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Chloride Transport across the Basolateral Cell Membrane of the *Necturus* Proximal Tubule: Dependence on Bicarbonate and Sodium

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Summary. The transport of chloride across the *Necturus* proximal tubule cell was studied in the doubly-perfused kidney using conventional, chloride-sensitive and pH-sensitive microelectrodes. Lowering chloride activity in the basolateral solution results in a reduction in intracellular Cl^- activity (a^i_{Cl}). This reduction in a^i_{Cl} is inhibited by removing either HCO_3^- or Na^+ from the perfusion solution, indicating that both HCO_3^- and Na^+ are required for Cl^- movement across the basolateral cell membrane. Reducing either HCO_3^- or Na^+ in the basolateral solution causes an increase in a^i_{Cl}. Thus changes in either Na^+ or HCO_3^- chemical gradients across the basolateral cell membrane significantly affect chloride movement. Changing intracellular pH by means of NH_4Cl exposure results in an increase in a^i_{Cl} followed by a sharp decrease when NH_4Cl is removed. These changes in intracellular chloride do not occur in the absence of HCO_3^-. Likewise, the decrease in a^i_{Cl} following NH_4Cl treatment requires the presence of Na^+ in the basolateral solution. We conclude that chloride is transported across the basolateral cell membrane in exchange for both Na^+ and HCO_3^-. Our results also support the presence of a Na^+/Cl^- cotransport mechanism on the apical cell membrane.

Key words basolateral chloride transport · *Necturus* proximal tubule

Introduction

A major fraction of chloride filtered by the kidney is reabsorbed by the proximal tubule by two pathways, the paracellular and transcellular pathways. The presence of a large paracellular chloride conductance across the proximal tubular epithelium in *Necturus* supports the view that the paracellular shunt pathway participates in net chloride reabsorption [15]. In addition, there is evidence that part of the proximal tubule chloride reabsorption also occurs via a transcellular route [18].

Concerning the transcellular route of proximal tubular chloride reabsorption, Spring and Kimura [27] have provided experimental evidence that chloride enters the cell of the *Necturus* proximal tubule across the *apical* cell membrane through an electrically silent Na^+/Cl^- cotransport system that raises the intracellular chloride activity above electrochemical equilibrium. Based mainly on experiments with transport inhibitors, Lucci and Warnock [20] have proposed that both $Na^+ - H^+$ and $Cl^- - HCO_3^-$ exchange pathways exist in the apical cell membrane of rat proximal tubules and that coupling of these two distinct transport mechanisms effectively appears as electroneutral cotransport of sodium and chloride.

Electrophysiological studies suggest that chloride transport across the *basolateral* cell membrane of proximal tubule cells may not be electrodiffusive, since intracellular chloride activity has been shown not to be sensitive to changes in basolateral cell membrane potential [26] and the chloride conductance of this cell membrane is quite low [15]. However, the precise nature of the nonconductive mechanism of chloride transport across the basolateral cell membrane, particularly its possible coupling to other ions, is poorly understood. Monitoring intracellular Cl^- activity during a number of changes in intracellular and extracellular ion activities, we obtained evidence for the presence of a chloride transport system in the basolateral cell membrane which translocates Cl^- out of the cell and HCO_3^- and Na^+ into the cell.

Materials and Methods

Kidney Preparation

Adult male and female specimens of *Necturus* were obtained from Connecticut Valley Biological Supply Co. (Southampton, Mass.), kept in an aquarium at 12 °C for at least one month prior to use, and fed live goldfish. The kidneys were doubly-perfused via the aortic and portal circulation as described previously [11]. The surface of the kidney was continuously superfused. The composition of the perfusion solutions is listed in Table 1. Changes in the peritubular perfusion were made with

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Table 1. Concentration of solutes in *Necturus* perfusion solutions (mM)

Ion	1 Control	2 Low Cl^-	3 Zero Cl	4 HCO_3^- free (pH 7.6)	5 HCO_3 free low Cl^- (pH 7.6)	6 Low HCO_3 (pH 6.8)	7 High K	8 High K, low Cl	9 Low Na^+	10 Low Na, low Cl	11 NH_4Cl
Na^+	100.5	93.0	93.9	100.5	89.5	100.5	83.0	84.3	0.5	0.5	80.5
K^+	2.5	2.5	2.5	2.5	2.5	2.5	20	20	2.5	2.5	2.5
Ca^{++}	1.8	7.6	7.6	1.8	7.6	1.8	1.8	7.6	1.8	1.8	1.8
Mg^{++}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cl^-	98.1	8.1	0	98.1	8.1	106.1	98.1	8.1	98.1	8.1	98.1
HCO_3^-	10.0	10.0	10.0	–	–	2.0	10.0	10.0	10.0	10.0	10.0
$H_2PO_4^-$	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SO_4^-	–	5.8	8.6	–	5.8	–	–	5.8	–	–	–
Gluconate	–	83.4	85.9	–	83.8	–	–	91.3	–	–	–
BDA^+	–	–	–	–	–	–	–	–	90	–	–
$Hepes^-$	–	–	–	5.6	5.6	–	–	–	–	–	–
Hepes	–	–	–	4.4	4.4	–	–	–	–	–	–
Mannitol	–	–	–	–	–	–	–	–	–	150	–
TMA^+	–	–	–	–	–	–	–	–	10.0	10.0	–
NH_4^-	–	–	–	–	–	–	–	–	–	–	20.0

All solutions were bubbled with 1% CO_2/99% O_2 except solutions # 4 and 5 which were bubbled with 100% O_2.

the aid of a four-way valve placed near the site where the caudal vein was cannulated. Identical changes were simultaneously made in the superfusion solutions. Changes of the luminal solution were accomplished by means of a double-barreled pipette placed in the glomerulus (Theta Style I, R and D Optical Systems, Spencerville, Md.). One barrel of the pipette contained the control solution to which 0.1% Hercules Green shade #2 (H. Kohnstamn & Co., N.Y.) was added. This was helpful in the identification of the perfused tubule. The other barrel was filled with the experimental perfusion solution. Only early proximal tubules [16] were chosen for study. In some experiments, the tubule lumen was injected with Sylgard 185 (Dow Corning Corp., Midland, Mich.).

Basolateral Cell Membrane Potential Differences

Conventional single-barreled microelectrodes were drawn on a horizontal microelectrode puller (Model PD-5, Narishige Scientific Instruments, Tokyo, Japan) from 1.2 mm OD and 0.5 mm ID fiber containing glass capillaries (Frederick Haer & Co., Brunswick, Me.) and filled with 1 M KCl. The resistance of the electrodes ranged from 50 to 80×10^6 Ω, and the tip potential was less than 5 mV.

Basolateral cell membrane potential differences (V_{bl}) were measured by means of one of the two channels of a very high input impedance electrometer (Model *F*223 Instruments, New Haven, Ct). V_{bl} was measured with reference to a 3 M KCl Agar bridge placed on the surface of the kidney in the superfusion solution and circuit asymmetries were cancelled. The microelectrode and reference electrode connections to the electrometer were made via Ag/AgCl half cells. Intracellular impalements were accepted only if (i) the change in potential from baseline was abrupt, (ii) shortly after the impalement, the intracellular voltage remained constant within 2 mV, and (iii) the voltage returned to the original baseline when the microelectrode was withdrawn from the cell.

Intracellular Chloride Activity

Chloride ion-specific electrodes were manufactured from the same glass capillaries as the conventional microelectrodes by means of a modification of the technique of Fujimoto and Kubota [10]. Electrodes were dipped for 3 sec in a 0.1% solution of a silicone polymer (1107 fluid, Dow Corning Corp., Midland, Mich.) in acetone and heated to 300 °C for one half hour. Electrodes were back-filled with a small quantity of Cl^- specific ion exchanger (Corning #477315).

Chloride electrodes were gently beveled using "the thick slurry technique" [19] and examined under a microscope to assess tip diameter. Only electrodes with tip diameters of <1 μm were used. After beveling, the electrodes were back-filled with 0.5 M KCl and their tips immersed in 100 mM NaCl for several hours before use.

