# Bicarbonate Secretion and Chloride Absorption by Rabbit Cortical Collecting Ducts

## Role of Chloride/Bicarbonate Exchange

**Robert A. Star, Maurice B. Burg, and Mark A. Knepper**
*Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205*

## Abstract

**Cortical collecting ducts (CCD) from rabbits treated with deoxycorticosterone (DOC) actively secrete bicarbonate at high rates. To investigate the mechanism of bicarbonate secretion, we measured bicarbonate and chloride transport in CCD from rabbits treated with DOC for 9–24 d. Removal of chloride (replaced with gluconate) from both perfusate and bath inhibited bicarbonate secretion without changing transepithelial voltage. Removal of chloride only from the bath increased bicarbonate secretion, while removal of chloride only from the perfusate inhibited secretion. In contrast to the effect of removing chloride, removal of sodium from both the perfusate and bath (replacement with *N*-methyl-D-glucamine) did not change the rate of bicarbonate secretion. The rate of bicarbonate secretion equaled the rate of chloride absorption in tubules bathed with 0.1 mM ouabain to inhibit any cation-dependent chloride transport. Under these conditions, chloride absorption occurred against an electrochemical gradient. Removal of bicarbonate from both the perfusate and bath inhibited chloride absorption. Removal of bicarbonate only from the bath inhibited chloride absorption, while removal of bicarbonate from the lumen stimulated chloride absorption. We conclude that CCD from DOC-treated rabbits actively secrete bicarbonate and actively absorb chloride by an electroneutral mechanism involving 1:1 chloride/bicarbonate exchange. The process is independent of sodium.**

## Introduction

Mammalian cortical collecting ducts perfused in vitro either secrete or absorb bicarbonate,[1] depending on the acid-base state of the animal (1, 2). Bicarbonate secretion and absorption are parallel, independently regulated active transport processes (1, 3, 4) that may occur via two distinct types of intercalated cells (5). Deoxycorticosterone (DOC)[2] administration to rats or rabbits fed an alkaline ash diet induces a very high rate of bicarbonate secretion (3, 4, 6). These observations have led to the view that bicarbonate secretion by cortical collecting ducts may be a physiologically important response to net alkali intake or to metabolic alkalosis (4).

The cellular mechanism of bicarbonate secretion is poorly understood. Conflicting conclusions have been reached about the ionic requirements for bicarbonate secretion. For example, the transepithelial chloride concentration gradient influences the rate of bicarbonate secretion (4, 7, 8) compatible with chloride bicarbonate exchange as in other bicarbonate secreting epithelia (9, 10). Yet, chloride replacement in both perfusate and bath with methyl sulfate, nitrate, or isethionate did not inhibit bicarbonate secretion (8, 11), indicating that bicarbonate secretion may not depend on chloride. The role of sodium is also unclear. Bicarbonate secretion persisted despite inhibition of the Na,K-ATPase with ouabain (3, 8, 11) and, therefore, was considered to be independent of active sodium transport. Yet, bicarbonate secretion was inhibited when choline replaced sodium in perfusate and bath solutions (11), raising the possibility that sodium may be involved in another way.

Because cortical collecting ducts from rabbits treated with DOC secrete bicarbonate at much higher rates than previously observed (3, 4), the rate of bicarbonate secretion now is more easily and accurately measured. Also, the rate of net bicarbonate secretion (15–20 pmol/min per mm [4]) is much greater than the rate of simultaneous proton secretion (4–5 pmol/min per mm [3, 4]), so that in DOC-treated tubules, the predominant flux is bicarbonate secretion. In this paper, we used the DOC model of bicarbonate secretion to investigate the mechanism of bicarbonate transport in rabbit cortical collecting ducts. We tested for chloride/bicarbonate exchange by studying the effect of chloride removal and chloride concentration gradients on bicarbonate secretion, as well as the effect of bicarbonate removal and bicarbonate concentration gradients on chloride transport. We tested for sodium involvement in bicarbonate secretion by studying the effect of replacing sodium with *N*-methyl-D-glucamine.

## Methods

Pathogen-free female New Zealand White rabbits (Small Animal Breeding Facility, National Institutes of Health, Bethesda, MD) were injected with DOC pivalate (12.5 mg/kg i.m.; Ciba-Geigy Corp., Pharmaceuticals Div., Summit, NJ). This depot preparation releases hormone for at least 4 wk. The rabbits were given unlimited access to rabbit chow (National Institutes of Health Feed A) and water until they were used 9–24 d later (weight 1.5–2.3 kg).

A preliminary report of this work was presented at the 17th Annual Meeting of the American Society of Nephrology, December 1984, in Washington, DC, and appeared in abstract form in 1985. *Kidney Int.* 27:289.

Address correspondence to Dr. Star, National Institutes of Health, Building 10, Room 6N307, Bethesda, MD 20205.

*Received for publication 1 February 1985 and in revised form 17 April 1985.*

1. In this paper, the terms bicarbonate and total $CO_2$ are used interchangeably since 95% of the total $CO_2$ is in the form of bicarbonate. The transported ion is referred to as bicarbonate; we recognize, however, that the techniques used cannot distinguish between the transport of $HCO_3^-$ per se and the combined transport of $OH^-$ plus $CO_2$.

2. *Abbreviations used in this paper:* CCD, cortical collecting ducts; DOC, deoxycorticosterone.

The rabbits were killed by decapitation. The left kidney was removed and sliced into coronal sections. Tubules were dissected at 15°C in control A solution (Table I) gassed continuously with 95% $O_2$ and 5% $CO_2$. Segments of cortical collecting ducts beginning at the last convergence of initial collecting tubules were dissected. The length of the tubules was always <2.5 mm.

After dissection, the tubules were transferred to a bath chamber and mounted on concentric pipettes for perfusion (12). The tubules were warmed to 37°C in a temperature-controlled chamber and bathed with solution kept at constant pH, $PCO_2$, and $PO_2$, using a continuously flowing bath system (13).

