et al., 1987; Aronson, 1989).

Our experiments provide six lines of evidence that argue against apical Cl-base exchange as a mechanism for Na-linked Cl^- transport across the apical membrane. First, the fall in a_i^{Cl} seen in control conditions after substrate or Na^+ removal from the lumen occurs in the nominal absence of formate or oxalate. Second, exchange of Cl^- for the organic anions lactate and glutamate cannot be responsible for the observed changes in a_i^{Cl} following removal of the substrates lactate and glutamate from the lumen. Indeed, lactate removal from the lumen decreases a_i^{Cl} which is opposite to the expected change if lactate were leaving the cell through Cl-lactate exchange. Moreover, the Cl-formate exchanger has little or no affinity for lactate (Karniski & Aronson, 1985; Karniski & Aronson, 1987). Third, bicarbonate removal from the lumen decreases the a_i^{Cl}, a change opposite to that expected if Cl-HCO_3 exchange were present. Fourth, the application of SITS in the lumen does not decrease steady-state a_i^{Cl}. Fifth, the decrease in a_i^{Cl} upon removal of luminal Na^+ is not significantly altered by the presence of SITS in the lumen. Sixth, the decrease of a_i^{Cl} is still very large when luminal Na^+ is removed in the presence of DIDS in lumen and bath in HCO_3^--free solutions.

Basolateral Na-Independent Cl-HCO_3 Exchange

Basolateral Na-independent Cl-HCO_3 or Cl-OH exchange was found in *Necturus*, rat and rabbit proximal tubules (Edelman et al., 1981; Alpern & Chambers, 1987; Grassl et al., 1987; Nakhoul et al., 1990). The presence of Cl-HCO_3 exchange on the basolateral membrane is supported by the observed changes in a_i^{Cl} in three experimental conditions of our study. First, in control conditions the change in free energy ΔG across the basolateral membrane for Cl-HCO_3 exchange is negative (Boron & Boulpaep, 1983*b*) predicting net Cl^- influx into the cell and HCO_3^- efflux. When HCO_3^- is removed from the bath, HCO_3^- exit from cell to bath in exchange for Cl^- entry into the cell is accelerated, and the a_i^{Cl} rises as shown in Table 6-II. Second, the observation that the addition of SITS to the bath decreases the a_i^{Cl} (Table 5-I) is also in agreement with the presence of Cl-HCO_3 exchange. However, it should be noted that SITS inhibition was unable to bring the a_i^{Cl} in electrochemical equilibrium across either cell membrane, suggesting that the exchanger may have a low affinity for SITS. Third, addition of DIDS to lumen and bath in the absence of HCO_3^- brings the a_i^{Cl} to a value not different from that predicted by equilibrium across the apical membrane. This finding suggests that DIDS blocks an active entry step for Cl^-, perhaps Cl-OH exchange.

Basolateral Na-Driven Cl-HCO_3 Exchange (Na/HCO_3-Cl/H)

Na-driven Cl-HCO_3 electroneutral exchange (Na/HCO_3-Cl/H) has been reported in the basolateral membrane of *Necturus* proximal tubule (Guggino et al., 1983) and mammalian proximal tubule (Alpern & Chambers, 1987; Sasaki & Yoshiyama, 1988). First, our experiments in *Ambystoma* (Table 2-III) confirm the observation in *Necturus* (Guggino et al., 1983), that removal of basolateral Na^+ causes the a_i^{Cl} to increase. These findings are consistent with the presence of Na-driven Cl-HCO_3 electroneutral exchange in the basolateral membrane of *Ambystoma* proximal tubule. However, because large depolarizations accompany the removal of basolateral Na^+, presumably resulting from the operation of the electrogenic Na/HCO_3 cotransporter, conductive Cl movement could explain the increase in a_i^{Cl} as well. Second, the effect of basolateral Na^+ removal on a_i^{Cl} can be completely blocked by SITS in the bath (Table 5-IV). This finding could argue in favor of basolateral Na-driven Cl-HCO_3 electroneutral exchange. However, SITS very much reduced the depolarization caused by removal of basolateral Na^+, as expected from the known SITS-sensitivity of the basolateral electrogenic Na/HCO_3 cotransporter. This leaves conductive Cl movement as an alternative explanation for the increase in a_i^{Cl}.