Cl^- electrodes were calibrated in pure solutions of 5, 10, 50, and 100 mM KCl solutions. The slope (*S*) of the electrode response was determined from the regression line of voltage against the logarithim of the Cl^- activity. The average slope of Cl^- electrodes used in this study was 54 mV/10-fold change in a_{Cl}.

The selectivity of the Cl^- electrode to anions ($k_{Cl,A}$) was determined from:

$$k_{Cl,A} = 10^{\left[\frac{V_{100\,A} - V_{100\,Cl}}{S}\right]} \quad (1)$$

where $V_{100\,A}$ is the voltage of the Cl^- electrode in 100 mM of the test solution and $V_{100\,Cl}$ the voltage in 100 mM KCl. The selectivity of the Cl^- electrodes to HCO_3^- and gluconate was 0.10 ± 0.01 and 0.03 ± 0.01, respectively. The low sensitivity of Cl^- electrode to gluconate made it a good replacement ion for Cl^- in the perfusion solutions. The activity of HCO_3^- in *Necturus* perfusion solution is low (7.6 mM), and because the contribution of HCO_3^- to the Cl^- signal in control solution is <1 mV, this error was neglected in our calculation of a_{Cl}^i.

Intracellular chloride activity, a_{Cl}^i was measured in the proximal tubule cells by means of cell impalements in the same proximal tubule with both a conventional KCl and a chloride specific microelectrode. Both electrodes were maintained within cells of the same tubule during perfusion of control solution and during changes in either apical or basolateral solutions. This technique allowed a continuous recording of both the voltage from the conventional and Cl^--sensitive microelectrodes throughout each experiment described in this study. Voltages

from the electrodes were subtracted by means of a dual, differential, very high input impedance electrometer (Model F-223 Dual Channel Electrometer).

The criteria for an acceptable impalement with a Cl^- electrode were as discussed above for conventional microelectrodes. In addition, at the end of each experiment the voltage recorded by the Cl^- electrode (V_{Cl}) in control perfusion solution was compared to V_{Cl} in 100 mM KCl. Because the activity Cl^- was nearly the same in both solutions, the similarity of V_{Cl} in 100 mM KCl and control solutions was also used to assess the electrode reliability. Intracellular Cl^- activity was calculated from the following equation:

$$a^i_{Cl} = a^{bl}_{Cl} 10^{\left[\frac{V_{Cl} - V_{bl}}{S}\right]} \quad (2)$$

where a^i_{Cl} and a^{bl}_{Cl} are intracellular and basolateral Cl^- activities, respectively, and V_{Cl} the voltage change in the Cl^- electrode from the basolateral solution to the inside of the cell. In all experiments intracellular Cl^- activity was measured continuously during a control period and following a 2–5 min exposure to an experimental solution. Steady-state intracellular Cl^- activity from the same tubule perfused with control solution was compared directly to the steady-state activity in experimental solutions.

Reference Liquid Ion Exchange Microelectrodes

Reference liquid ion exchange microelectrodes (RLIE) of the type described by Thomas and Cohen [30] were manufactured by a technique similar to those of Cl^- electrodes. They displayed equal selectivity to both Na^+ and K^+ and resistances of 10^{10} Ω. Since the sum of intracellular and extracellular Na^+ and K^+ activities are approximately equal, the RLIE microelectrode with the same selectivity for both cations was used to record the basolateral cell membrane potential. The basolateral cell membrane potentials were measured in the same tubule with both the RLIE and conventional microelectrodes.

Intracellular pH

Intracellular pH was measured in *Necturus* proximal tubule cells with recessed-tip, glass microelectrodes, manufactured according to the technique of Thomas [29].

Electrodes were calibrated in rapidly flowing perfusion solutions at pH 7.60 (Solution 1) and pH 6.80 (Solution 6). Intracellular pH was measured by means of impalements in different cells of the same tubule with a pH electrode and a conventional 1 M KCl microelectrode. Both electrodes were maintained in the cells throughout changes in basolateral perfusion solutions. Intracellular pH was calculated from the following equation:

$$pH^i = pH^o - \left[\frac{V_{pH} - V_{bl}}{S}\right] \quad (3)$$

where pH^i and pH^o are intracellular and extracellular pH and V_{pH} is the voltage recorded by the pH electrode, and S is the slope. The slopes of the electrodes ranged from 55 to 58 mV/pH unit. The 95% response time of electrodes to an 0.8 pH unit change in solution was about 25 sec.

Results

Accuracy of Intracellular Cl^- Measurements

The accuracy of the results of this study depends upon the reliable continuous measurement of intracellular Cl^- activities in control perfusions and during changes in either apical or basolateral solutions using both conventional and Cl^- sensitive microelectrodes. Since the validity of a^i_{Cl} depends upon precise readings of V_{bl} and V_{Cl}, several possible sources of error in our techniques were evaluated; (i) the accuracy of measuring V_{bl} with 1 M KCl microelectrodes, (ii) the validity of measuring V_{Cl} and V_{bl} in two different cells of the same tubule, and (iii) the precision of measuring V_{Cl} in the presence of interfering intracellular anions. These sources of error were tested by the experiments described below.

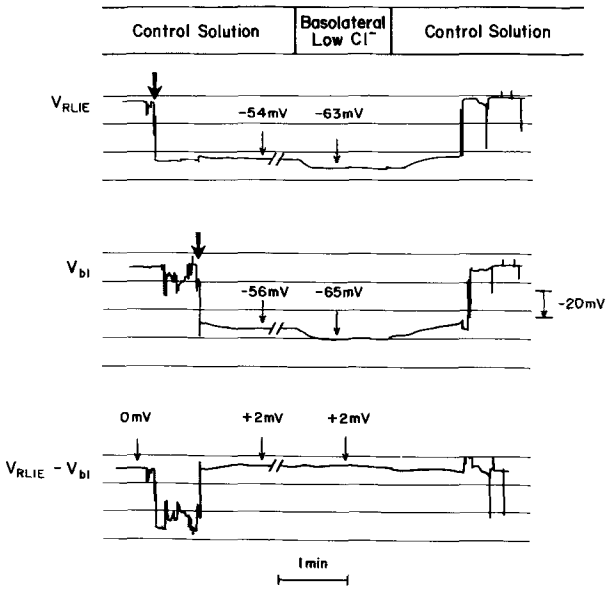

Fig. 1. Experiment to determine if a liquid ion exchange microelectrode and a conventional microelectrode record the same basolateral cell membrane potential in control perfusion solutions and after a reduction of basolateral Cl^-. The upper and middle tracings are the voltages recorded by the reference liquid ion exchange microelectrode (V_{RLIE}) and the conventional 1-M KCl microelectrode (V_{bl}), respectively. The lower tracing is the difference in voltages ($V_{RLIE} - V_{bl}$). Dark arrows mark the time at which the electrode was placed in a cell. Note that both the conventional and the reference liquid ion exchange microelectrodes record the same intracellular voltage, within 2 mV, even during a reduction in the Cl^- activity of the basolateral solution

The first two sources of error were evaluated by means of a reference liquid ion exchange microelectrode [30]. The reference liquid ion exchange microelectrode has an equal selectivity for Na^+ and K^+, thus making it possible to record the basolateral cell membrane potential. An experiment in which both a RLIE and a conventional microelectrode were placed and maintained in cells of the proximal tubule is shown in Fig. 1. At the beginning of the experiment, a RLIE microelectrode (upper tracing) and a conventional 1 M KCl micro-

electrode (middle tracing) were placed on the surface of the kidney in the superfusion solution and the voltages recorded. The difference in the voltages sensed by the two microelectrodes was also recorded (lower tracing). Cell impalements were made in two cells of the same tubule, first with the RLIE (upper tracing) and then with a conventional 1-M KCl microelectrode (middle tracing). Note that the basolateral cell membrane potentials recorded by both electrodes are within 2 mV (lower tracing). The basolateral solution was then switched to a low Cl solution (Solution 2), and both electrodes recorded a hyperpolarization of the same magnitude. The identity of the voltage changes recorded during solution changes is shown by the absence of any voltage change in $V_{RLIE} - V_{Cl}$ (lower tracing). This experiment shows that liquid ion exchange microelectrodes record the same basolateral cell membrane potentials as conventional microelectrodes and provides a check of the validity of using V_{Cl} and V_{bl} in different cells of the same tubule to calculate intracellular Cl^- activity.