During a 15–30-min equilibration period, the perfusion rate was adjusted to about 1 nl/min per mm. Fluid leaving the tubule was collected under water-equilibrated mineral oil into calibrated volumetric constriction pipettes (5–13 nl). The flow rate was determined by measuring the length of time needed to fill the volumetric pipette. The length of the tubules was measured with an eyepiece micrometer.

*Solutions and protocols.* The composition of the solutions used is shown in Table I. All bicarbonate-containing solutions were equilibrated with 95% $O_2$/5% $CO_2$. Bicarbonate-free solutions were equilibrated with 100% $O_2$. *N*-methyl-D-glucamine bicarbonate was made by bubbling *N*-methyl-D-glucamine (Aldrich Chemical Co., Inc., Milwaukee, WI) with 5% $CO_2$. In solutions containing sodium gluconate (Aldrich Chemical Co., Inc.), the ionic calcium activity (measured by a calcium ion electrode model 93-20; Orion Research Inc., Cambridge, MA) was matched to the control A solution by adding additional calcium salts. Six protocols were performed.

*Symmetric chloride removal (protocol 1).* Control measurements were made while the tubules were perfused and bathed with the control A solution. Either before or after the control measurements, the perfusate and bath were exchanged to the chloride-free solution or with a similar solution containing 2 mM or 10 mM chloride. Gluconate replaced chloride in all cases.

*Bath chloride removal (protocol 2).* After control measurements were made as in protocol 1, the bath solution was switched to the chloride-free solution.

*Symmetric sodium removal (protocol 3).* Tubules were perfused and bathed with the sodium-free solution. *N*-Methyl-D-glucamine replaced sodium. To ensure complete sodium removal, the perfusion and bath exchange systems were washed exhaustively with 160 mM KCl followed by the perfusion solutions; the tubules were set up in the sodium-free solution. Samples of fluid obtained during the experiments from the drain of the perfusate exchange system and from the bath chamber confirmed that the sodium concentration was <0.20 mM. After experimental measurements were made, the perfusate and bath were switched to the sodium-containing (i.e. control A) solutions; the control period always followed the sodium-free period.

*Symmetric bicarbonate removal (protocol 4).* Control measurements were made while the tubules were perfused and bathed with the control B solution. In this and subsequent protocols, the bath always contained 0.1 mM ouabain (Sigma Chemical Co., St. Louis, MO) to inhibit active sodium- or potassium-dependent chloride transport. The chloride concentration was reduced (50 mM) so that a given absolute change in chloride concentration would produce a larger, and therefore more accurately measurable fractional change. After control measurements, both the perfusate and bath solutions were switched to the bicarbonate-free solution. The perfusion chamber was suffused with 100% $O_2$ while the tubule was bathed with the bicarbonate-free solution.

*Perfusate bicarbonate removal (protocol 5).* After control measurements were made as in protocol 4, the perfusate was exchanged to the bicarbonate-free solution.

*Bath bicarbonate removal (protocol 6).* After control measurements were made as in protocol 4, the bath was switched to the bicarbonate-free solution.

*Measurements.* In bulk solutions, sodium was measured by a flame photometer (model 143; Instrumentation Laboratory, Inc., Lexington, MA); osmolality by a vapor pressure osmometer (model 5100C; Wescor Inc., Logan, UT); pH by a glass electrode (Radiometer America Inc., Westlake, OH); and total $CO_2$ by an Ericsen $CO_2$ analyzer (model E-100; Corning Medical, Corning Glass Works, Medfield, MA). The radioactivity of [$^{14}$C]inulin was measured by a liquid scintillation counter (model LS350; Beckman Instruments, Inc., Fullerton, CA), using Aquasol scintillation fluid (New England Nuclear, Boston, MA).

The transepithelial potential difference was measured using calomel cells connected to the perfusate and bath solutions by NaCl bridges (14). When perfusate and bath solutions differed, a correction was made for the liquid junction potentials, which were measured with a flowing KCl junction replacing the perfusion pipette. The following values were added to the measured potential to obtain the corrected potential: chloride-free vs. control A, +6.2 mV; bicarbonate-free vs. control B, +0.6 mV.

In some experiments, net fluid transport was determined by adding [*carboxy*-$^{14}$C]inulin (New England Nuclear) to the perfusate (20 μCi/ml) and measuring radioactivity in perfusate and collected fluid. Fluid transport rate ($J_v$, in nanoliters per minute per millimeter) was calculated as $J_v = V_L (C_L/C_0 - 1)$, where $V_L$ is the fluid collection rate (nanoliters per minute per millimeter) and $C_L/C_0$ is the ratio of $^{14}$C activities in collected and perfusate fluid.

The total $CO_2$ concentration in perfusate, bath, and collected fluid was determined by microcalorimetry (15). A linear standard curve was obtained for each experiment. The precision of the instrument is sufficient to resolve concentration differences between samples of 1–2 mM. The net transport rate of total $CO_2$ was calculated as $J_{TCO_2} = (C_0 - C_L) \times V_L$, where $C_0$ is the total $CO_2$ concentration measured in the perfusate, $C_L$ is the total $CO_2$ concentration measured in the collected fluid, and $V_L$ is the tubule flow rate normalized per millimeter of tubule length. This equation is valid when there is no net fluid absorption, as confirmed in this study (see Results).

Chloride concentrations were measured in collected fluid, perfusate,

*Table I. Composition of Solution*

| Component | Control A | Sodium-free | Chloride-free*‡ | Control B‡ | Bicarbonate-free* |
|---|---|---|---|---|---|
| | *mM* | *mM* | *mM* | *mM* | *mM* |
| $Na^+$ | 146 | 0 | 146 | 146 | 146 |
| $K^+$ | 5 | 5 | 5 | 5 | 5 |
| $NMDG^+$ | | 145.5 | | | |
| $Ca^{++}$ | 2 | 2 | 7 | 5 | 6 |
| $Mg^{++}$ | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| $Cl^-$ | 118 | 118 | 0 | 50 | 50 |
| $HCO_3^-$ | 25 | 25 | 25 | 25 | 0 |
| Gluconate | | | 128 | 74 | 101 |
| Phosphate | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Lactate | 4 | 4 | 4 | 4 | 4 |
| Sulfate | 1.2 | 1.25 | 1.2 | 1.2 | 1.2 |
| Citrate | 1 | 0.8 | 1 | 1 | 1 |
| Glucose | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| Alanine | 6 | 6 | 6 | 6 | 6 |
| Ouabain (Bath only) | | | | 0.1 | 0.1 |

Solutions containing bicarbonate were equilibrated with 5% $CO_2$/95% $O_2$. Bicarbonate-free solutions were gassed with 100% $O_2$, then titrated to 7.46 with 0.1 N NaOH. NMDG, *N*-methyl-D-glucamine.