Apical and Basolateral Cl^- Channels

A chloride conductance was described in rabbit renal microvillus membrane vesicles (Warnock & Yee, 1981; Seifter et al., 1984), and in rat basolateral membrane vesicles (Grassl et al., 1987). Patch-clamp studies have detected single chloride channels in the basolateral membrane of isolated cells of *Ambystoma* proximal tubule (Segal & Boulpaep, 1990) and of rabbit proximal straight tubule (Gögelein & Greger, 1986; Segal et al., 1993). Chloride channels in the basolateral membrane of *Ambystoma* proximal tubule can be blocked in part by DPC (Segal & Boulpaep, 1992).

Several lines of evidence in the present work favor the existence of Cl^- channels. First, because prevailing electrochemical driving forces predict passive Cl^- exit across both apical and basolateral membrane, blocking Cl^- channels should result in an increase in a_i^{Cl}. Our finding that a_i^{Cl} increases after addition of DPC to the lumen and/or to bath (Table 9-I and 9-II), is consistent with the presence of Cl^- channels at the apical and basolateral membrane of the cell.

Second, when the contribution of all anion exchangers to a_i^{Cl} homeostasis or to transcellular Cl^- transport was prevented by bathing the tubule on both sides in DIDS and nominally HCO_3^--free solutions, a_i^{Cl} achieved electrochemical equilibrium across the apical membrane and departed only slightly from equilibrium across the basolateral membrane.

Third, when in the presence of DIDS and HCO_3^--free solutions large a_i^{Cl} changes were induced by the blockade of transepithelial Na^+ absorption, the Cl^- distribution remained in electrochemical equilibrium across the basolateral membrane. Because DIDS fails to block the Cl-conductive pathway in rat basolateral membrane vesicles (Grassl et al., 1987), the present data are consistent with the presence of Cl^- channels at both the apical and basolateral membrane.

Fourth, all experimental maneuvers, that caused a hyperpolarization of V_1 or V_2, resulted in a decrease of a_i^{Cl}. Similarly, experiments that caused depolarizations of V_1 or V_2 resulted in an increase of a_i^{Cl}. In the present experiments the largest membrane potential changes resulted either from alterations of the current carried by Na/substrate symport across the apical membrane or from alterations of the current carried by the electrogenic Na/HCO_3 cotransporter across the basolateral membrane by Na/substrate symport. The resulting changes in V_1 or V_2 observed during the 23 experimental maneuvers of Tables 2–8,10 and the concomitant changes in a_i^{Cl} are shown in Fig. 8*A* and *B*. The a_i^{Cl} before an experimental maneuver ($a_i^{Cl\ pre}$) and after the maneuver ($a_i^{Cl\ post}$) are calculated as $\log(a_i^{Cl\ post}/a_i^{Cl\ pre})$ and plotted in Fig. 8*A* against the change in basolateral membrane potential ΔV_1 expressed as ($V_1^{post} - V_1^{pre}$). In Fig. 8*B* the values of $\log (a_i^{Cl\ post}/a_i^{Cl\ pre})$ are plotted against the change in apical membrane potential ΔV_2 expressed as ($V_2^{post} - V_2^{pre}$). The logarithmic changes in a_i^{Cl} are correlated to the concomitant changes in basolateral membrane potential ΔV_1, yielding a slope of 9.13 V^{-1}, or a 3.4-fold change in a_i^{Cl} induced by a ΔV_1 of 58 mV. The logarithmic changes in a_i^{Cl} are correlated with the concomitant changes in apical membrane potential ΔV_2, yielding a slope of $-7.82\ V^{-1}$, or a 2.8-fold change in a_i^{Cl} induced by a ΔV_2 of 58 mV. The slope of both lines is less than the Nernstian slope of 1/58 mV or 17.2 V^{-1}. However, with two membranes in series and identical external Cl^- concentrations bathing each membrane, it is physically impossible to satisfy electrochemical equilibration across each membrane, unless the ΔV_2 and ΔV_1 are of equal magnitude. Indeed, if both membranes contain Cl^- channels, the relative Cl^- conductance and potential change at a particular membrane will determine how closely a_i^{Cl} approximates equilibrium across that membrane. As shown by the intercepts of the regression lines the reversal potentials for ΔV_1 and ΔV_2 do not differ from zero. The data of Fig. 8 suggest that changes in a_i^{Cl} are mediated, at least in part, by movement of Cl^- through Cl^- channels at either the apical or basolateral membrane.