The magnitude of the third source of error was estimated by measuring intracellular Cl^- activity in tubules which were perfused for one hour with zero Cl^- (Solution 3) in both aortic and peritubular perfusions. After one hour perfusion with zero Cl^- solution V_{bl} was -70 ± 2 mV [12] and a^i_{Cl}, 1.8 ± 0.2 mM [12]. It should be noted that the detection of an apparent activity of Cl^- in the cell after Cl^- removal from the bathing solutions does not necessarily imply that the value can be used as a blank. (i) There is no way to know if the apparent interfering ions are present in the same activity in control and experimental perfusions. (ii) The choice of the replacement ion for Cl^- could contribute to the blank signal if after one hour perfusion the replacement ion is present inside the cell. Consequently, the replacement ion itself could generate an additional interference not present in control solutions. (iii) Finally, it is possible that a small quantity of Cl^- remains inside of the cell after perfusion with Cl^--free solution for one hour. Because of the uncertainties of the measurement of very low Cl^- activities, and the small activity signal remaining after zero Cl^- perfusion, the intracellular Cl^- values after one hour perfusion with zero Cl^- were not subtracted from the measured intracellular Cl^- activities.

During control perfusions the mean intracellular Cl^- activity for all the experiments ($n = 140$) described in this study is 12.6 ± 0.36 mM and the basolateral cell membrane potential -66 ± 4 mV. This mean intracellular Cl^- activity is higher than the equilibrium activity of 5 mM predicted by the basolateral cell membrane potential. A higher than predicted intracellular Cl^- activity in the *Necturus* proximal tubule is well known [5, 13, 17, 26, 27]. Hence, there is an electrochemical gradient favoring the efflux of Cl^-.

Dependence of Cl^- Movement across the Basolateral Cell Membrane on External Bicarbonate and SITS Treatment

Reducing the Cl^- activity of the basolateral solution from 74 mM (Solution 1) to 6 mM (Solution 2) causes a reduction in intracellular Cl^-, a hyperpolarization of the basolateral cell membrane potential, and a large increase in the electrochemical driving force favoring Cl^- exit from the cell (Table 2). The time course of the reduction in intracellular Cl^- and of the hyperpolarization of V_{bl} is shown in Fig. 2.

To determine if HCO_3^- in the perfusion solutions is required for the efflux of Cl^- from the cell across the basolateral cell membrane, HCO_3^- was first removed from both apical and basolateral perfusion solutions at constant pH (Solution 4). When similarly to the previous experiment Cl^- activity in the basolateral solution was reduced from 74 to 6 mM in a HCO_3^- free solution at constant pH (Solution 5), there was no significant change in the mean intracellular chloride activity or basolateral cell membrane potential despite a large increase in the electrochemical driving force favoring the efflux of Cl^- from the cell (Table 2). This experiment suggests (i) that HCO_3^- in the basolateral solution is required for the efflux of Cl^- from the

Table 2. Dependence of Cl^- movement across the basolateral cell membrane on external HCO_3^- and inhibition by SITS

	a^i_{Cl} (mM)	V_{bl} (mV)	$V_{bl} - E_{Cl}$ (mV)
Control	15.3 ± 1.0	-62 ± 1	-22 ± 2
Basolateral low Cl^-	9.9 ± 0.7	-69 ± 1	-80 ± 2
P ($n = 13$)	<0.001	<0.001	<0.001
HCO_3^- free pH 7.6[a]	11.8 ± 0.84	-65 ± 3	-23 ± 2
HCO_3^- free pH 7.6 and basolateral low Cl^-	11.3 ± 1.0	-63 ± 4	-81 ± 3
P ($n = 13$)	NS	NS	$P < 0.001$
SITS[b]	9.0 ± 0.9	-76 ± 2	-19 ± 3
SITS[b] and basolateral low Cl	9.9 ± 0.9	-75 ± 3	-86 ± 3
P ($n = 11$)	NS	NS	$P < 0.001$

[a] HEPES was used to replace HCO_3^- in both apical and basolateral solutions and the pH was kept constant at control levels.
[b] SITS was applied at a concentration of 5×10^{-4} M to both apical and basolateral solutions for 15 min prior to the experiment.

cell across the basolateral cell membrane and (ii) that intracellular Cl^- activity appears to be insensitive to a change in the electrochemical gradient.

To characterize further the nature of the interaction of HCO_3^- with the transport of Cl^- across the basolateral cell membrane, SITS, a known inhibitor of anion exchange in red blood cells [3], was applied to the apical and basolateral control perfusion solutions at a concentration of 5×10^{-4} M for 15 min prior to the experiment. Since the signal of the Cl^- electrode was SITS sensitive (about 5 mV), in SITS experiments the reference voltage of Control Ringer was used to calculate a^i_{Cl}. Reducing the Cl^- activity of the basolateral solution from 74 to 6 mM in the presence of SITS (Table 2) had no significant effect on either intracellular Cl^- or basolateral cell membrane potential despite a large increase in the electrochemical gradient favoring Cl^- efflux from the cell.

Dependence of Intracellular Cl^- and pH on Bicarbonate in the Basolateral Solution

Removing HCO_3^- from the basolateral solution only, at constant pH of 7.6 (Solution 4), results in an increase in intracellular Cl^- activity, and a decrease in both the basolateral cell membrane potential and the electrochemical driving force across the basolateral cell membrane (Table 3). A representative example of another series of experiments in which the HCO_3^- concentration of the basolateral solution was lowered from 10 to 2 mM and the pH reduced from 7.6 to 6.8 is shown in Fig. 3. Perfusion with a solution containing 2 mM HCO_3^-, at pH 6.8 (Solution 6), on the basolateral side results in an increase in intracellular Cl^- and a decrease in both basolateral cell membrane potential and electrochemical driving force (Table 3). The increase in intracellular Cl^- following HCO_3^- reductions either at constant pH or at low pH cannot occur by a simple electrodiffusive pathway, because an electrochemical gradient still favors the efflux of Cl^- across the basolateral cell membrane despite a decrease in V_{bl} in these low HCO_3^- solutions.

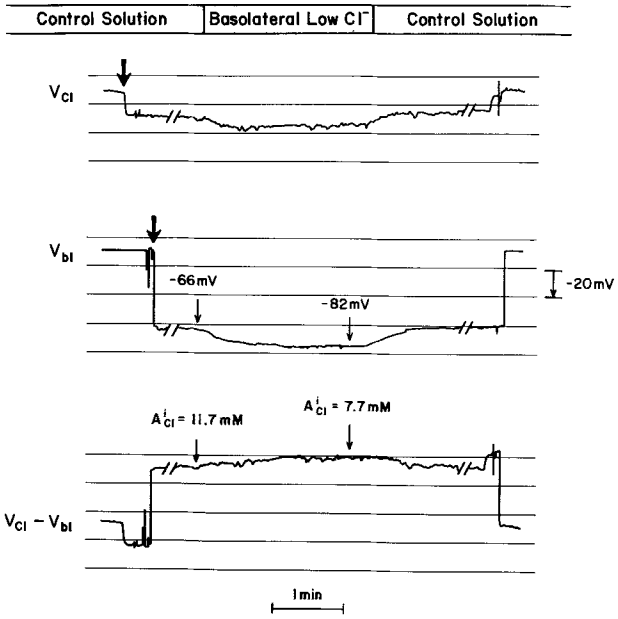

Fig. 2. Experiment in which the Cl^- activity of the basolateral solution was lowered. The upper and middle tracings are the voltages recorded by the Cl^--sensitive microelectrode (V_{Cl}) and the conventional microelectrode (V_{bl}), respectively. Dark arrows mark the time at which the cell was impaled with the microelectrodes. The lower tracing, the difference $V_{Cl} - V_{bl}$, directly proportional to a^i_{Cl}. A reduction of Cl^- in the basolateral solution causes a rapid reduction in a^i_{Cl} from 11.7 mM in control to 7.7 mM in low Cl^- solution (lower tracing) and a simultaneous depolarization of V_{bl} (middle tracing). When control solution is replaced in the basolateral solution both a^i_{Cl} and V_{bl} return to control values

