The gastrointestinal tract (GIT) of the domesticated horse (*Equus caballus*) is a voluminous organ that allows the horse to obtain nutrients from a high-fiber, low-energy diet and still perform as a highly athletic domesticated animal. With that complexity comes a remarkable predisposition and sensitivity to GIT disturbances, ironically attributed to some drugs intended to preserve athletic potential. As a species with hindgut fermentation, the equine large colon is the site of microbial digestion of plant fiber, which produces large quantities of short-chain fatty acids (SCFAs). Neutralization of colon contents is critical to the normal digestive process in equids, and the consequences of failed neutralization are severely altered microbial fermentation and mucosal surface damage.

Three distinct mechanisms of HCO$_3^-$ secretion previously described in a murine model were confirmed in the equine colon. The RDC had a greater capacity for electrogenic, Cl$^-$-independent HCO$_3^-$ secretion than the RVC ($P = 0.04$). In the RDC, all HCO$_3^-$ secretion was decreased by PBZ ($P < 0.02$) but was not studied in the RVC because of low baseline secretion.

Secretion of HCO$_3^-$ by the RDC could play a pivotal role in equine colon physiology, because intense microbial fermentation in this site could require HCO$_3^-$ secretion to buffer short-chain fatty acids. Inhibition of this secretion by PBZ could interfere with mucosal buffering and predispose to changes associated with right dorsal colitis.

#### OBJECTIVE
To examine bicarbonate (HCO$_3^-$) secretion ex vivo in the equine large colon to determine any differences between the right dorsal colon (RDC) and right ventral colon (RVC). The effect of phenylbutazone (PBZ) on HCO$_3^-$ secretion was examined in the RDC.

#### ANIMALS
14 healthy horses.

#### PROCEDURES
In anesthetized horses ($n = 10$), segments of mucosa from RDC and RVC were harvested to measure HCO$_3^-$ secretion ex vivo with the pH Stat method. The effect of PBZ on HCO$_3^-$ secretion in the RDC was studied in 4 additional horses.

#### RESULTS
Three distinct mechanisms of HCO$_3^-$ secretion previously described in a murine model were confirmed in the equine colon. The RDC had a greater capacity for electrogenic, Cl$^-$-independent HCO$_3^-$ secretion than the RVC ($P = 0.04$). In the RDC, all HCO$_3^-$ secretion was decreased by PBZ ($P < 0.02$) but was not studied in the RVC because of low baseline secretion.

#### CLINICAL RELEVANCE
Secretion of HCO$_3^-$ by the RDC could play a pivotal role in equine colon physiology, because intense microbial fermentation in this site could require HCO$_3^-$ secretion to buffer short-chain fatty acids. Inhibition of this secretion by PBZ could interfere with mucosal buffering and predispose to changes associated with right dorsal colitis.
However, its association with NSAIDs such as PBZ suggests that suppression of prostaglandin production could be a key element. Regional differences in \( \text{HCO}_3^- \) secretion could explain why certain portions of the colon are more susceptible to localized hindgut acidosis.

Much of our current understanding of important physiological roles in the equine colon has been extrapolated from research in laboratory animals. The secretion of \( \text{HCO}_3^- \) is notoriously difficult to quantitate, so this anion is largely understudied. The \( \text{pH Stat} \) system is an indirect measurement of \( \text{HCO}_3^- \) secretion ex vivo that operates continuously through a servomechanism that delivers the necessary volume of sulfuric acid (\( \text{H}_2\text{SO}_4 \)) to titrate the mucosal solution pH to a selected value (pH 7.0). Stat refers to the principle that the pH remains around the selected pH value throughout the measurement period, which is achieved by acid infusion into the bathing medium to offset the pH change induced by \( \text{HCO}_3^- \) secretion. The volume of acid required is regarded as equivalent to the amount of \( \text{HCO}_3^- \) secreted by the tissue for the time period studied, under conditions that can enhance or reduce \( \text{HCO}_3^- \) secretion.