Na-Linked Transcellular Cl Transport

Two maneuvers were used to test for Na-linked transcellular Cl^- transport: the removal from the lumen of or-

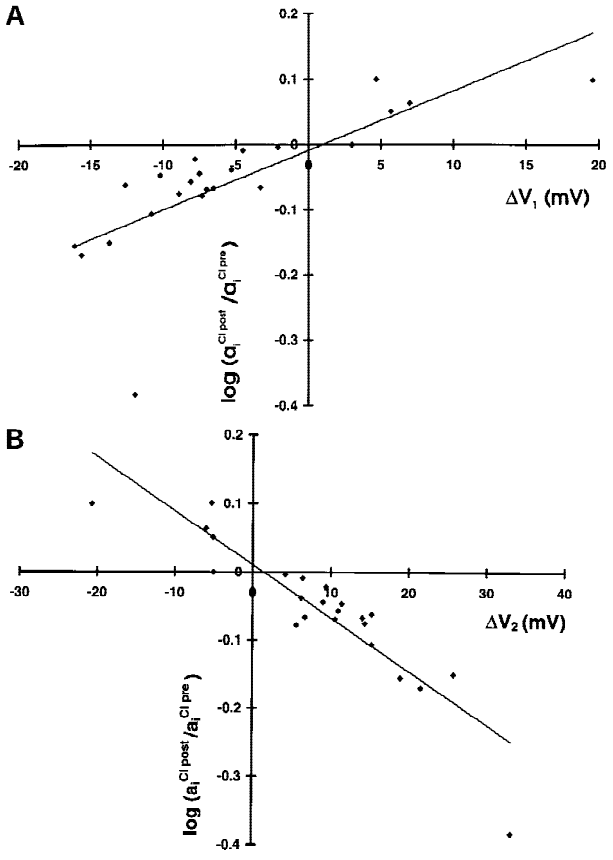

Fig. 8. (*A*) Changes in a_i^{Cl} plotted as log (a_i^{Cl} in 0 Na/a_i^{Cl} in 100 Na) against the concomitant changes in basolateral membrane potential ΔV_1. Data of Tables 2–8,10. The regression equation is log (a_i^{Cl} in 0 Na/a_i^{Cl} in 100 Na) = −0.009 + 0.00913 ΔV_1 ($P < 0.0001$). The intercept does not differ significantly from 0. $r = 0.76$. (*B*) Changes in a_i^{Cl} plotted as log (a_i^{Cl} in 0 Na/a_i^{Cl} in 100 Na) against the concomitant changes in apical membrane potential ΔV_2 (calculated as V_2 in 0 Na − V_2 in 100 Na). Data of Tables 2–8,10. The regression equation is log (a_i^{Cl} in 0 Na/a_i^{Cl} in 100 Na) = 0.011 − 0.00782 ΔV_2 ($P < 0.0001$). The intercept does not differ significantly from 0. $r = 0.90$.

ganic substrates taken up by the cell together with Na^+, and the removal of Na^+ itself from the lumen. Multiple pathways can be responsible for the apparent linkage between Na^+ and Cl^- transcellular absorption.

At least two immediate effects of substrate removal in HCO_3 containing solutions are the source of altered Cl^- transport: (i) a decrease in a_i^{Na} and (ii) hyperpolarization of V_2 and V_1. Activation of basolateral Na/HCO_3-Cl/H exchange by a lowered a_i^{Na}, and increased potential differences across Cl^- channels would each favor Cl^- extrusion and thus explain the observed fall in a_i^{Cl}. Substrate removal in HCO_3-free solutions provides an additional source of altered Cl^- transport: a decrease of pH_i caused by the one-sided removal of lactate. In turn a low pH_i would activate basolateral Na-independent Cl-OH exchange.