* Solution 2 Cl, 1 mM $CaCl_2$ replaced 1 mM $CaGluconate_2$ in chloride-free solution. 10 Cl, 5 mM $CaCl_2$ replaced 5 mM $CaGluconate_2$ in chloride-free solution.

‡ Gluconate-containing solutions were titrated with calcium gluconate to yield a calcium activity equal to that of the control A solution.

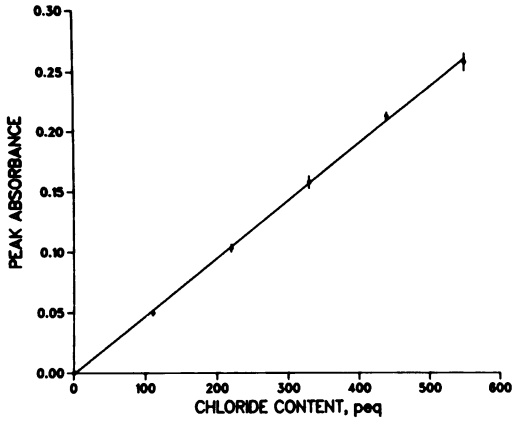


*Figure 1.* Standard curve for chloride measurements using a continuous flow ultramicrocolorimeter. Each point is the mean±SD for four determinations.

and bath with a continuous flow ultramicrocolorimeter (16). In this analytic method, chloride displaces thiocyanate from mercuric thiocyanate, forming ferric thiocyanate, which absorbs light in the region 440–540 nM (diagnostic kit 460; Sigma Chemical Co.). Two optical filters (CS 4-76, Corning Medical; and Wratten 65, Kodak Laboratory and Specialty Chemicals, Eastman Kodak Co., Rochester, NY) isolate a 50% transmission band between 470 and 535 nm. A linear standard curve (Fig. 1) was obtained for samples containing up to 550 pmol of chloride. With 6-nl samples, a concentration difference of <1 mM can be resolved at 50 mM absolute chloride concentration. The net transport rate of chloride was calculated using an equation analogous to that used for total $CO_2$.

Total $CO_2$ or chloride in collected fluid was measured immediately after the collection. In experiments in which both measurements are reported, the collected fluid sample was split as follows: The flow rate was determined with a 13-nl pipette. This sample was then redeposited into the mineral oil in the original collection pipette. Two smaller pipettes were then used to sample the larger volume for total $CO_2$ and chloride measurements.

*Statistics.* For each perfusion condition a mean value for collected total $CO_2$, chloride, inulin concentration, tubule flow rate, and/or net flux was calculated from the results of two or three measurements. When available, control and recovery period values were averaged. This average control value was compared with the experimental value using a paired *t* test. Regression analysis was carried out using the method of least squares. A *P* value of <0.05 was regarded as indicating a statistically significant difference. All results are reported as mean±1 SE.

## Results

### *Net fluid transport*

In previous studies of cortical collecting ducts, net fluid transport was negligibly low or absent without vasopressin (3, 17). We confirmed the absence of fluid transport in cortical collecting tubules from DOC-treated rabbits under three specific conditions used in this study, i.e., control (control A solution in perfusate and bath), bath chloride removal (control A solution in perfusate and chloride-free solution in bath), and sodium removal (sodium-free solution in perfusate and bath) (Table II).

### *Net total $CO_2$ transport*

*Effect of chloride removal (protocol 1).* Replacement of all chloride ($n$ = 2) or all but 2 mM chloride ($n$ = 2) with gluconate in the perfusate and bath completely inhibited net total $CO_2$ secretion without a significant change in the transepithelial potential difference (Table III, Fig. 2). The inhibition was reversible. In four additional tubules perfused and bathed with the 10-mM chloride solution, the mean total $CO_2$ flux was reduced from −7.9±2.2 to −4.8±1.1 pmol/min per mm.

*Effect of imposed transepithelial chloride gradients (protocol 2).* A recent study showed that removal of perfusate chloride (replaced with sulfate or gluconate) inhibited total $CO_2$ secretion in DOC-treated rabbits (4). These results were confirmed in four tubules perfused with the chloride-free solution; the transepithelial potential difference became more negative (−6.0±5.0 to −22.8±5.8 mV, $P < 0.05$), while bicarbonate secretion was inhibited (−9.1±1.8 to −0.0±1.6 pmol/min per mm, $P < 0.05$). The change in transepithelial potential difference was most likely due to a chloride diffusion potential through the chloride selective tight junction (18). To investigate whether bath chloride removal affects total $CO_2$ secretion, tubules were perfused with control A solution and bathed with chloride-free solution (Table III, Fig. 3). Complete bath chloride removal increased net total $CO_2$ secretion (−16.1±3.2 to −30.8±6.4 pmol/min per mm), and the transepithelial voltage changed significantly from −17.9±6.4 to +5.2±10.6 mV.

*Effect of sodium removal (protocol 3).* In eight tubules, sodium was replaced in both perfusate and bath with *N*-methyl-D-glucamine (Table IV, Fig. 4). Sodium removal significantly changed the transepithelial potential difference (−22.0±7.3 to 14.5±3.9 mV), but the rate of total $CO_2$ secretion remained unchanged (−12.7±3.2 to −14.6±3.1 pmol/min per mm).