Table 3. The response of intracellular chloride activity, pH, and estimated HCO_3^- activity to basolateral HCO_3^- reductions

	a^i_{Cl} (mM)	pH^i	$a^i_{HCO_3}$ (mM)[a]	V_{bl} (mV)	$V_{bl}-E_{Cl}$ (mV)	$V_{bl}-E_H$ (mV)
Control	13.7±1.8	–	–	−68±3	−24±2	–
Basolateral-HCO_3^- free						
pH 7.6	16.6±2.0	–	–	−55±3	−16±3	–
n	9	–	–	9	9	
P	<0.005			<0.001	<0.001	
Control	12.7±1.3	7.30±0.03	3.9±0.3	−66±2	−21±2	−49±2
Basolateral low HCO_3^-						
pH 6.8	17.7±2.2	7.09±0.05	3.1±0.4	−48±2	−8±2	−65±3
n	12	7	7	19	12	7
P	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001

[a] Intracellular HCO_3^- activity was calculated from pH^i according to Eq. (4)

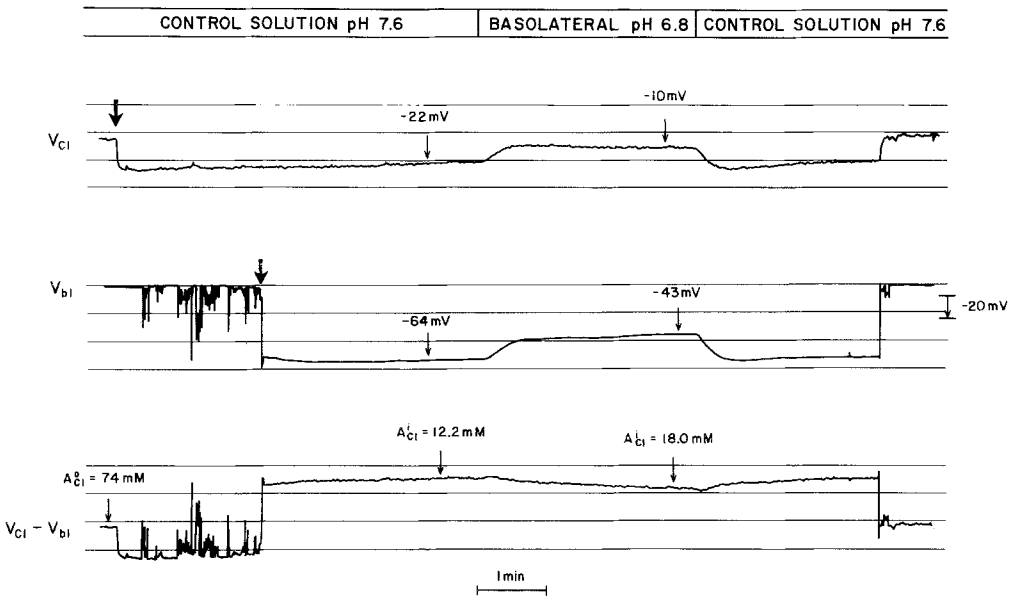

Fig. 3. An experiment to determine the effect of lowering the pH of the basolateral solution on a^i_{Cl}. V_{Cl}, V_{bl}, and $V_{Cl}-V_{bl}$ are the same as shown in Fig. 2. A reduction of the pH of the basolateral solution results in an increase in a^i_{Cl} from 12.2 to 18.0 mM (lower tracing) and a depolarization of V_{bl} from −64 to −43 mV (middle tracing)

In order to define the gradient for HCO_3^- (Table 3), intracellular pH was measured in proximal tubule cells. Intracellular pH measured in control perfusion solutions is lower than extracellular pH but higher than predicted from the basolateral cell membrane potential. As a result, there is an electrochemical gradient which favors the influx of protons and the efflux of HCO_3^- across the basolateral cell membrane. This result has been observed in many cells of different organisms [23]. In contrast, intracellular pH measured in 2 mM HCO_3^- solutions at pH 6.8, on the basolateral side only, is greater than extracellular pH.

Assuming the same intracellular and extracellular carbonic acid activities and pK, intracellular HCO_3^- activities $a^i_{HCO_3^-}$ were estimated from the following equation:

$$pH^o - pH^i = \log \frac{a^o_{HCO_3^-}}{a^i_{HCO_3^-}} \quad (4)$$

where pH^o, and $a^o_{HCO_3^-}$ are the pH and HCO_3^- activity of the basolateral perfusion solution, respectively. The results are given in Table 3. Estimated intracellular HCO_3^- activity in control perfusions is less than extracellular as expected from intracellular pH being lower than extracellular. Acidifying the basolateral solution from pH 7.6 to 6.8 results in a reduction of intracellular HCO_3^-.

The experiments described above show that a reduction in the activity of HCO_3^- in the basolateral solution results in an increase in intracellular Cl^- and a decrease in intracellular HCO_3^-. These observations suggest the presence in the basolateral cell membrane of a mechanism for the countermovement of Cl^- and HCO_3^-.

Dependence of Cl^- Movement Across the Basolateral Cell Membrane on Na^+ and K^+ in the Basolateral Solution

In three groups of experiments Na^+ activity was reduced in the basolateral solution from 76 to 0.4 mM (Solution 7). The results are given in Table 4. In the first group a reduction of Na^+ in the basolateral solution causes an increase in intracellular Cl^- and a reduction in both basolateral cell membrane potential and electrochemical driving force for Cl^-. However, the electrochemical driving force in low Na^+ solution still favors the efflux of Cl^- across the basolateral cell membrane.

A possible explanation for the increase in intracellular chloride following a reduction in basolateral Na^+ is an increase in apical Cl^- entry. Since in the *Necturus* proximal tubule cell lowering basolateral Na^+ lowers intracellular Na^+ [4] thus increasing the Na^+ gradient across the apical cell membrane, an increase in the transport of chloride through an apical Na^+ and Cl^- cotransport mechanism could occur. To test this possibility, in a

Table 4. Dependence of Cl movement across the basolateral cell membrane on Na^+ and K^+ in the basolateral solution

	a^i_{Cl} (mM)	V_{bl} (mV)	$V_{bl}-E_{Cl}$ (mV)
Control	11.5±0.6	−63±2	−16±2
Basolateral low Na^+	16.8±1.4	−45±3	−6±2
$P(n=27)$	<0.001	<0.001	<0.001
Lumen oil filled	14.2±1.1	−47±2	−5±2
Basolateral low Na^+ Lumen oil filled	18.4±1.5	−32±5	0±2.7
$P(n=9)$	<0.001	<0.001	<0.001
Control	12.3±1.3	−62±4	−15±3
Basolateral low Na^+ and low Cl	12.1±1.5	−57±3	−74±2
$P(n=10)$	NS	NS	<0.001
Control	12.3±0.8	−66±1	−19±1
Basolateral high K	11.8±1.2	−46±1	+1±3
$P(n=10)$	NS	<0.001	<0.001
Control	12.4±0.8	−68±2	−21±1
Basolateral high K and low Cl	9.6±0.8	−59±2	−69±3
$P(n=15)$	<0.001	<0.001	<0.001

second group of experiments the lumen of the tubule was filled by means of a pipette containing Sylgard 185. Intracellular Cl^- activity was then measured during a reduction in basolateral Na^+. The results are given in Table 4. Filling the lumen of the tubule with oil results in a decrease in V_{bl}. Under this condition the electrochemical driving force for Cl^- across the basolateral cell membrane is only slightly negative. Reducing Na^+ in the basolateral solution causes intracellular Cl^- to increase and abolishes the electrochemical potential for Cl^-. This increase is similar to the rise in intracellular chloride following basolateral Na^+ reduction in free flow tubules. The fact that the increase in intracellular Cl^- resulting from a decrease in basolateral Na^+ occurs even if luminal Cl^- entry is blocked is strong evidence that the Na^+ chemical gradient across the basolateral cell membrane can influence intracellular Cl^- activity by a mechanism located completely on the basolateral cell membrane.