In contrast, Na^+ removal from the lumen has at least three immediate effects that cause altered Cl^- transport: (i) a decrease in a_i^{Na}, (ii) a fall in pH_i, and (iii) hyperpolarization of V_2 and V_1. Operation of SITS-inhibitable basolateral Na/HCO_3-Cl/H exchange and Cl-HCO_3 (or -OH) exchange together with increased driving forces across Cl^- channels would all favor Cl^- extrusion and thus explain the observed fall in a_i^{Cl}. The relative contribution of each of these pathways cannot be fully established from the present experiments. Indeed, bilateral DPC does not blunt the fall in a_i^{Cl} upon removal of luminal Na^+. However, the starting a_i^{Cl} was higher in the presence of DPC (17.5 mM) than in control (9.9 mM). Any transport system that extrudes Cl^- could be activated by an elevated a_i^{Cl}. For example, the high a_i^{Cl}, combined with a low a_i^{Na} and pH_i in response to the removal of luminal substrates and Na^+, would activate the basolateral Na/HCO_3-Cl/H exchange, driving Cl^- out of the cell and Na^+ and HCO_3^- in. Moreover, Cl^- channels insensitive to DPC (Segal & Boulpaep, 1992) may still contribute to the a_i^{Cl} response. Conversely, in the presence of bilateral DIDS, the removal of Na^+ from lumen still decreases a_i^{Cl}, indicating that basolateral Na/HCO_3-Cl/H or Cl-HCO_3 (OH) exchange is not the sole mechanism by which luminal Na^+ removal decreases a_i^{Cl}.

Finally, it should be pointed out that whereas the logarithmic changes in a_i^{Cl} correlated well with induced changes in basolateral membrane potential, the absolute values of a_i^{Cl} generally did not come into electrochemical equilibrium. The changes in a_i^{Cl} may therefore in part be caused by some crosstalk between the apical and the basolateral membrane, e.g., low or high states of apical uptake of Na and Cl may cause an unknown transduction mechanism to modulate basolateral chloride conductance.

Cell Model of Transcellular Cl Pathways

Figure 9 incorporates the cellular transport pathways for Cl^- in proximal tubules perfused with control solutions that contain bicarbonate and lactate but no other carboxylic acids. Cl^- channels are shown at the apical membrane. Na-independent Cl-HCO_3 (or OH) exchange, Na-driven Cl-HCO_3 exchange, and Cl^- channels are shown at the basolateral membrane. Passive driving forces for Cl^- favor the exit of Cl^- from the cell across the basolateral membrane when ($V_1 - E_{Cl}$) is negative and across the apical membrane when ($V_2 - E_{Cl}$) is positive. The present results show the existence of electrochemical gradients favoring Cl^- exit in all experimental conditions, except during some maneuvers in the presence of DIDS where electrochemical equilibrium was attained. In contrast, because a negative ($V_2 - E_{Cl}$) was never detected, apical Cl^- uptake through Cl^- channels is not expected. Moreover, when the lumen does not contain bases such as formate or oxalate, Cl^- is not expected to enter the cell through apical Cl-base exchange, except for

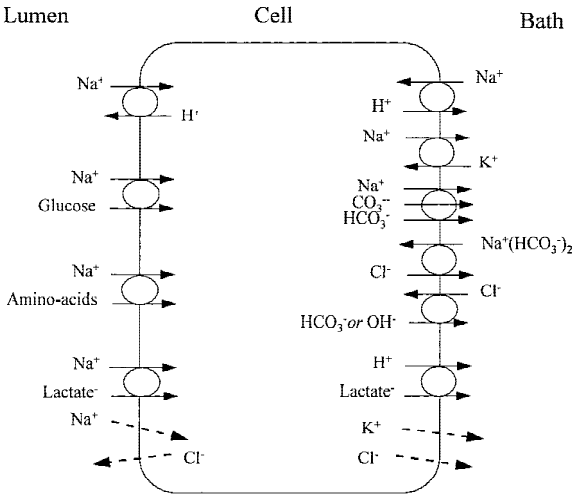

Fig. 9. Cell model of Cl^- transport pathways in solutions containing no anionic bases other than bicarbonate and lactate. Exchangers and cotransporters are represented by arrows linked by a circle. Ion channels are represented by dashed arrows. The Cl^- pathways are apical Cl^- channels, basolateral Cl^- channels, basolateral Na-independent Cl-HCO_3 or Cl-HO exchange, and basolateral Na-driven Cl-HCO_3 exchange. Additional pathways involving acid or base transport are apical Na-H exchange, basolateral Na-H exchange, basolateral $Na/HCO_3/CO_3$ cotransport, apical Na/Lactate cotransport, and basolateral H^+/monocarboxylate cotransport. Pathways involving Na^+ transport include apical Na-H exchange, apical Na/Glucose cotransport, apical Na/amino acid cotransport, apical Na/Lactate cotransport and apical Na^+ channels, basolateral Na-H exchange, basolateral Na-K-ATPase, basolateral $Na/HCO_3/CO_3$ cotransport, and basolateral Na-driven Cl-HCO_3 exchange.

unknown moieties of intracellular base (e.g., monocarboxylate) generated by cell metabolism. Thus, in our experimental conditions transepithelial Cl^- movement should have proceeded via a paracellular route (Boulpaep, 1971; Sackin & Boulpaep, 1981*b*).