Previous \( \text{pH Stat} \) studies have identified three distinct mechanisms of \( \text{HCO}_3^- \) secretion in the rat distal colon:

1. A chloride (\( \text{Cl}^- \))-dependent \( \text{HCO}_3^- \) secretion mediated by apical membrane \( \text{Cl}^-/\text{HCO}_3^- \) anion exchanger;
2. SCFA-dependent \( \text{HCO}_3^- \) secretion mediated by an apical membrane SCFA-/\( \text{HCO}_3^- \) exchanger of unestablished molecular identity;
3. cAMP-stimulated \( \text{HCO}_3^- \) secretion mediated by apical membrane cystic fibrosis transmembrane conductance regulator (CFTR) (Figure 2). The term "electrogenic" is used to describe the secretion of \( \text{HCO}_3^- \) in the third mechanism, in the absence of luminal \( \text{Cl}^- \), SCFA, or other ions capable of electroneutral exchange. The purpose of this study was to determine if the previously described systems of \( \text{HCO}_3^- \) secretion in rat colon exist in the equine colon and to determine if there is a difference in \( \text{HCO}_3^- \) secretion between the RDC and right ventral colon (RVC).

**Figure 1**—Equine large intestines, labeled from oral to aboral, cecum (A), right ventral colon (RVC) (B), left ventral colon (C), left dorsal colon (D), right dorsal colon (RDC) (E), and transverse colon (F). Orange squares indicate where tissue segments were harvested at surgery for ex vivo experiments.
The pH Stat technique used to measure HCO$_3^-$ secretion in this study does not provide measurements of electrical current and conductance, the mucosal properties typically studied in Ussing chambers. Previous work by our group$^8$ has demonstrated that NSAIDs (PBZ and indomethacin) decrease short circuit current in the equine RDC, possibly by suppressing the high prostaglandin tone that predominates in this tissue after it is dissected and mounted in the chambers.$^{22}$

The hypothesis of this study was predicated on the premise that the RDC has different and more active mechanisms for HCO$_3^-$ secretion than in the RVC. This feature of colonic physiology could render the RDC prone to some of the more devastating colonic diseases, especially if these diseases alter HCO$_3^-$ secretion. If HCO$_3^-$ secretion involves a CAMP-mediated anion channel that is stimulated by proinflammatory mediators, such as prostaglandins,$^8$ this and other systems for HCO$_3^-$ secretion could be altered by inflammation.$^{24,25}$ If so, recovery of the RDC from inflammatory diseases could require manipulation of HCO$_3^-$ secretion to overcome loss of this anion or to preserve its secretion as a protective mechanism against acid-induced mucosal injury.$^{25}$

Materials and Methods

Horses

This study was approved by the Institutional Animal Care and Use Committee of the University of Florida. Fourteen, clinically normal, adult horses were included, based on history from caretakers and owners of no GIT disorders within 6 months and no history of NSAID administration within 3 weeks of donation. Horses were of various breeds, including Thoroughbred ($n = 6$), American Quarter Horse (4), Tennessee Walking Horse (2), Dutch Warmblood (1), and Arabian (1), were included in the study. Median age of horses was 7 years (range, 2 to 25 years), and median body weight was 485 kg (range, 369 to 630 kg). Seven mares and 7 geldings were included in the study. For 48 hours before the start of the study, horses were kept on pasture, were fed grass hay (2% of body weight/day), and had unlimited access to water. An intravenous 14-gauge, 13.3-cm Teflon catheter was inserted aseptically into the right jugular vein for administration of anesthetic drugs, immediately before induction of general anesthesia.$^{28}$

General anesthesia

Horses were sedated with xylazine hydrochloride (0.5 mg/kg, IV) and butorphanol tartrate (0.02 mg/kg, IV). Anesthesia was induced with diazepam (0.1 mg/kg, IV) and ketamine hydrochloride (2.2 mg/kg, IV). The trachea of each horse was intubated. Horses were positioned in left lateral recumbency, and anesthesia was maintained with isoflurane (1% to 3%) in oxygen. Heart rate, respiratory rate, fraction of inspired oxygen, and inspiratory isoflurane concentrations were monitored continuously. Horses were euthanatized (see below) at the end of anesthesia and after tissue collection (approx 30 minutes after induction).

Intestinal preparation

Anesthetized horses in left lateral recumbency were prepared for an aseptic right flank celiotomy. The RDC and RVC were accessed through this incision and partly exteriorized.$^8$ Full-thickness sections of mucosa 5 X 15 cm were taken from both the RDC and RVC$^{23,26}$ (Figure 1) and processed as described below. After the tissues were harvested, the horses were euthanatized with an overdose of pentobarbital sodium with phenytoin (88 mg/kg, IV). Each tissue segment was immediately submerged in plain Kreb’s Ringer buffer solution at 37°C after harvesting and kept in a warming bath during transport (< 25 minutes).$^{27,28}$ In the laboratory, mucosal sheets were harvested by dissection through the submucosal plane and mounted in Ussing chambers (P2304; Physiologic Instruments, San Diego, CA),$^{15}$ with a circular aperture of 1.26 cm$^2$.