In addition, in a third group of experiments, reducing both Na^+ *and* Cl^- activities to 0.4 and 6 mM (Solution 8), respectively, results in no significant change in intracellular Cl^- activity or basolateral cell membrane potential despite a large increase in the electrochemical gradient favoring Cl^- exit across the basolateral cell membrane (Table 4). These results sharply contrast those shown above where a significant decrease in intracellular Cl^- occurred following a reduction of basolateral Cl^- in the presence of control levels of Na^+ (Table 2). These experiments indicate that the basolateral chemical gradient of Na^+ affects the movement of Cl^- across the basolateral cell membrane.

In two groups of experiments the effect of the K^+ gradient on a^i_{Cl} was tested by increasing the activity of K^+ in the basolateral solution from 1.9 to 15.2 mM (Solution 9). The results are given in Table 4. Raising K^+ in the basolateral solution had no significant effect on a^i_{Cl}. In contrast, simultaneously raising K^+ activity and decreasing Cl^- activity of the basolateral solution (Solution 10) caused a decrease in intracellular Cl^-. The decrease in intracellular Cl^- observed in high K solution is similar to that reported in Table 2 where Cl^- was lowered in the basolateral solution in control K^+. From these two groups of experiments we conclude that changes in the chemical gradient of K^+ do not interfere with Cl^- movement across the basolateral cell membrane.

Influence of Basolateral NH_4Cl Treatment on Intracellular Cl^- Activity

The experiments described in previous sections of this paper were designed to study intracellular Cl^- activity during changes in the ionic composition of the basolateral perfusion solution. In contrast, the experiments reported in this section deal with the effects of changing intracellular pH at constant extracellular pH on intracellular Cl^-.

Exposure of a cell to NH_4Cl results in alkalinization of intracellular pH followed by a slow fall in intracellular pH. When the cell is returned to a NH_4Cl free solution, there is a rapid decline in intracellular pH below control levels followed by a gradual return to control pH. This pattern of response of intracellular pH to NH_4Cl treatment has been observed in many different cell types [23], including proximal tubule cells [1, 22].

Intracellular Cl^- activities in the *Necturus* proximal tubule were measured continuously during a 2 to 5 min exposure to 20 mM NH_4Cl in the basolateral solution (Solution 11) and during the return to an NH_4Cl-free basolateral control solution. A sample experiment is shown in Fig. 4, and mean values of intracellular Cl^- activities and basolateral cell membrane potentials, at 12-sec intervals, are plotted in Fig. 5.

During an exposure of the basolateral side of seven *Necturus* proximal tubules there is a large increase in intracellular Cl^- which reaches a peak value of 22.1±3.5 mM from a control of 14.5±1.5 mM. This is followed by a slow decline in intracellular Cl^-, reaching a plateau value of intracellular Cl^- of 19.4±1.5 mM. When NH_4Cl

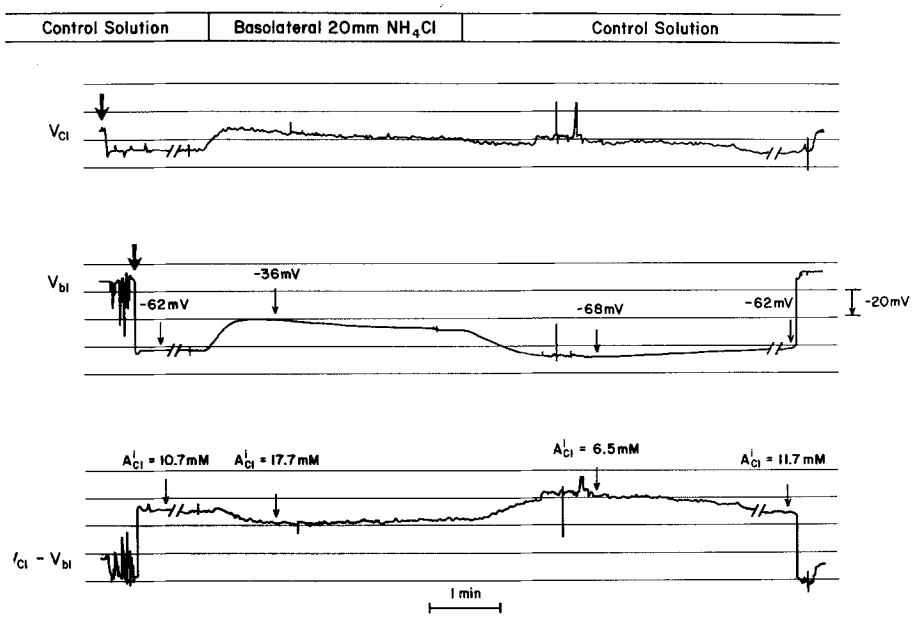

Fig. 4. The response of V_{bl} and a^i_{Cl} to NH_4Cl treatment V_{Cl}, V_{bl}, and $V_{Cl} - V_{bl}$ are the same as shown in Fig. 2. When NH_4Cl is perfused in the basolateral solution there is a transient increase in a^i_{Cl} from 10.7 mM in control to a peak of 17.7 mM in NH_4Cl (lower tracing) and a transient depolarization in V_{bl} from -62 mV to peak low value of -36 mV (middle tracing). After NH_4Cl treatment there is a large reduction in a^i_{Cl} to 6.5 mM (lower tracing) and a hyperpolarization of V_{bl} to -68 mV (middle tracing), followed by a gradual return to control values

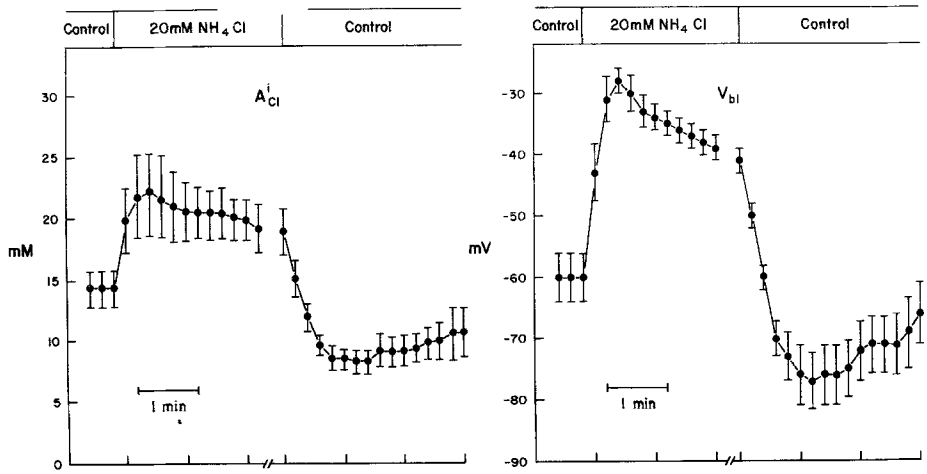

Fig. 5. Average response of intracellular Cl^- activity (a^i_{Cl}) and basolateral cell membrane potential (V_{bl}) to exposure of the proximal tubule cell to 20 mM NH_4Cl. Both a^i_{Cl} and V_{bl} were measured continuously during the experiment. Points represent the average values at 12-sec intervals from seven continuous experiments on seven tubules

is removed from the basolateral solution, there is a rapid fall in intracellular Cl^- to a minimum of 8.2 ± 0.9 mM.

The basolateral cell membrane potential falls during NH_4Cl treatment to a minimum value of -28 ± 2 mV from a control potential of -60 ± 4 mV. When NH_4Cl-free solution is returned to the basolateral side there is a hyperpolarization of the basolateral cell membrane potential to a peak of -77 ± 5 mV.