What are the active transport mechanisms that keep intracellular chloride activity in the *Ambystoma* proximal tubule above that predicted by electrochemical equilibrium across either the apical or the basolateral membrane of the cell? The model of Fig. 9 suggests that in the absence of anionic bases in the tubule lumen, the principal cause of an elevated a_i^{Cl} is Cl-HCO_3 or Cl-OH exchange at the basolateral membrane. The electrochemical gradients for Cl^-, Na^+ and HCO_3^- in HCO_3-containing solutions predict Cl^- to leave the cell across the apical membrane via Cl^- channels and across the basolateral membrane via both Cl^- channels and Na-driven Cl-HCO_3 exchange. In contrast, in nominally HCO_3-free solutions, apical and basolateral Cl^- exit proceeds via Cl^- channels only.

This work was supported by grants DK-13844 and DK-17433 from the National Institutes of Health.

References

Abdulnour-Nakhoul, S., Boulpaep, E.L. 1986. Apical membrane transport mechanisms for Na entry in *Ambystoma* kidney proximal tubule. *Kidney Int.* **29:**403

Alpern, R.J., Chambers, M. 1987. Basolateral membrane Cl/HCO_3 exchange in the rat proximal convoluted tubule: Na-dependent and independent modes. *J. Gen. Physiol.* **89:**581–598

Andreoli, T.E., Schafer, J.A., Troutman, S.L., Watkins, M.L. 1979. Solvent drag component of Cl^- flux in superficial proximal straight tubules: evidence for a paracellular component of isotonic fluid absorption. *Am. J. Physiol.* **237:**F455–F462

Aronson, P.S. 1989. The renal proximal tubule: a model for diversity of anion exchanger and stilbene-sensitive anion transporters. *Annu. Rev. Physiol.* **51:**419–441

Aronson, P.S., Giebisch, G. 1997. Mechanisms of chloride transport in the proximal tubule. *Am. J. Physiol.* **273:**F197–F192

Boron, W.F., Boulpaep, E.L. 1983*a*. Intracellular pH regulation in the renal proximal tubule of the salamander: Na-H exchange. *J. Gen. Physiol.* **81:**29–52

Boron, W.F., Boulpaep, E.L. 1983*b*. Intracellular pH regulation in the renal proximal tubule of the salamander: Basolateral HCO_3 transport. *J. Gen. Physiol.* **81:**53–94

Boulpaep, E.L. 1971. Electrophysiological properties of the proximal tubule. Importance of cellular and intercellular pathways. *In:* Electrophysiology of Epithelial Cells, edited by G. Giebisch, p. 91–118. Stuttgart: Schattauer

Burg, M., Grantham, J., Abramow, M., Orloff, J. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* **210:**1293–1298

Cabantchik, Z.I., Rothstein, A. 1972. The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulfonic stilbene derivatives. *J. Membrane Biol.* **10:**311–330

Cassola, A.C., Mollenhauer, M., Frömter, E. 1983. The intracellular chloride activity of rat kidney proximal tubular cells. *Pfluegers Arch.* **399:**259–265

Chao, A.C., Armstrong, W., McD. 1987. Cl^--selective microelectrodes: sensitivity to anionic Cl transport inhibitors. *Am. J. Physiol.* **253:**C343–C347

Costanzo, L.S., Windhager, E.E. 1978. Calcium and sodium transport by the distal convoluted tubule of the rat. *Am. J. Physiol.* **235:**F492–F506

Di Stefano, A., Wittner, M., Schlatter, E., Lang, H.J., Englert, H., Greger, R. 1985. Diphenylamine-2-carboxylate, a blocker of the Cl^--conductive pathway in Cl-transporting epithelia. *Pfluegers Arch.* **405:**S95–S100

Edelman, A., Bouthier, M., Anagnostopoulos, T. 1981. Chloride distribution in the proximal convoluted tubule of *Necturus* kidney. *J. Membrane Biol.* **62:**7–17

Eveloff, J., Warnock, D.G. 1987. K-Cl transport systems in rabbit renal basolateral membrane vesicles. *Am. J. Physiol.* **252:**F883–F889

Fernandez, P.C., Puschett, J.B. 1973. Proximal tubular actions of metolazone and chlorothiazide. *Am. J. Physiol.* **225:**954–961