### *Net chloride transport*

We evaluated the effect of bicarbonate removal on chloride and bicarbonate transport. The tubules were studied in solutions containing 50 mM chloride (control B solution and bicarbonate-free solution, Table I). Ouabain (0.1 mM) was added to the bath to inhibit active sodium and potassium transport, and hence, any chloride transport coupled to the active cation transport.

*Effect of bicarbonate (protocol 4).* Cortical collecting ducts studied with the control B solution in the perfusate and bath absorbed chloride (13.8±3.3 pmol/min per mm) and secreted

*Table II. Net Fluid Transport Rate in Cortical Collecting Ducts from DOC-treated Rabbits*

| Experimental condition | *n* | Length | Flow rate | $V_{te}$ | $J_v$ |
|---|---|---|---|---|---|
| | | *mm* | *nl/min per mm* | *mV* | *nl/min per mm* |
| Control A | 4 | 2.0±0.1 | 1.12±0.02 | −22.8±6.2 | −0.02±0.05 |
| Bath chloride removal | 4 | 2.0±0.1 | 1.16±0.06 | −24.4±10.9 | −0.00±0.02 |
| Sodium removal | 5 | 1.6±0.2 | 1.22±0.04 | 15.5±13.3 | −0.04±0.02 |

Values are mean±SE. $V_{te}$, transepithelial voltage. $J_v$ net fluid transport rate.

*Table III. Effect of Chloride on Total $CO_2$ Transport in Cortical Collecting Ducts from DOC-treated Rabbits*

| Protocol | n | Length | $V_{te}$ C | $V_{te}$ E | $V_{te}$ C vs. E | $TCO_2$ flux C | $TCO_2$ flux E | $TCO_2$ flux C vs. E |
|---|---|---|---|---|---|---|---|---|
| | | *mm* | *mV* | *mV* | *P* | *pmol/min per mm* | | *P* |
| Symmetric chloride removal* | 4 | 1.6±0.3 | −23.2±9.5 | −26.5±8.9 | NS | −8.6±1.8 | 0.2±0.3 | <0.05 |
| Bath chloride removal | 7 | 2.0±0.2 | −17.9±6.4 | 5.2±10.6 | <0.05 | −16.1±3.2 | −30.8±6.4 | <0.05 |

Values are mean±SE. Negative fluxes indicate net secretion. Positive fluxes indicate net absorption. Mean perfusion flow rates were 1.1 to 1.2±0.1 nl/min per mm and did not differ significantly among groups. The transepithelial voltage ($V_{te}$) was corrected for liquid junction potentials (see Methods). C, control. E, experimental. NS, not statistically significant, $P > 0.05$. $TCO_2$, total carbon dioxide. * During experimental period, perfusate and bath contained 0 or 2 mM chloride.

total $CO_2$ (−12.6±3.5 pmol/min per mm) at nearly equal rates (Table V, Figs. 5 and 6). Replacement of bicarbonate by gluconate in perfusate and bath significantly inhibited both chloride absorption (to 3.5±1.4 pmol/min per mm) and total $CO_2$ secretion (to −3.9±0.9 pmol/min per mm). Note that despite complete absence of exogenous bicarbonate and carbon dioxide from both perfusate and bath, the tubules still secreted significant amounts of total $CO_2$, which presumably was derived from metabolism (see Discussion).

### *Simultaneous total $CO_2$ and chloride transport*

Under the conditions presented above, we found bicarbonate secretion and chloride absorption were highly correlated. To further determine the quantitative relationship between the rates of total $CO_2$ and chloride transport, tubules were perfused, in five additional experiments, with bicarbonate removed either from the perfusate or the bath (protocols 5 and 6, Table VI). Bicarbonate removal from the perfusate increased both the rate of total $CO_2$ secretion and the rate of chloride absorption in three individual experiments. Bicarbonate removal from the bath decreased the rate of total $CO_2$ secretion and the rate of chloride absorption in two experiments. These data are plotted with the previous results in Fig. 6. The net total $CO_2$ flux and net chloride flux were highly correlated ($r^2 = 0.92$, $P < 0.05$). The relationship between the two fluxes, as analyzed by linear regression, is shown by the dashed line. The slope (−0.96) was not significantly different from −1.0, a consistent with 1:1 coupling of chloride and bicarbonate transport. The transepithelial voltage did not correlate significantly with the rate of bicarbonate secretion ($r^2 = 0.2$, $P > 0.05$).

## Discussion

The major conclusions from the present studies are *a*) cortical collecting ducts actively secrete bicarbonate and actively absorb chloride by 1:1 chloride/bicarbonate exchange; *b*) the overall transepithelial process is electroneutral; and *c*) bicarbonate secretion does not require sodium. The basis for these conclusions follows:

*Cortical collecting ducts actively secrete bicarbonate and actively absorb chloride.* It was shown in earlier studies (3, 8, 11, 19) and confirmed in the present ones that cortical collecting ducts can actively secrete bicarbonate. Bicarbonate secretion occurred against both chemical and electrical potential gradients since the lumen bicarbonate concentration was increased above the bath when the electrical potential gradient was lumen negative (Table III).

Active chloride absorption was also demonstrated. In the presence of ouabain, the transepithelial voltage was lumen positive and chloride was absorbed, decreasing its concentration in the lumen below that in the bath (Table V). Normally, much of the chloride absorption by cortical collecting ducts is passive, driven by the lumen-negative voltage produced by active sodium transport (17). Even under normal conditions, however, part of the chloride absorption must be active, because the chloride concentration in the lumen may become lower than can be accounted for by the transepithelial voltage (20). Active chloride absorption was also previously found in micropuncture studies of rat superficial distal tubules (21, 22) that contain cortical collecting duct cells in their terminal portions (23).

*Bicarbonate secretion requires chloride.* Replacement of chloride with gluconate in the perfusate and bath completely

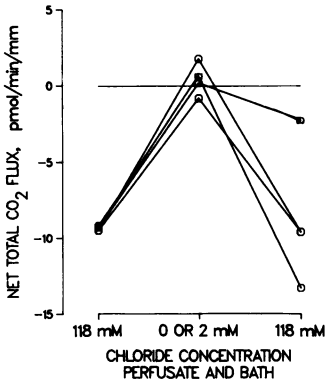


*Figure 2.* Effect of symmetric chloride replacement with gluconate on total $CO_2$ flux in cortical collecting ducts from DOC-treated rabbits. Open circles are means of two to three collections taken from a single tubule. Solid lines connect paired measurements made in individual tubules. $P < 0.05$, 0–2 mM chloride vs. 118 mM chloride by paired *t* test.