The influence of HCO_3^- on the movement of Cl^- across the basolateral cell membrane resulting from NH_4CL treatment was tested by exposing the basolateral side of the proximal tubule to 20 mM NH_4Cl in the absence of HCO_3^- in both apical and basolateral solutions. The results are illustrated in Fig. 6. In contrast to the previous experiments summarized in Fig. 5, NH_4Cl treatment and removal in the absence of extracellular HCO_3^- and CO_2 produces no significant change in intracellular Cl^- activity. During NH_4Cl treatment in HCO_3^--free solutions the basolateral cell membrane potential depolarizes from -60 ± 4 mV in control to a minimum of -53.1 ± 5 mV and following NH_4Cl treatment hyperpolarizes to a maximum of -63 ± 3 mV. The changes in V_{bl} in HCO_3^- free solutions are smaller in magnitude than in HCO_3^--containing solutions. The lack of response of intracellular Cl^- activity in HCO_3^--free solution suggests that HCO_3^- is required for the movement of Cl^- across the basolateral cell membrane.

An additional experiment was performed to ascertain if Na^+ in the basolateral solution is required for the response of intracellular Cl^- to

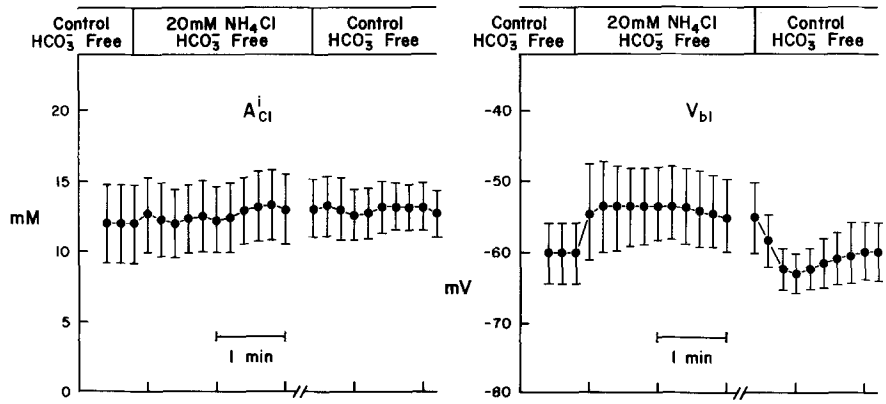

Fig. 6. An experiment similar to the one described in Fig. 5 except that all solutions were bicarbonate free. Note that in HCO_3^--free solutions NH_4Cl treatment has no effect on a^i_{Cl}

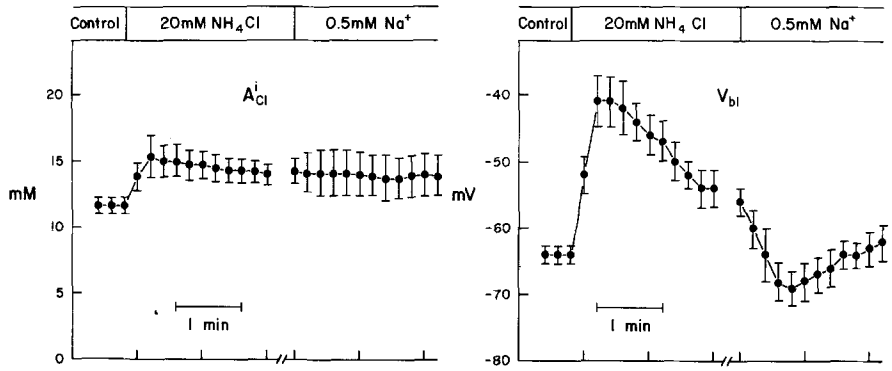

Fig. 7. An experiment similar to Fig. 5. In this case, following NH_4Cl treatment, a solution with low Na^+ was perfused on the basolateral side only. Note that the reduction of a^i_{Cl} following NH_4Cl treatment shown in Fig. 5 is inhibited when a solution low in Na^+ is perfused on the basolateral side

NH_4Cl treatment. Similar to the experiments described above, the proximal tubule cell was exposed to 20 mM NH_4Cl for 2 to 5 min on the basolateral side only and intracellular Cl^- measured continuously during the experiment. However, following NH_4Cl treatment, a low Na^+ solution was present in the basolateral solution. The results are shown in Fig. 7. The first two maneuvers depicted in Fig. 7, namely control and NH_4Cl exposure are similar to those of Fig. 5. In contrast, in the third period shown in Fig. 7, there is no significant change in intracellular Cl^- when, following NH_4Cl treatment the basolateral side is exposed to a low Na^+ solution. Thus, Na^+ is required for the fall in intracellular Cl^- following the removal of NH_4Cl.

Dependence of Intracellular Cl^- on the Composition of the Luminal Perfusion Solution

Intracellular Cl^- activity was measured during alterations of the luminal perfusion solution to characterize the movement of Cl^- across the apical cell membrane. Lowering chloride in the luminal perfusion solution results in a reduction of intracellular chloride activity and a hyperpolarization of the basolateral cell membrane potential without a change in the electrochemical driving force for chloride across the basolateral cell membrane (Table 5).

Table 5. Effects of low luminal Cl^-, HCO_3, or Na^+ on intracellular Cl^- activity and basolateral cell membrane potential

	a^i_{Cl} (mM)	V_{bl} (mV)	$V_{bl}-E_{Cl}$ (mV)
Control	14.3±1.7	−56±3	−12±2
Lumen low Cl^-	7.6±1.1	−69±3	−8±2
$P(n=14)$	<0.001	<0.001	NS
Basolateral Low Na^+	27.6±4.4	−35±5	−6±2
Basolateral low Na^+ and lumen low Cl^-	23.9±3.5	−38±6	−6±3
$P(n=13)$	NS	NS	NS
Control	11.8±1.9	−62±4	−14±3
Lumen low HCO_3^- pH 6.8	11.3±1.8	−62±5	−12±4
$P(n=7)$	NS	NS	NS
Control	12.4±2	−60±3	−13±3
Lumen low Na^+	6.6±1	−75±4	−12±3
$P(n=10)$	<0.001	<0.001	NS

In order to show that the reduction of intracellular Cl^- resulting from lowering luminal Cl^-

results from the efflux of Cl^- across the basolateral cell membrane, luminal Cl^- was also lowered in the absence of basolateral Na^+. The results in Table 5 show that prior lowering of Na^+ in the basolateral solution inhibits the drop of Cl^- following a reduction of luminal chloride. Thus the response of intracellular chloride activity to a given reduction in luminal chloride is controlled by Na^+ in the basolateral solution.

In another series of experiments, a low HCO_3^-, pH 6.8, solution (Solution 6) was used to perfuse the lumen. Whereas lowering basolateral HCO_3^- caused a significant increase of a^i_{Cl} and a decrease in V_{bl} (Table 3), there was no effect on either parameter when a similar solution change was performed in the lumen (Table 5). Hence the results indicate that the luminal cell membrane does not have an apparent HCO_3^- permeability and that HCO_3^- does not play a role in the regulation of the Cl^- distribution across the apical cell membrane.

Finally, lowering sodium in the luminal perfusion solution had the same effect as lowering chloride in the lumen, i.e. a reduction of intracellular chloride activity and a hyperpolarization of the basolateral cell membrane without a change in electrochemical driving force. The dependence of intracellular chloride on luminal Na^+ confirms the observations of Spring and Kimura [27] and is consistent with the operation of a Na^+/Cl^- cotransport system in the apical cell membrane.

Discussion

Regulation of Intracellular Chloride Activity

The intracellular chloride activity of the *Necturus* proximal tubule, as that of many epithelial cells [8], is above electrochemical equilibrium. This could result from specific transport mechanisms across either the apical or basolateral cell membranes. In the present study, we confirm the presence of Na^+/Cl^- cotransport on the apical cell membrane of the *Necturus* proximal tubule. In addition, we now show that the transport of Cl^- across the basolateral cell membrane is also essential in regulating intracellular chloride activity. Cl^- transport across the basolateral cell membrane appears to involve at least two transport systems.

Transport Mechanisms for Cl^- across the Basolateral Cell Membrane

The key observation of the present study is the dependence of intracellular Cl^- activity upon basolateral extracellular activity of sodium and bicarbonate.