Forster, J., Steels, P.S., Boulpaep, E.L. 1980. Organic substrate effects on and heterogeneity of *Necturus* proximal tubule function. *Kidney Int.* **17:**479–490

Frömter, E.E., Gessner, K. 1974. Active transport potentials, membrane diffusion potentials and streaming potentials across the rat kidney proximal tubule. *Pfluegers Arch.* **351:**85–98

Gögelein, H., Greger, R. 1986. A voltage-dependent ionic channel in the basolateral membrane of late proximal tubules of the rabbit kidney. *Pfluegers Arch.* **407:**S142–S148

Grassl, S.M., Holohan, P.D., Ross, C.R. 1987. Cl^--HCO_3^- exchange in

rat renal basolateral membrane vesicles. *Biochim. Biophys. Acta* **905:**475–484

Greger, R., Schlatter, E. 1981. Presence of luminal K^+, a prerequisite for active NaCl transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pfluegers Arch.* **392:**92–94

Grinstein, S., McCulloch, L., Rothstein, A. 1979. Transmembrane effects of irreversible inhibitors of anion transport in red blood cells. *J. Gen. Physiol.* **73:**495–514

Guggino, W.B., Boulpaep, E.L., Giebisch, G. 1982. Electrical properties of chloride transport across the *Necturus* proximal tubule. *J. Membrane Biol.* **65:**185–196

Guggino, W.B., London, R., Boulpaep, E.L., Giebisch, G. 1983. Chloride transport across the basolateral cell membrane of the *Necturus* proximal tubule: dependence on bicarbonate and sodium. *J. Membrane Biol.* **71:**227–240

Ishibashi, K., Rector, F.C., Berry, C.A. 1990. Chloride transport across the basolateral membrane of rabbit proximal convoluted tubules. *Am. J. Physiol.* **258:**F1569–F1578

Ishibashi, K., Sasaki, S., Yoshiyama, N. 1988. Intracellular chloride activity of rabbit proximal straight tubule perfused in vitro. *Am. J. Physiol.* **255:**F49–F56

Karniski, L.P., Aronson, P.S. 1985. Chloride/formate exchange with formic acid recycling: A mechanism for active chloride transport across epithelial membranes. *Proc. Natl. Acad. Sci. USA* **82:**6362–6365

Karniski, L.P., Aronson, P.S. 1987. Anion exchange pathways for Cl^- transport in rabbit renal microvillus membranes. *Am. J. Physiol.* **253:**F513–F521

Khuri, R.N., Agulian, S.K., Bogharian, K., Aklanjian, D. 1975. Electrochemical potentials of chloride in proximal renal tubule of *Necturus maculosus. Comp. Biochem. Physiol.* **50A:**695–700

Kimura, G., Spring, K.R. 1978. Transcellular and paracellular tracer chloride fluxes in *Necturus* proximal tubule. *Am. J. Physiol.* **235:**F617–F625

Larson, M., Spring, K.R. 1983. Bumetanide inhibition of NaCl transport by *Necturus* gallbladder. *J. Membrane Biol.* **74:**123–129

Lawrence, P.K., Aronson, P.S. 1987. Anion exchange pathways for Cl^- transport in rabbit renal microvillus membranes. *Am. J. Physiol.* **253:**F513–F521

Lucci, M.S., Warnock, D.G. 1979. Effects of anion-transport inhibitors on NaCl reabsorption in the rat superficial proximal convoluted tubule. *J. Clin. Invest.* **64:**570–579

McConnell, K.R., Aronson, P.S. 1994. Effects of inhibitors on anion exchangers in rabbit renal brush border membrane vesicles. *J. Biol. Chem.* **269:**21489–21494

Morgunov, N., Boulpaep, E.L. 1987. Electrochemical analysis of renal Na^+-glucose cotransport in salamander proximal tubule. *Am. J. Physiol.* **252:**F154–F169

Nakhoul, N.L., Chen, L.K., Boron, W.F. 1990. Intracellular pH regulation in the rabbit S_3 proximal tubule. Basolateral Cl-HCO_3 exchange and Na-HCO_3 cotransport. *Am. J. Physiol.* 258:F371–F381

Oberleithner, H., Giebisch, G., Lang, F., Wang, W. 1982. Cellular mechanism of the furosemide sensitive transport system in the kidney. *Klin. Wochenschr.* **60:**1173–1179