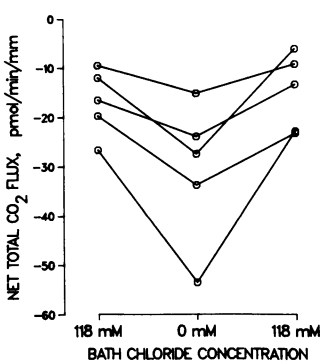


*Figure 3.* Effect of bath chloride replacement with gluconate on total $CO_2$ flux in cortical collecting ducts from DOC-treated rabbits. $P < 0.05$, 118 mM chloride vs. 0 mM chloride by paired *t* test.

*Table IV. Effect of Sodium on Total $CO_2$ Transport in Cortical Collecting Ducts from DOC-treated Rabbits*

| Protocol | n | Length | $V_{te}$ | | | $TCO_2$ flux | | |
|---|---|---|---|---|---|---|---|---|
| | | | E | C | E vs. C | E | C | E vs. C |
| | | mm | mV | mV | P | pmol/min per mm | | P |
| Symmetric sodium removal | 8 | 1.8±0.2 | 14.5±3.9 | −22.0±7.3 | <0.05 | −14.6±3.1 | −12.7±3.2 | NS |

Values are mean±SE. Negative fluxes indicate net secretion. Positive fluxes indicate net absorption. Mean perfusion flow rates were 1.1 to 1.2±0.1 nl/min per mm and did not differ significantly between groups. C, control. E, experimental. $TCO_2$, total carbon dioxide. $V_{te}$, transepithelial voltage.

and reversibly inhibited bicarbonate secretion (Table III). We interpret this inhibition as an effect of chloride removal, per se. Other possibilities can be ruled out. The transepithelial voltage, a possible driving force for bicarbonate transport, did not change significantly (Table III). A direct inhibition by the added gluconate is unlikely for a number of reasons. First, bicarbonate secretion persisted despite the presence of 118 mM gluconate when perfusate and bath contained 10 mM chloride. Second, bicarbonate secretion increased when 128 mM gluconate replaced all the chloride in the bath (Table III). Third, replacement of luminal chloride by sulfate instead of gluconate (4) also inhibited bicarbonate secretion. Fourthly, the inhibition of bicarbonate secretion by symmetric (chloride-free) gluconate solutions was reversible (Fig. 2), ruling out any irreversible toxic effect of gluconate. We conclude that bicarbonate secretion requires chloride, as in the turtle urinary bladder (9).

In previous experiments, bicarbonate secretion was not significantly inhibited by complete replacement of chloride with nitrate or methylsulfate (11) and replacement of all but 2 mM of chloride with isethionate (8). Although there were many differences in the details of the experiments, a possible explanation for the apparent disagreement of these results with ours is that the anion specificity of the chloride/bicarbonate exchanger is low and that certain anions other than gluconate anions can substitute for chloride (or bicarbonate). Certainly, there are examples of rather low selectivity in other anion transport systems. For example, sulfate and many other anions substituted for chloride on the red blood cell anion exchanger (24), sulfate substituted for chloride in bicarbonate transport by Amphiuma jejunum (10) and rabbit colon (25), and nitrate was transported by the chloride transport system in shark rectal glands (26). We have not directly investigated the anion selectivity of the chloride/bicarbonate exchanger in this study, but the information would be of interest.

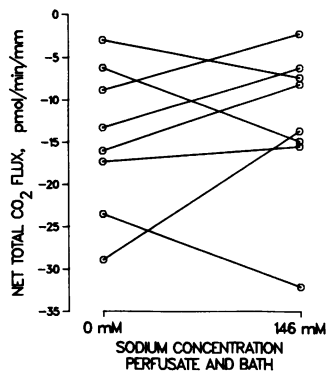


*Figure 4.* Effect of symmetric sodium replacement with *N*-methyl-D-glucamine on total $CO_2$ flux in cortical collecting ducts from DOC-treated rabbits. $P > 0.05$, 0 mM sodium vs. 146 mM sodium by paired *t* test.

*Chloride absorption requires bicarbonate.* When ouabain was present to inhibit chloride transport linked to active sodium and potassium transport, replacement of bicarbonate by gluconate in the perfusate and bath virtually completely inhibited chloride absorption (Table V). Following arguments similar to those above, we interpret this inhibition as an effect of bicarbonate removal, per se. The small amount of chloride transport that persisted can be accounted for by residual transport of bicarbonate derived from metabolic $CO_2$.[3]

Similar results have been reported in the turtle urinary bladder. In the presence of ouabain, chloride absorption required bicarbonate (27), and removal of bicarbonate from the serosal side inhibited the chloride absorption (9, 27).

*The transepithelial chloride gradient affects bicarbonate secretion.* Removal of chloride from the perfusate inhibited bicarbonate secretion (4), while removal of chloride from the bath increased bicarbonate secretion (Table III). Although the transepithelial voltage changed in these experiments (due to a chloride diffusion potential via the tight junction [18]), the bicarbonate permeability of cortical collecting duct is so low (28) that any resultant changes in passive bicarbonate transport should be too small to account for the observed effects.

*The transepithelial bicarbonate gradient affects chloride absorption.* When ouabain was present, removal of bicarbonate from the perfusate stimulated chloride absorption, while removal of bicarbonate from the bath inhibited chloride absorption (Table VI).