pH Stat recordings

HCO$_3^-$ secretion was quantified using Bi-burette TIM 856 (Radiometer Analytical S.A., Villeurbanne, France) that titrates both above and below a Stat pH 7.0, as previously described.$^{29,30}$ Briefly, luminal solution pH was continuously maintained at a constant (or stat) pH by the addition of 0.05 M H$_2$SO$_4$, PHC 4000 pH Electrode Combs (Hach, Loveland, CO) were used to continuously monitor pH in real time. Pumps were programmed to operate in real time to the changes in luminal pH by delivering $\geq 0.005 \mu$L at a given interval. The pH electrode and H$_2$SO$_4$ pipette were placed in the mucosal chamber. The acid used for the titration was diluted in the same ionic solution as used in that particular experiment to give a final concentration of 0.05 M. Based on this methodology, HCO$_3^-$ secretion is equivalent to the amount of acid required to maintain pH at 7.0. All experiments were performed in an open circuit. HCO$_3^-$-free solutions were gassed with 100% O$_2$, while HCO$_3^-$-containing solutions were gassed with 95% O$_2$ and 5% CO$_2$. HCO$_3^-$ secretion was expressed relative to time and mucosal surface area as $\mu$Eq-cm$^{-2}$·h$^{-1}$. Inhibitors were added to the mucosal solution during the initial steady-state period, and pH was adjusted and allowed to equilibrate for 5 to 10 minutes until a steady rate of HCO$_3^-$ secretion was again observed. Different solutions (Table 1) and additives were used in the experiments (Table 2).

Inhibition and stimulation studies

Experiments were performed in sequence to measure HCO$_3^-$ secretion under various conditions. Three general protocols were followed to determine the basic physiological mechanisms of HCO$_3^-$ secretion in the equine colon, as outlined in Figure 3. Horses were not randomly assigned to each protocol because each protocol provided information needed for subsequent protocols. Instead, a sequence was followed to assess basic mechanisms and intensity
of HCO₃⁻ secretion in RDC versus RVC (protocol 1), to further assess secretion in RDC (protocol 2), and then to assess the response to PBZ (protocol 3).

**Protocol 1**

This protocol examined the basic mechanisms of HCO₃⁻ secretion in RVC and RDC in the absence of luminal SCFA to eliminate anion exchange and then to assess anion channel blockers (n = 5 horses). The pH Stat equipment allowed for one sample of RDC and one sample of RVC to be tested per horse so duplication was not possible. Tissues were mounted in Ussing chambers with the mucosal and serosal chambers containing the HCO₃⁻ and Cl⁻-free solutions (HCO₃⁻ [-], Cl⁻ [-]), and gassed with 100% oxygen. Luminal pH was continuously monitored until steady state was achieved and therefore endogenous secretion of HCO₃⁻ was expended. The HCO₃⁻-free solution on the serosal surface of the chamber was then exchanged for a HCO₃⁻-containing Ringer solution to produce baseline or control secretion. Baseline HCO₃⁻ secretion is representative of the electrogenic HCO₃⁻ secretion produced by the tissues gassed with 95% O₂ and 5% CO₂. After 30 minutes of steady-state secretion, inhibitors were added to the mucosal chamber (Figure 3). In the initial experiment, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), a nonspecific anion channel blocker, was added to the luminal surface. In subsequent experiments, more specific anion channel blockers such as CFTR inhibitor-172 (CF-172) and calcium activated Cl⁺ channel inhibitor A01 (CaCCinh-A01) (Table 2) were used to further characterize the electrogenic HCO₃⁻ secretion. Next, the HCO₃⁻-free, Cl⁻-free unbuffered solution on the mucosal surface of the chamber was replaced with a HCO₃⁻-free, Cl⁻-containing, unbuffered solution. Any HCO₃⁻ secretion under these conditions was attributed to an electroneutral anion exchange (AE) mechanism. Finally, the AE inhibitor 4,4'-disothiocyanato-stilbene-2,2'-disulfonic acid (DIDs) was added to the luminal surface to inhibit the electroneutral exchange (Figure 3 and Table 2).

**Protocol 2**

Mechanisms of HCO₃⁻ secretion in the RVC and RDC were examined in the presence of luminal SCFA and luminal Cl⁻ to determine the role of anion exchangers (n = 5 horses). The pH Stat equipment allowed for one sample of RDC and one sample of RVC to be tested per horse so duplication was not possible.