O'Regan, M.G., Malnic, G., Giebisch, G. 1982. Cell pH and luminal acidification in *Necturus* proximal tubule. *J. Membrane Biol.* **69:**99–106

Pusch, M., Jentsch, T.J. 1994. Molecular physiology of voltage-gated chloride channels. *Physiol. Rev.* **74:**813–827

Rosenberg, S.O., Fadil, T., Schuster, V.L. 1993. A basolateral lactate/H^+ cotransporter in Madin-Darby Canine Kidney (MDCK) cells. *Biochemical J.* **289:**263–268

Sackin, H., Boulpaep, E.L. 1981*a*. Isolated perfused salamander proximal tubule: methods, electrophysiology, and transport. *Am. J. Physiol.* **241:**F39–F52

Sackin, H., Boulpaep, E.L. 1981*b*. Isolated perfused salamander proximal tubule. II. Monovalent ion replacement and rheogenic transport. *Am. J. Physiol.* **241:**F540–F555

Sasaki, S., Yoshiyama, N. 1988. Interaction of chloride and bicarbonate transporters across the basolateral membrane of rabbit proximal straight tubule: evidence for Na coupled chloride/bicarbonate exchange. *J. Clin. Invest.* **81:**1004–1111

Schild, L., Aronson, P.S., Giebisch, G. 1991. Basolateral transport pathways for K^+ and Cl^- in rabbit proximal tubule: effects on cell volume. *Am. J. Physiol.* **260:**F101–F109

Schild, L., Giebisch, G., Karniski, L.P., Aronson, P.S. 1987. Effect of formate on volume reabsorption in the rabbit proximal tubule. *J. Clin. Invest.* **79:**32–38

Schlatter, E., Greger, R., Weidtke, C. 1983. Effect of ''high ceiling'' diuretics on active salt transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pfluegers Arch.* **396:**210–217

Segal, A.S., Boulpaep, E.L. 1990. Basolateral chloride channels in single isolated proximal tubule cells of *Ambystoma. J. Am. Soc. Nephrol.* **1:**691

Segal, A.S., Boulpaep, E.L. 1992. cAMP-activated chloride channel on the basolateral membrane of renal proximal tubule. *J. Am. Soc. Nephrol.* **3:**819

Segal, A.S., Geibel, J., Boulpaep, E.L. 1993. A chloride channel resembling CFTR on the basolateral membrane of rabbit proximal tubule. *J. Am. Soc. Nephrol.* **4:**879

Seifter, J.L., Aronson, P.S. 1984. Cl^- transport via anion exchange in *Necturus* renal microvillus membranes. *Am. J. Physiol.* **241:**F888–F895

Seifter, J.L., Knickelbein, R., Aronson, P.S. 1984. Absence of Cl-OH exchange and NaCl cotransport in rabbit renal microvillus membrane vesicles. *Am. J. Physiol.* **247:**F753–F759

Siebens, A.W., Boron, W.F. 1987. Effect of electroneutral luminal and basolateral lactate transport on intracellular pH in salamander proximal tubules. *J. Gen. Physiol.* **90:**799–831

Spring, K.R., Kimura, G. 1978. Chloride reabsorption by renal proximal tubules of *Necturus. J. Membrane Biol.* **38:**233–254

Steels, P.S., Boulpaep, E.L. 1987. pH dependent electrical properties and buffer permeability of the *Necturus* proximal tubule cell. *J. Membrane Biol.* **100:**165–182

Stokes, J.B. 1984. Sodium chloride absorption by the urinary bladder of the winter flounder. *J. Clin. Invest.* **74:**7–16

Wang, T., Giebisch, G., Aronson, P.S. 1992. Effects of formate and oxalate on volume absorption in rat proximal tubule. *Am. J. Physiol.* **263:**F37–F42

Wang, T., Segal, A.S., Giebisch, G., Aronson, P.S. 1995. Stimulation of chloride transport by cAMP in rat proximal tubules. *Am. J. Physiol.* **268:**F204–F210

Wang, X., Levi, A.J., Halestrap, A.P. 1996. Substrate and inhibitor specificities of the monocarboxylate transporters of single rat heart cells. *Am. J. Physiol.* **270:**H476–484

Warnock, D.G., Yee, V.J. 1981. Chloride uptake by brush border membrane vesicles isolated from rabbit renal cortex. *J. Clin. Invest.* **67:**103–115