*The flux ratio is 1:1 in the presence of ouabain.* We deter-

3. The measured rate of total $CO_2$ secretion in the absence of exogenous bicarbonate and $CO_2$ was somewhat greater than the rate of $^{14}C$ carbon dioxide production (0.6 pmol/min per mm) previously measured directly in rat cortical collecting ducts (36). It was also somewhat higher than the rate of $CO_2$ production inferred from other measurements such as oxygen consumption of rabbit cortical tubule suspensions in the presence of ouabain (2.5 pmol/min per mm) (37) and the antimycin A-stimulated lactate production of cortical collecting ducts (0.6 pmol/min per mm) (38). In the present study, however, the collecting ducts were perfused, which presumably increased the rate of transport and the metabolism necessary to support the transport. Because these numbers are of the same order of magnitude as the rate of secretion of bicarbonate by the cortical collecting ducts in bicarbonate-free solutions, we believe that metabolism is a plausible source of the secreted bicarbonate. Along the same lines, enough $CO_2$ was apparently produced by metabolism of turtle bladder cells in $CO_2$-free solutions to sustain acidification that otherwise would have required exogenous $CO_2$ (39).

*Table V. Total $CO_2$ and Chloride Transport in Cortical Collecting Ducts from DOC-treated Rabbits*

| Protocol | n | Length | $V_{te}$ C | $V_{te}$ E | $V_{te}$ C vs. E | $TCO_2$ flux C | $TCO_2$ flux E | $TCO_2$ flux C vs. E | Chloride flux C | Chloride flux E | Chloride flux C vs. E |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *mm* | *mV* | *mV* | *P* | *pmol/min per mm* | | *P* | *pmol/min per mm* | | *P* |
| Symmetric bicarbonate removal | 7 | 1.9±0.2 | 10.5±3.5 | 5.4±2.4 | NS | −12.6±3.5 | −3.9±0.9 | <0.05 | 13.8±3.3 | 3.5±1.4 | <0.05 |

All values are mean±SE. All solutions contained 50 mM chloride. All baths contained 0.1 mM ouabain. Mean perfusion rates were 1.1–1.2 nl/min per mm and did not differ significantly between groups. C, control. E, experimental. $TCO_2$, total carbon dioxide. $V_{te}$, transepithelial voltage.

mined the relationship between chloride and bicarbonate fluxes in the presence of ouabain (Fig. 6). Under these conditions, net sodium, potassium, and sodium-coupled chloride fluxes were eliminated. Bicarbonate secretion and chloride absorption were highly correlated with a slope of ~ −1.0. In this open-circuited state, net transepithelial transfer of charge must be zero. Because a 1:1 coupling ratio completely satisfies this requirement, it is unnecessary to assume that any other ion is involved in the anion exchange process. From this correlation alone, we cannot be certain that the coupling is direct via an exchanger. The lack of any consistent relation between bicarbonate and chloride transport and the transepithelial voltage (see below), however, suggests to us that the coupling is likely to be direct.

*The overall transepithelial process is electroneutral.* We did not find any consistent relation between the rate of bicarbonate secretion and the transepithelial voltage. Removal of chloride from the perfusate and bath inhibited bicarbonate secretion without any change in the transepithelial voltage (Table III). Also, removal of sodium from the perfusate and bath (Table IV), addition of ouabain to the bath (3, 8, 11), or removal of potassium from the bath (3) inhibited the lumen-negative voltage without significantly affecting the rate of bicarbonate secretion. Finally, comparing all of the individual collections under various conditions, there was no significant relationship between bicarbonate transport rate and transepithelial voltage (results not shown). We conclude that under the conditions of our study, bicarbonate secretion is electroneutral, that is, does not generate a transepithelial voltage. Similarly, in the untreated turtle urinary bladder, bicarbonate secretion is electroneutral (9), although an electrogenic mechanism was induced by treatments that increased intracellular cyclic AMP (29, 30).

*Bicarbonate secretion does not require sodium.* It was shown in previous studies (3, 8, 11) and confirmed in the present ones that ouabain, an inhibitor of the Na,K-ATPase, does not prevent bicarbonate secretion by cortical collecting ducts. Therefore, sodium gradients generated by the Na,K-ATPase cannot be the energy source for the active bicarbonate transport. By the same reasoning the Na,K-ATPase cannot drive the active chloride transport. Although we are not certain what primary active transport is ultimately responsible for active bicarbonate and chloride transport, we believe it likely to be a proton ATPase, as recently proposed for the turtle urinary bladder (31).

We tested for dependence on sodium other than through the Na,K-ATPase by measuring bicarbonate transport in the absence of sodium. Bicarbonate secretion continued in the absence of sodium in the perfusate and bath (Table II). The measured sodium concentration was <0.2 mM, which is well below the Michaelis constant reported for sodium in other sodium-dependent transport processes such as a basolateral sodium/hydrogen exchanger in rabbit cortical collecting duct (~15 mM) (32), bicarbonate secretion in Amphiuma intestine (5 mM) (10), or sodium chloride absorption in rabbit thick ascending limb (3.6 mM) (33). Also, the sodium concentration was low enough to eliminate the lumen-negative transepithelial voltage associated with active sodium absorption.

In previous studies, replacement of sodium with a different cation, choline, inhibited bicarbonate secretion in rabbit cortical collecting ducts (11). In view of the lack of effect of sodium replacement with *N*-methyl-D-glucamine in the present study, we speculate that choline could have inhibited net bicarbonate secretion. This is supported by the observation in the turtle urinary bladder that choline inhibited net bicarbonate secretion (34).

*Summary.* These results provide evidence that cortical collecting ducts actively secrete bicarbonate and actively absorb chloride by a process involving direct chloride/bicarbonate ex-

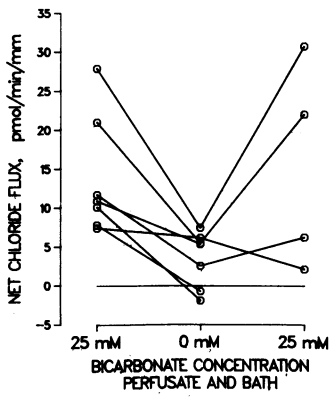


*Figure 5.* Effect of symmetric bicarbonate replacement with gluconate on net chloride flux in cortical collecting ducts from DOC-treated rabbits. Ouabain (0.1 mM) was present in bath. $P < 0.05$, 25 mM bicarbonate vs. 0 mM bicarbonate, by paired *t* test.