Several of our results lead to the conclusion that sodium is involved in the transport of chloride across the basolateral cell membrane. They include:

1. an increase in a^i_{Cl} when Na^+ is reduced in the basolateral solution (Table 4);
2. an increase in a^i_{Cl} when Na^+ is reduced in the basolateral solution is independent of the presence of luminal chloride (oil filled lumen, Table 4, or perfusions of the lumen with low chloride solutions, Table 5);
3. the fall in a^i_{Cl} following a reduction of basolateral chloride activity is inhibited by Na^+ removal from the basolateral solution (Table 4);
4. the fall of a^i_{Cl} following a reduction of luminal chloride activity is inhibited by the removal of basolateral Na^+ (Table 5);
5. the fall of a^i_{Cl} following sudden intracellular acidification (NH_4Cl treatment) is inhibited by the removal of basolateral Na^+ (Fig. 7).

Results indicating that bicarbonate also plays a role in the transport of chloride across the basolateral cell are:

1. an increase in a^i_{Cl} when bicarbonate is removed from the basolateral solution (Table 2). This effect of HCO_3^- is independent of basolateral pH (Table 3),
2. the fall in a^i_{Cl} following basolateral chloride reduction is inhibited by the absence of basolateral bicarbonate (Table 2);
3. the rise and fall of a^i_{Cl} during intracellular pH transients (NH_4Cl treatment) is inhibited by the absence of bicarbonate (Fig. 6).

These results can best be explained by a transport system in the basolateral cell membrane which moves both sodium and bicarbonate into the cell in exchange for chloride leaving the cell[1]. Transport systems of this type have been identified in many invertebrate nonepithelial cells including the snail neuron [28], the barnacle muscle [2], and the squid giant axon [25]. They function mainly to regulate intracellular pH and their mode of transport appears to be electroneutral. The stoichiometry would be one of the following: Na^+/HCO_3^--

[1] Linkage of Na, HCO_3^- and Cl^- different from the model proposed in Fig. 8, which is also thermodynamically favorable, is possible. Such alternative exchange systems might include Na^+/HCO_3^--K^+/Cl^-. In this discussion we have chosen a model involving only Na^+/HCO_3^--H^+/Cl^- because transporters of this type have been described previously (*see* ref. 23 for a review).

H^+/Cl^-; $Na^+/2\,HCO_3^-$-Cl^-; Na^+/CO_3^{--}-Cl^-. These three are thermodynamically indistinguishable.

The energy for the electroneutral transport of chloride across the basolateral cell membrane can be described by the following equation:

$$\frac{\Delta G}{F}=\frac{RT}{F}\ln\frac{(a^i_{Na})(a^i_{HCO_3})^2(a^o_{Cl})}{(a^o_{Na})(a^o_{HCO_3})^2(a^i_{Cl})} \tag{5}$$

where ΔG is the free energy change. $\Delta G<0$ denotes sodium and bicarbonate influx and chloride efflux from the cell.

In control conditions the chloride chemical gradient, a^o_{Cl}/a^i_{Cl} is approximately 6:1 (from $a^o_{Cl}=$ 74 mM and $a^i_{Cl}=12.6$ mM) and the sodium chemical gradient a^o_{Na}/a^i_{Na} is also about 6:1 ($a^o_{Na}=76$ mM and $a^i_{Na}=12$ mM; *see* ref. 4). Thus the Na^+ and Cl^- chemical gradients are approximately equal in magnitude and direction. The bicarbonate chemical gradient is about 2:1 ($a^o_{HCO_3}=7.6$ mM and $a^i_{HCO_3}=3.9$ mM). The calculated $\Delta G/F$ calculated from Eq. (5) is -35 mV. Hence, in control conditions ΔG is negative and chloride is transported out of the cell in exchange for sodium and bicarbonate. Similarly it can also be shown that when either bicarbonate or sodium is lowered in the basolateral solution (Tables 3 and 4) $\Delta G/F$ is positive at $+17$ and $+92$ mV, respectively. Therefore, the reversal of either bicarbonate or sodium gradients across the basolateral cell membrane results in the inward movement of chloride as evidenced by an increase in intracellular chloride (Tables 3 and 4).

In contrast to the electroneutral transport system identified in the invertebrate nerve and muscle cells, the basolateral transport system in our study may also involve the transfer of charge. For example, all experimental maneuvers which result in an increase in intracellular chloride are accompanied by a reduction in basolateral cell membrane potential. They include:

1. lowering Na^+ in the basolateral solution (Table 4)
2. lowering HCO_3^- in the basolateral solution (Tables 2 and 3)
3. alkalinizing intracellular pH with NH_4Cl (Fig. 4).

Likewise, experiments which cause a decrease in intracellular chloride result in an increase in basolateral cell membrane potential. They include:

1. lowering basolateral Cl^- (Table 2)
2. lowering either apical Na^+ or Cl^- (Table 5)
3. acidifying intracellular pH following NH_4Cl treatment (Fig. 5).

Importantly, inhibiting chloride movements by either removing basolateral HCO_3^- (Table 2 and Fig. 6) or by removing basolateral Na^+ (Table 4 and Fig. 7) also diminishes the changes in basolateral cell membrane potential. Thus the chloride movement across the basolateral cell membrane can be accompanied by the opposite movement of negative change.

Although the present study does not exclude other possibilities, including changes in the relative permeability of ions other than chloride, one explanation for the observed membrane potential changes could be the operation of an electrogenic transport system. Recently, Boron and Boulpaep have identified, in the *Ambystoma* proximal tubule, an electrogenic Na^+/HCO_3^- transport system that transports sodium, bicarbonate, and negative charge in the same direction [1]. One possible stoichiometry of this system is $Na^+/2\,HCO_3^-$. This system does not require Cl^-, whereas in the present study chloride movements are tightly linked to those of sodium and bicarbonate. It is possible, that in the *Necturus* proximal tubule cell, a combination of electrogenic $Na^+/2\,HCO_3^-$ and electroneutral $Na^+/2\,HCO_3^-$-CL^- transport are present. The simplest case involving one neutral cycle of the hypothetical transport system for every electrogenic cycle would have the stoichiometry $2\,Na^+/4\,HCO_3^-$-Cl^- and would result in one negative charge entering the cell during chloride exit and one negative charge leaving as chloride enters. The free energy charge of such a system is described by the following equation:

$$\frac{\Delta G}{F}=\frac{RT}{F}\ln\frac{(a^i_{Na})^2(a^i_{HCO_3})^4(a^o_{Cl})}{(a^o_{Na})^2(a^o_{HCO_3})^4(a^i_{Cl})}-V_{bl} \tag{6}$$

where $\Delta G<0$ predicts sodium and bicarbonate influx along with one negative charge and chloride efflux. Using control values for intracellular ion activities given above and a basolateral cell membrane potential of -62 mV, the calculated $\Delta G/F$ for this system is -55 mV. Since in control conditions ΔG is smaller than zero, this system would be expected to transfer chloride out of the cell in exchange for sodium, bicarbonate, and one negative charge.

Using cable analysis we have shown in a recent paper that the chloride conductance of the basolateral cell membrane is immeasurably low [13]. The chloride conductance was assessed from the resistance of the basolateral cell membrane (R_{bl}) in control conditions and following chloride removal from both apical and basolateral solutions.

This observation is not inconsistent with the

possible presence of the two described transport systems involving both an electrogenic and an electroneutral mode in the basolateral cell membrane since the electrogenic mode does not require chloride.

Another apparent contradiction concerns the finding of a correlation between steady-state intracellular chloride activity and basolateral cell membrane potential [13, 26] despite a very low electrodiffusive chloride conductance [13]. However, such a correlation between intracellular chloride activity and basolateral cell membrane potential could be accounted for by the operation of a charged carrier for chloride in the basolateral cell membrane as discussed above. Since the Na^+/HCO_3^--Cl^- carrier is responsible for chloride exit from the cell and entry of negative charge, a high turnover rate would result in low intracellular chloride activities and high basolateral cell membrane potentials. In contrast, a less active carrier would result in high intracellular chloride activities and lower basolateral membrane potentials.