<table>
<thead>
<tr>
<th>Table 1—Composition of solutions.</th>
<th>Basolateral</th>
<th>Luminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constituent</td>
<td>HCO₃⁻ (-), Cl⁻ (-)</td>
<td>HCO₃⁻ (-), Cl⁻ (-)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>119.8</td>
<td>119.8</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Cyclamide</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Isethionate</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>SCFA (+), Cl⁻ (+)</td>
<td>140</td>
<td>140</td>
</tr>
</tbody>
</table>

Compositions of constituents of all solutions are given in mM; pH of all Cl⁻-containing solutions were adjusted to 7.0 with H₂SO₄. HCO₃⁻-containing solutions were gassed with 95% O₂ and 5% CO₂; (-) indicates solution does not contain the listed anions; (+) indicates solution does contain the listed anions; HCO₃⁻ (-) solutions were gassed with 100% O₂. Both lumens and bath solutions also contained 5.2 K⁺, 1.2 Ca²⁺, and 1.2 Mg²⁺. All lumens solutions were HCO₃⁻ free and contained 0.1 mM HEPES (pH 7.0).

<p>| Table 2—Additives used in Ussing chambers studies |</p>
<table>
<thead>
<tr>
<th>Full name</th>
<th>Abbreviation</th>
<th>Use</th>
<th>Chamber</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺-activated Cl⁻ channel inhibitor A01</td>
<td>CaCCinh-A01</td>
<td>Specific inhibitor of the calcium activated chloride channels located on the apical surface of mucosal cells</td>
<td>Luminal</td>
<td>10 μM</td>
</tr>
<tr>
<td>CFTR inhibitor-172</td>
<td>CF-172</td>
<td>Specific inhibitor of the CFTR channels located on the apical surface of mucosal cells</td>
<td>Luminal</td>
<td>10 μM</td>
</tr>
<tr>
<td>4,4'-Disothiocyanato-stilbene-2,2'-disulfonic acid</td>
<td>DIDs</td>
<td>AE inhibitor that specifically inhibits the electroneutral secretion of HCO₃⁻ through apical HCO₃⁻/Cl⁻ exchangers such as SLC26A3</td>
<td>Luminal</td>
<td>100 μM</td>
</tr>
<tr>
<td>5-Nitro-2-(3-phenylpropylamino)benzoic acid</td>
<td>NPPB</td>
<td>Nonspecific inhibitor of apical chloride/HCO₃⁻ channels</td>
<td>Luminal</td>
<td>100 μM</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>PBZ</td>
<td>NSAID (nonspecific cyclo-oxygenase inhibitor)</td>
<td>Basolateral and luminal</td>
<td>1 μg/ml</td>
</tr>
</tbody>
</table>

Different additives used in the Ussing chambers studies for protocols 1-3.

AJVR
Figure 3—A—Flow chart for protocol 1 (mechanisms of HCO$_3^-$ secretion in the absence of luminal SCFAs in the inhibition and stimulation studies). Solutions free of the designated anion are indicated by (−) and for anion inclusion by (+). After each change in either serosal or mucosal solution, or the addition of any inhibitor, luminal pH was continuously monitored until steady state was achieved. HCO$_3^-$ secretion was then determined using pH Stat methodology for approximately 30 minutes. Times of addition are approximate because they varied between horses according to different rates of achieving steady state. B—Flow chart for protocol 2 (mechanisms of HCO$_3^-$ secretion in the presence of the luminal SCFA isobutyrate). After each change in either serosal or mucosal solution, or the addition of any inhibitor, luminal pH was continuously monitored until steady state was achieved. HCO$_3^-$ secretion was then determined using pH Stat methodology for approximately 30 minutes. Times of addition are approximate because they varied between horses according to different rates of achieving steady state. C—Flow chart for protocol 3 (HCO$_3^-$ secretion in the presence of PBZ). After each change in either serosal or mucosal solution, luminal pH was continuously monitored until steady-state was achieved. HCO$_3^-$ secretion was then determined using pH Stat methodology for approximately 30 minutes. Times of addition are approximate because they varied between horses according to different rates of achieving steady state.

Solutions containing Cl$^-$ and the SCFA isobutyrate were added sequentially to the luminal solution after baseline (control) HCO$_3^-$ secretion was determined. The nonspecific anion channel inhibitor NPPB was added to the luminal surface in the final step of the experiment at the concentration described to inhibit electrogenic HCO$_3^-$ secretion (Figure 3 and Table 2). Any HCO$_3^-$ secretion under these conditions was attributed to an electroneutral anion exchange mechanism.