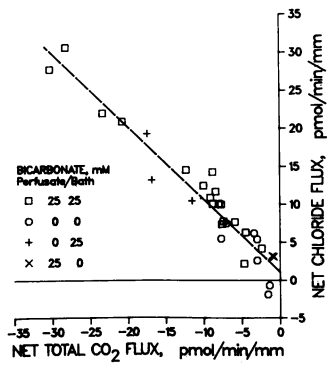


*Figure 6.* Relationship between net total $CO_2$ flux and net chloride flux under control, bicarbonate-free, and bicarbonate gradient conditions. Ouabain (0.1 mM) was present in bath. Correlation was highly significant ($r^2 = 0.92$, $P < 0.05$). Dashed line obtained by regression analysis of all four conditions has slope of −0.96.

*Table VI. Effect of Bicarbonate Gradients on Chloride Flux and Total $CO_2$ Flux in Cortical Collecting Ducts*

| Protocol | Length | $V_{te}$ | | $TCO_2$ flux | | Chloride flux | |
|---|---|---|---|---|---|---|---|
| | | C | E | C | E | C | E |
| | *mm* | *mV* | *mV* | *pmol/min per mm* | | *pmol/min per mm* | |
| 5 | 2.4 | 11.0 | 4.2 | −5.9 | −11.5 | 7.7 | 10.4 |
| | 1.4 | 5.5 | 5.5 | −5.6 | −16.8 | 7.1 | 13.3 |
| | 1.7 | 6.3 | 9.0 | −8.2 | −17.4 | 12.1 | 19.4 |
| Mean | 1.8 | 7.6 | 6.2 | −6.6 | −15.2 | 9.0 | 14.4 |
| 6 | 2.4 | 11.2 | 12.8 | −8.0 | −.8 | 10.1 | 3.1 |
| | 1.7 | 4.0 | 2.6 | −10.0 | −1.0 | 12.3 | 3.2 |
| Mean | 2.1 | 7.4 | 7.7 | −9.0 | −0.9 | 11.1 | 3.2 |

All values are mean±SE. All solutions contained 50 mM chloride. All baths contained 0.1 mM ouabain. Mean perfusion rates were 1.1–1.2 nl/min per mm and did not differ significantly among groups. C, control. E, experimental. $TCO_2$, total carbon dioxide. $V_{te}$, transepithelial voltage.

change.[4] The evidence for direct coupling of chloride and bicarbonate fluxes is that bicarbonate secretion required the presence of chloride (Table III), that chloride absorption required the presence of bicarbonate (Table V), and that the transport of bicarbonate and chloride each was affected by transepithelial gradients of the other (Tables III and VI). The lack of any consistent relation between anion fluxes and the transepithelial voltage rules out the possibility that the two fluxes are coupled via the transepithelial voltage, as has been proposed for chloride and proton secretion in the outer medullary collecting duct (35). The 1:1 stoichiometry in the absence of net sodium and potassium transport provides evidence that no other ions are involved in the electroneutral exchange process. The bicarbonate secretion and chloride absorption apparently are linked by direct chloride/bicarbonate exchange.

## References


1. McKinney, T. D., and M. B. Burg. 1977. Bicarbonate transport by rabbit cortical collecting tubules. *J. Clin. Invest.* 60:766–768.

2. Atkins, J. L., and M. B. Burg. 1983. Secretion and absorption of bicarbonate by rat collecting ducts. *Clin. Res.* 31:423*a*. (Abstr.)

3. Knepper, M. A., D. W. Good, and M. B. Burg. 1984. Mechanism of ammonia secretion by cortical collecting ducts of rabbits. *Am. J. Physiol.* 247:729–738.

4. Garcia-Austt, J., D. W. Good, M. B. Burg, and M. A. Knepper. 1985. Deoxycorticosterone-stimulated bicarbonate secretion in rabbit cortical collecting ducts: effects of luminal chloride removal and in vivo acid loading. *Am. J. Physiol.* 249:F205–F212.

5. Schwartz, G. J., and Q. Al-Awqati. 1985. Two functionally distinct types of mitochondria-rich cells in cortical collecting tubule as determined by changes in cell $pH_i$ in individually identified cells. *Kidney Int.* 27: 288. (Abstr.)

6. Knepper, M. A., D. W. Good, J. Garcia-Austt, and M. B. Burg. 1984. Deoxycorticosterone-stimulated bicarbonate secretion in cortical collecting ducts from rabbits and rats. *Kidney Int.* 25:278. (Abstr.)

7. Boyer, J., and M. Burg. 1981. Bicarbonate secretion in isolated perfused rabbit cortical collecting ducts. *Kidney Int.* 29:233 (Abstr.)

8. Laski, M. E., D. G. Warnock, and F. C. Rector, Jr. 1983. Effects of chloride gradients on total $CO_2$ flux in the rabbit cortical collecting tubule. *Am. J. Physiol.* 244:F112–F121.

9. Leslie, B. R., J. H. Schwartz, and P. R. Steinmetz. 1973. Coupling between $Cl^-$ absorption and $HCO_3^-$ secretion in turtle urinary bladder. *Am. J. Physiol.* 225:610–617.

10. White, J. F., and M. A. Imon. 1982. Intestinal $HCO_3^-$ secretion in Amphiuma: stimulation by mucosal $Cl^-$ and serosal $Na^+$. *J. Membr. Biol.* 68:207–214.

11. McKinney, T. D., and M. B. Burg. 1978. Bicarbonate secretion by rabbit cortical collecting tubules in vitro. *J. Clin. Invest.* 61:1421–1427.

12. Burg, M. B. 1972. Perfusion of isolated renal tubules. *Yale J. Biol. Med.* 45:321–326.

13. Tomita, K., J. J. Pisano, and M. A. Knepper. 1985. Control of $Na^+$ and $K^+$ transport in the cortical collecting duct of the rat: effects of bradykinin, vasopressin, and deoxycorticosterone. *J. Clin. Invest.* 76: 132–136.