The possibility that a neutral Cl^--HCO_3^- exchange pathway exists in the basolateral cell membrane has been recently suggested by Edelman, Bouthier and Anagnostopolous [5]. These investigators showed that SITS applied to the basolateral side of the *Necturus* proximal tubule causes a small reduction in intracellular chloride. They concluded that SITS was inhibiting the movement of chloride into the cell across the basolateral cell membrane via a Cl^- and HCO_3^- exchange mechanism. From a consideration of chemical gradients presented above, the $\Delta G/F$ for such a neutral Cl^--HCO_3^- exchange in control conditions is +28 mV. Such a Cl^--HCO_3^- exchange mechanism would result in movement of Cl^- *into* the cell and of bicarbonate out of the cell. If such Cl^--HCO_3^- exchange is a significant transport pathway for chloride to enter the cell from the basolateral side then it should be able to maintain intracellular chloride above electrochemical equilibrium. However, we have shown that filling the lumen with oil reduces intracellular chloride to a value close to equilibrium. This observation suggests that *luminal* rather than basolateral chloride entry is the major mechanism for keeping intracellular chloride above electrochemical equilibrium. It should also be noted that SITS treatment, both in this study (Table 2) and that of Edelman et al. [5] is ineffective in lowering intracellular chloride to electrochemical equilibrium. Although the possibility of simple Cl^--HCO_3^- exchange on the basolateral cell membrane cannot be eliminated, it is clearly not a major pathway for chloride to enter the cell.

Transport of Chloride across the Apical Cell Membrane

Based on microperfusion studies in which the cellular chloride activity was monitored during exposure to sodium-free solutions in the lumen, Spring and Kimura [27] suggested that chloride moves across the apical cell membrane through a Na^+/Cl^- cotransport pathway. In contrast, Edelman, Bouthier and Anagnostopolous [5] perfused the lumen of the *Necturus* proximal tubule with low Na^+ solutions and observed no change in intracellular chloride activity. In the present study we have shown that there is a strong dependence of intracellular chloride on luminal Na^+ (Table 5) consistent with the presence of luminal Na^+/Cl^- cotransport. In contrast, there appears to be little dependence of intracellular chloride on luminal bicarbonate activity (Table 5). Thus the presence of a sizeable apical chloride entry mechanism which involves exchange for bicarbonate or an equivalent anion is unlikely. It is also noteworthy that the apical cell membrane potential is not sensitive to changes in apical bicarbonate activity, a finding that is consistent with a low luminal relative conductance to HCO_3^- or an equivalent anion. A similar absence of any change in apical cell membrane following luminal changes in HCO_3^- has also been observed in rat proximal [9] and *Ambystoma* proximal tubules [1].

Possible Effects of Cell Volume

The conclusions of this paper are based on experiments in which it is assumed that intracellular chloride activity following alterations in the gradients of either chloride, bicarbonate, or sodium reflect a net flux of chloride. Yet changes in intracellular chloride activity could also result from modifications in either activity coefficient or in cell volume. To address this problem experiments were carried out in which we made large increases in osmolality of the basolateral solution from 210 to 290 mOsmol by adding raffinose. It was observed that intracellular chloride activity did not change despite the expected cell shrinkage. Similar results were obtained by Fisher, Persson and Spring in the *Necturus* gallbladder [7]. In contrast to our observations just described, raising the osmolality of the basolateral solution in the absence of HCO_3^-, after basolateral SITS treatment, or after basolateral Na^+ reduction, resulted in significant increases in intracellular chloride [15]. These findings contribute strong additional support for our hypothesis that a Na^+/HCO_3^--Cl^- mechanism exists on the basolateral cell membrane which is

activated by cell shrinkage and which regulates intracellular chloride. Since changes in cell volume in control conditions did not result in alterations in intracellular chloride, we believe it safe to conclude that the significant changes of intracellular chloride that we observed in the present study were not induced by changes in cell volume.

A Model for the Transport of Cl^- across the Necturus Proximal Tubule

The data from this and previous studies allow us to give a description of the pathways for transepithelial chloride transport across the *Necturus* proximal tubule (Fig. 8). It is well known that the paracellular shunt pathway in the *Necturus* proximal tubule is highly Cl^- conductive and that the transepithelial electrochemical gradient favors the passive reabsorption of chloride from the lumen (*see* 13 for a review). Thus the major fraction of Cl^- reabsorbed, may cross the tubule via the paracellular pathway. In addition, Kimura and Spring [18] using combined radioisotope and compartmental analysis techniques concluded that about 2/3 of net Cl^- reabsorption was paracellular and 1/3 transcellular. However the estimate of 2/3 of the net reabsorption occuring via the paracellular pathway could be an underestimate since in the compartmental analysis employed by Spring and Kimura, unidirectional influxes across the shunt and across the apical cell membrane were assumed to be an estimate of net flux. Therefore, the exact fractional contribution of each pathway to the transcellular movement of chloride is not certain. In contrast to paracellular chloride movement, transcellular chloride transport is mainly nonelectrodiffusive. Chloride enters the cell across the apical cell membrane through a Na^+/Cl^- cotransport mechanism and leaves the cell across the basolateral cell membrane by way of a Na^+/HCO_3^--Cl^- countertransport system.

The presence of multiple transport systems for chloride have been identified in other cell membrane systems [8]. In the squid axon for example, intracellular pH is regulated by a mechanism involving Na^+/HCO_3^--Cl^- [25]. Recently, Russell [24] has shown by measuring the unidirectional fluxes of Na^+ and Cl^-, that, in addition, the movements of these two ions are tightly coupled and furosemide sensitive. It was concluded that a Na^+ and Cl^- coupled uptake mechanism was present in the axolemma of the squid axon. Thus two mechanisms exist for Cl^- movement, one which transports chloride into the cell raising intracellular chloride above electrochemical equilibrium,

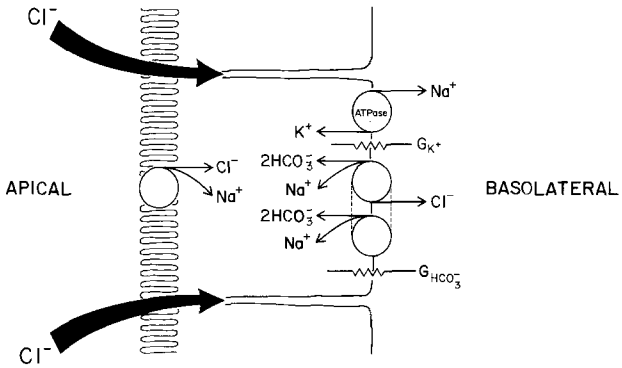

Fig. 8. Model for the transport of Cl^- across the *Necturus* proximal tubule cell. A large fraction of chloride transport occurs by way of the paracellular pathway. Chloride enters the cell across the apical cell membrane via a Na^+/Cl^- cotransporter. Chloride leaves the cell across the basolateral cell membrane by means of a $Na^+/HCO_3^- - Cl^-$ transport system. It is possible that this transport system is variably coupled to an electrogenic Na^+/HCO_3^- transporter and thus carries net negative charge into the cell. The energy for both apical and basolateral transport systems is derived from electrochemical ion gradients generated by the $Na^+ - K^+$ ATPase pump on the basolateral cell membrane. Both a bicarbonate and a potassium conductance may exist on the basolateral cell membrane [21]. These conductances would result in the recycling of bicarbonate and potassium. It should be noted that this model does not include pathways for sodium transport across the apical cell membrane which do not involve chloride. Hence, establishing any stoichiometry between transcellular sodium and chloride transport based on the transport components in the model is unwarranted

and another one which results in chloride efflux during a response to an acid load. Likewise, Fisher, Persson and Spring [7], using optical techniques in combination with chloride sensitive microelectrodes, have postulated the presence of separate Na-H and Cl-HCO_3 exchange mechanisms in the apical cell membrane of the gallbladder. These double exchange mechanisms are thought to be activated by cell shrinkage, they are amiloride and SITS sensitive, and they appear to operate mainly to regulate cell volume. In addition to these exchangers, a Na^+/Cl^- cotransport mechanism has also been shown to be present in the apical cell membrane. In contrast to the other transport system it is sensitive to bumetanide but insensitive to SITS or amiloride [6]. Our experiments indicate that renal tubule cell membranes share in common with these cell membranes the presence of carrier-mediated sodium-sensitive chloride transport systems that involve exchange with bicarbonate ions.

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