Protocol 3

Effect of PBZ on HCO$_3^-$ secretion was examined in the absence of luminal SCFA in RDC only (n = 4 horses), with two samples of RDC to be tested per horse. The initial steps were similar to protocols 1 and 2, but RVC was excluded because baseline endogenous HCO$_3^-$ secretion was considered too low to detect inhibition. Endogenous HCO$_3^-$ secretion was expended and then a HCO$_3^-$-containing Ringer solution was added to the basolateral chamber and gassed with 5% CO$_2$ and 95% O$_2$. The pH Stat methodology was used to measure possible. Baseline HCO$_3^-$ secretion was determined using the same sequence as described in protocol 1. Baseline HCO$_3^-$ secretion was considered representative of electrogenic HCO$_3^-$ secretion by the tissues.
baseline (electrogenic) HCO$_3^-$ secretion, as previously described. The HCO$_3^-$–free, Cl$^-$–free unbuffered solution on the mucosal side of the chamber was then replaced with a HCO$_3^-$–free, Cl$^-$–containing, unbuffered solution. PBZ was then added to the luminal and apical surface (3.24 X 10$^{-6}$ M [1 μg/ml]) (Figure 3 and Table 2) and HCO$_3^-$ secretion measured.

**Statistical methods**

For HCO$_3^-$ secretion in protocols 1 and 2, tissues from each horse yielded one set of observations for the RDC, and one set of data for the RVC. Data are expressed as medians. A statistical software program was used for analysis (SPSS, IBM, Armonk, NY). Shapiro-Wilks tests for normality were performed on all data sets for both the RDC and RVC and demonstrated that data was not normally distributed. Therefore, nonparametric statistical analyses were performed, with a significance level set at $P < 0.05$. Wilcoxon signed rank test was used to compare HCO$_3^-$ secretion between the RDC and RVC. To compare the effect of anion transport inhibitors and different luminal solutions on HCO$_3^-$ secretion, a Kruskal-Wallis analysis was performed, with pairwise comparison between each successive step in the experiment. Protocols 1, 2, and 3 were analyzed separately, and $P < 0.05$ was considered significant. The number of subjects (horses) chosen was based on previous studies$^{8,20,28}$ using similar techniques by the same investigators with a power value of 0.80, as determined by software on www.statistics.com/content/javastat.html#Power. A mean difference of 1.5 μEq·cm$^{-2}$·h$^{-1}$ of HCO$_3^-$ secretion between the two segments of colon, with a margin of error of 0.8 μEq·cm$^{-2}$·h$^{-1}$, could be detected with this sample size. In protocols 1 and 2, 5 horses were used in each because the Stat pH system could accommodate only 2 tissues per horse (RDC and RVC) and steps in each protocol affected transporters in a way that prevented consecutive use of the other. However, comparisons between RVC and RDC were performed in parallel in tissues from the same horses, except for PBZ experiments, which were only performed on RDC.

**Results**

**pH Stat studies**

**Protocol 1**

The baseline, Cl$^-$–independent, electrogenic HCO$_3^-$ secretion of the RDC mucosa was significantly higher than in the RVC ($P = 0.04$; Figure 4). The rate of electrogenic HCO$_3^-$ secretion observed in the RVC was so low (or absent) in some tissues that it was not practical to add inhibitors to attempt to further elucidate the mechanism of HCO$_3^-$ secretion. The nonspecific anion channel blocker NPPB was then used to confirm that anion channels were involved in apical HCO$_3^-$ secretion in the horses’ colonic mucosa. This blocker decreased the HCO$_3^-$ secretion from 3.32 μEq·cm$^{-2}$·h$^{-1}$ to 0.69 μEq·cm$^{-2}$·h$^{-1}$ in the RDC. In subsequent experiments, the addition of CF-172 significantly decreased HCO$_3^-$ secretion in the RDC compared to baseline HCO$_3^-$ secretion ($P < 0.01$), which indicates the involvement of CFTR in apical HCO$_3^-$ secretion. In the RDC, no further inhibition of HCO$_3^-$ secretion was noted upon the addition of CaCCinh-A01, an inhibitor of the calcium-activated Cl$^-$ channels.