14. Burg, M. B., and N. Green. 1973. Function of the thick ascending limb of Henle's loop. *Am. J. Physiol.* 224:659–668.

15. Vurek, G., D. Warnock, and R. Corsey. 1975. Measurement of picomole amounts of carbon dioxide by calorimetry. *Anal. Chem.* 47: 765–767.

16. Vurek, G. G. 1981. Flow-through nanocolorimeter for measurement of picomole amounts of magnesium and phosphate. *Anal. Lett.* 14:261–269.

17. Stoner, L. C., M. B. Burg, and J. Orloff. 1974. Ion transport in cortical collecting tubule; effect of amiloride. *Am. J. Physiol.* 227:453–459.

18. Sansom, S. C., E. J. Weinman, and R. G. O'Neil. 1984. Microelectrode assessment of chloride-conductive properties of cortical collecting duct. *Am. J. Physiol.* 247:F291–F302.

19. Lombard, W. E., J. P. Kokko, and H. R. Jacobson. 1983. Bicarbonate transport in cortical and outer medullary collecting tubules. *Am. J. Physiol.* 244:F289–F296.

20. Hanley, M. J., J. P. Kokko, J. B. Gross, and H. R. Jacobson. 1980. Electrophysiologic study of the cortical collecting tubule of the rabbit. *Kidney Int.* 17:74–81.

21. Rector, F. C., and J. R. Clapp. 1962. Evidence for active chloride reabsorption in the distal renal tubule of the rat. *J. Clin. Invest.* 41:101–107.

22. Velazquez, H., D. W. Good, and F. S. Wright. 1984. Mutual dependence of sodium and chloride absorption by renal distal tubule. *Am. J. Physiol.* 247:F904–F911.

23. Woodhall, P. B., and C. C. Tisher. 1973. Response of the distal tubule and cortical collecting duct to vasopressin in the rat. *J. Clin. Invest.* 52:3095–3108.

24. Knauf, P. A. 1979. Erythrocyte anion exchange and the band 3 protein: transport kinetics and molecular structure. *Curr. Top. Membr. Transp.* 12:249–363.

25. Sullivan, S. K., and P. L. Smith. 1984. Bicarbonate (hydrogen) transport by rabbit descending colon. *Fed. Proc.* 43:1085. (Abstr.)

26. Silva, P., M. Myers, and F. H. Epstein. 1984. Efficiency of anion cotransport by shark rectal gland. *Clin. Res.* 32:565*a*. (Abstr.)

27. Husted, R. F., L. H. Cohen, and P. R. Steinmetz, 1979. Pathways


4. We did not attempt to determine whether the exchanger is in the apical or basolateral membrane. An apical location has been proposed for the exchanger responsible for bicarbonate secretion in rabbit cortical collecting duct (5) and turtle urinary bladder (31). This localization is supported by our observation of equal rates of net bicarbonate secretion and net chloride absorption in the absence of exogenous bicarbonate and $CO_2$ (Table V). The source of the secreted total $CO_2$ was likely to be cellular metabolism, as discussed above. Because the rates of total $CO_2$ secretion into the lumen and chloride absorption from the lumen remained equal while the only source of bicarbonate was $CO_2$ from within the cells, we infer that direct chloride/bicarbonate exchange occurred at the apical membrane.

for bicarbonate transfer across the serosal membrane of turtle urinary bladder: studies with a disulfonic stilbene. *J. Membr. Biol.* 47:27–37.

28. Schuster, V. L. 1985. Cyclic-AMP stimulated bicarbonate secretion in rabbit cortical collecting tubules. *J. Clin. Invest.* 75:2056–2064.

29. Satake, N., J. H. Durham, G. Ehrenspeck, and W. A. Brodsky. 1983. Active electrogenic mechanisms for alkali and acid transport in turtle bladders. *Am. J. Physiol.* 244:C259–C269.

30. Durham, J. H., and C. Matons. 1984. Chloride-induced increment in short-circuiting current of the turtle bladder: effects of in-vivo acid-base state. *Biochim. Biophys. Acta.* 769:287–310.

31. Palmisano, J., D. L. Stetson, R. Beauwens, P. Mitchell, and P. R. Steinmetz. 1985. Modification of urinary bicarbonate secretion by cyclic AMP and 9-anthroic acid. *Kidney Int.* 27:286. (Abstr.)

32. Chaillet, J. R., and W. F. Boron. 1984. Basolateral Na-H exchange in the rabbit cortical collecting tubule. *Fed. Proc.* 43:1089. (Abstr.)

33. Greger, R. 1981. Chloride reabsorption in the rabbit cortical thick ascending limb of the loop of Henle. A sodium dependent process. *Pfluegers Arch.* 390:38–43.

34. Arruda, J. A., G. Dytko, R. Mola, and N. A. Kurtzman. 1980. On the mechanism of lithium-induced renal tubular acidosis: studies in the turtle bladder. *Kidney Int.* 17:196–204.

35. Jacobson, H. L. 1984. Medullary collecting duct acidification. Effects of potassium, $HCO_3$ concentration, and $pCO_2$. *J. Clin. Invest.* 74:2107–2114.

36. Le Bouffant, F., A. Hus-Citharel, and F. Morel. 1984. Metabolic $CO_2$ production by isolated single pieces of rat distal nephron segments. *Pfluegers Arch.* 401:346–353.

37. Balaban, R. S., L. J. Mandel, S. P. Soltoff, and J. M. Storey. 1980. Coupling of active ion transport and aerobic respiratory rate in isolated renal tubules. *Proc. Natl. Acad. Sci. USA* 77:447–451.

38. Bagnasco, S., D. Good, R. Balaban, and M. Burg. 1985. Lactate production in isolated segments of the rat nephron. *Am. J. Physiol.* 248. 248:F522–F526.

39. Schwartz, J. H., and P. R. Steinmetz. 1971. $CO_2$ requirements for $H^+$ secretion by the isolated turtle bladder. *Am. J. Physiol.* 220:2051–2057.