With the addition of the HCO$_3^-$–free, Cl$^-$–containing solution to the luminal chamber, there was no significant difference ($P = 0.50$) in rate of Cl$^-$–dependent HCO$_3^-$ secretion between the RDC and the RVC. In the RDC, the rate of Cl$^-$–independent electrogenic HCO$_3^-$ secretion was higher than the Cl$^-$–dependent electroneutral HCO$_3^-$ secretion, although the difference was not significant ($P = 0.3$) (Figure 4). In the RVC, there was Cl$^-$–dependent electroneutral HCO$_3^-$ secretion, compared to the low activity of Cl$^-$–independent electrogenic HCO$_3^-$ secretion in the RVC.

In both the RDC and RVC, the addition of the inhibitor DIDS significantly decreased HCO$_3^-$ secretion compared to the Cl$^-$–dependent HCO$_3^-$ secretion of the previous step ($P < 0.05$; for both), evidence of a DIDS-sensitive anion exchanger such as solute carrier family 26 (anion exchanger) member 3 (SCL26A3). There was no difference in responsiveness to DIDS between the RDC and the RVC ($P = 1.0$).

**Protocol 2**

As with protocol 1, the baseline, Cl$^-$–independent, electrogenic HCO$_3^-$ secretion of the RDC mucosa was significantly higher than the RVC ($P = 0.04$; Figure 4). In the presence of luminal Cl$^-$ (119.8 mM), HCO$_3^-$ secretion of the RDC mucosa was similar to that in the RVC ($P = 0.1$). In the RDC, there was no significant difference ($P = 0.8$) between the rate of HCO$_3^-$ secretion with or without luminal Cl$^-$ (Figure 4). In the RVC, HCO$_3^-$ secretion was significantly higher ($P < 0.01$) with luminal Cl$^-$ than without, evidence of a Cl$^-$–dependent anion exchanger in RVC.

In the presence of luminal isobutyrate (25 mM), the RDC had significantly greater ($P < 0.05$) HCO$_3^-$ secretion than the RVC mucosa (Figure 4). In both the RDC and RVC, the addition of luminal isobutyrate, in the presence of luminal Cl$^-$, did not significantly increase HCO$_3^-$ secretion ($P = 0.5$) compared to HCO$_3^-$ secretion in the presence of luminal Cl$^-$ alone. Similarly, the addition of the anion channel blocker NPPB did not significantly reduce HCO$_3^-$ secretion in the RDC or the RVC, in the presence of luminal Cl$^-$ and luminal isobutyrate ($P = 0.3$). Therefore, the role of the apical Cl$^-$– HCO$_3^-$– secretion, in the presence of luminal substrates utilized in electroneutral HCO$_3^-$ exchange (ie. Cl$^-$ and isobutyrate), is low. However, the rate of HCO$_3^-$ secretion after the addition of NPPB was still significantly higher in the RDC than the RVC ($P < 0.05$). This may indicate that the RDC has a greater capacity for that mechanism of SCFA absorption than the RVC.

**Protocol 3**

The effect of PBZ on HCO$_3^-$ secretion in the RDC was examined in 4 horses. Relative to baseline electrogenic HCO$_3^-$ secretion, the addition of Cl$^-$ to the luminal surface at a concentration of 119.8 mM did
not significantly change the rate of HCO$_3^-$ secretion (Figure 4; $P = 0.6$), as demonstrated in protocol 2. The addition of PBZ to the luminal and basolateral surfaces significantly decreased ($P < 0.02$) HCO$_3^-$ secretion in the presence of luminal Cl$^-$, indicating that both electrogenic and electroneutral HCO$_3^-$ secretion in the RDC are possibly inhibited by PBZ.

**Discussion**

Results of this study provide new information about the potential role of a GIT-buffering system in the development of right dorsal colitis in horses and a possible explanation for the RDC as the predilection site for this disease. Adequate buffering of digesta in the equine large intestine is critical to normal digestion, and the consequences of inadequate buffering could include mucosal damage.
or alterations to the normal colonic microflora. The most important finding in this study was the difference in HCO$_3^-$ secretory patterns between the RDC and RVC (Figure 4). The RDC had a greater electrogenic, lumen Cl$^-$-independent HCO$_3^-$ secretion compared with the RVC. This is consistent with secretion of this anion through CFTR in RDC, which was supported by the response to the CFTR inhibitor CF-172. CFTR is activated by prostaglandins via signaling pathways and therefore potentially affected by treatment with NSAIDs. Both RDC and RVC expressed a capacity for electroneutral, lumen Cl$^-$-dependent HCO$_3^-$ secretion, presumably through an AE. The significance of this segmental diversity of physiological activity and CFTR expression between the RDC and RVC is unclear, although there are some potential explanations based on what is already known about equine hindgut physiology and electrolyte transport.

The low rate of HCO$_3^-$ secretion in the RVC raises the question about buffering microbial fermentation products in this segment. A unique feature of equine pancreatic secretion is a high concentration of Cl$^-$ in the final product, whereas other mammals produce a secretion high in HCO$_3^-$, presumably to buffer gastric acid leaving the stomach. Such buffering is required to protect the duodenal mucosa and to support small intestinal digestive processes. A possible teleological explanation for the robust Cl$^-$ secretion by the equine pancreas is to provide this anion to the distal jejunum and ileum, where it could take part in an anion exchange mechanism that would drive HCO$_3^-$ secretion into the lumen. This influx of HCO$_3^-$ could provide a buffer to support microbial fermentation in the cecum and subsequent parts of the large colon. However, any HCO$_3^-$ from the ileum is likely to be consumed before reaching the RDC. This would explain the need for endogenous secretion of HCO$_3^-$ in the RDC to maintain that steady, relatively neutral pH along the length of the large intestine.4

Electroneutral, Cl$^-$-dependent HCO$_3^-$ secretion in this study was not significantly different between the RDC and RVC (Figure 4). Therefore, an electroneutral secretion could drive HCO$_3^-$ produced in the cell into the lumen of the RVC and compensate for the regional lack of electrogenic HCO$_3^-$ secretion in that segment. Under normal conditions, such redundancy of transport systems could maintain optimum mucosal pH and solute concentrations, which are critical to intestinal health and function.

Electroneutral secretion of intracellular HCO$_3^-$ is accomplished also through exchange with another intraluminal substrate, such as a SCFA. These exchange mechanisms have been demonstrated in the rat colon and have now been confirmed in the equine RDC and RVC (Figure 4). Both Cl$^-$ and isobutyrate downregulated the electrogenic, Cl$^-$-independent secretion in the RDC. A similar effect has been described in the mouse colon through butyrate inhibition of Cl$^-$-dependent HCO$_3^-$, possibly caused by the interaction of butyrate with apical membrane Cl$^-$/HCO$_3^-$ exchange at a luminal site. Incubation with two substrates (Cl$^-$ and SCFAs) involved in electroneutral HCO$_3^-$ secretion did not have an additive effect on HCO$_3^-$ secretion, suggesting that the same exchanger was used for both anions. Isobutyrate was the only SCFA examined in the current study, because it was examined in the murine studies, and it is not metabolized in vitro. Butyrate is metabolized by colonocytes, and such metabolism could alter HCO$_3^-$ secretion.

The in vitro concentration of PBZ used in this study was 1 μg/ml, based on previous work on anion transport in the equine RDC. This is a concentration similar to that achieved in the plasma after IV administration, corrected for 96 to 98% protein binding. This concentration of PBZ had no effect on conductance in equine RDC in a previous study, but did cause some apoptosis of surface epithelial cells without direct histological evidence of mucosal necrosis. Tissue injury might be expected to blunt or even abolish a secretory response, which was not observed previously or in the current study. Also, the addition of PBZ to the chambers did not cause immediate notable fluctuations in the pH of the bathing solution. As described in a previous study, PBZ was added to both sides of the tissue, the serosal side to deliver through the lamina propria and the mucosal side to ensure that any luminal water crossing the paracellular route would contain PBZ and thereby maintain rather than dilute the concentration in the lamina propria.

The effects of PBZ were only examined in the RDC because the anion of interest and possible clinical relevance, HCO$_3^-$, was secreted by that segment, and baseline HCO$_3^-$ secretion was low in the RVC. Our results indicated that PBZ decreased both Cl$^-$-independent electrogenic HCO$_3^-$ secretion and Cl$^-$-dependent electroneutral HCO$_3^-$ secretion in the equine RDC (Figure 4). Decreased HCO$_3^-$ secretion in response to PBZ could interfere with the buffering capacity of the colonic mucosa and thereby contribute to the pathogenesis of right dorsal colitis in the horse. In rats, systemically administered NSAIDs can rapidly decrease pH within the gastric mucoid cap, followed by a progression of damage from the superficial epithelium to deeper layers of the mucosa. In murine models of cystic fibrosis, reduced Cl$^-$ and HCO$_3^-$ secretion can induce gross and histological changes in the colon that could be relevant to colonic diseases of horses, including right dorsal colitis. It is possible that inhibition of Cl$^-$ and HCO$_3^-$ secretion can lead to mucosal inflammation that is then perpetuated by secondary colonization of damaged epithelium by colonic bacteria. Further work investigating mechanisms of HCO$_3^-$ secretion in horses affected with chronic right dorsal colitis or on protracted treatment with PBZ should provide some insight on this issue.

Findings of this study demonstrated that the RDC of the horse has the capacity for active, electrogenic HCO$_3^-$ secretion ex vivo without exposure to a secretagogue (baseline secretion) and in contrast to the rat colon. In the current study, basal ion transport in equine RDC and RVC in Ussing chambers seems to be dominated by endogenous prostanoids, presumably produced by tissue dissection...
and related trauma during preparation.\textsuperscript{22} This has been previously reported in the equine RVC\textsuperscript{23} and was attributed to inhibition of Na\textsuperscript{+} and Cl\textsuperscript{−} absorption and stimulation of near-maximal electrogenic Cl\textsuperscript{−} secretion in response to endogenous prostanooids. As in the current study, such prostanoid release was attributed to tissue trauma during collection.

The role of prostaglandins on HCO\textsubscript{3}− secretion in the RDC in vivo is unknown, but such a role could explain NSAID-induced right dorsal colitis. The endogenous release of prostaglandins is a recognized stimulatory factor for HCO\textsubscript{3}− secretion.\textsuperscript{30,33} The role of prostaglandins could be studied by inhibiting their synthesis during tissue dissection with an NSAID given in vivo.\textsuperscript{27} The effect of added prostaglandins could then be studied with additional inhibitors to better define their effect on CFTR. Horses were anesthetized before tissue collection to maintain viability of the colon up to the time of harvesting. Possibly dissection and handling of the live tissue might have produced a pronounced endogenous prostanoid reaction compared with studies on tissues harvested post mortem. However, similar prostaglandin-induced results were obtained in our laboratory in studies\textsuperscript{30} on Cl\textsuperscript{−} secretion by equine colonic mucosa harvested post mortem. Despite similar collection methods for the RDC and RVC in the current study, significant and probably real differences were identified between these anatomically distinct segments.

Further investigations of HCO\textsubscript{3}− secretion in the equine colon should include techniques such as immunohistochemistry, immunofluorescence, or Western blot analysis to identify and quantify candidate protein transporters in the tissues. Commercial antibodies were tested and used in preliminary experiments as part of the current study. However, the CFTR staining pattern was weak and nonspecific compared with the apically enhanced CFTR staining pattern in colonic crypts in other species.\textsuperscript{39} Low levels of CFTR are expressed in many tissues and with low sensitivity/specificity to anti-CFTR antibodies.\textsuperscript{40} Subsequent work in our laboratory has demonstrated that this might apply more to equine colon than to equine small intestine, lung, and other tissues.

Based on results of this study, a major pathophysiological consequence of protracted treatment of a horse with an NSAID could be disruption of buffering by HCO\textsubscript{3}− within the microclimate of the horse’s colonic epithelium. Similar loss of anion secretion in murine models of cystic fibrosis has demonstrated pathologic changes in the intestinal tract,\textsuperscript{13,19,37,41,44} comparable to those identified in horses with right dorsal colitis.\textsuperscript{42} Presumably such changes arise from a lack of anion secretion critical for hydration and/or buffering of luminal contents and within the unstirred layer.

Results of the current study combined with recent studies on dietary or oral provision of HCO\textsubscript{3}− have also identified a potential method to replace HCO\textsubscript{3}− lost by impaired secretion in the RDC. Although HCO\textsubscript{3}− can reach the proximal part of the equine large colon in substantial amounts,\textsuperscript{9} a protected version of this anion might be required to deliver it in effective concentrations to the RDC.\textsuperscript{46} Enteral administration of NaHCO\textsubscript{3}− has been shown to increase cecal pH in horses\textsuperscript{47} and may be a potential treatment option for colonic acidosis, even in segments as distal as the RDC. The marked difference in the physiological process of HCO\textsubscript{3}− secretion between the RDC and RVC suggests that impaired secretion of this important anion could play a role in equine right dorsal colitis, a disease that is unique in its consistent localization to the RDC.

In conclusion, the three distinct mechanisms of HCO\textsubscript{3}− secretion that have previously been described in the murine colon\textsuperscript{20} have now been demonstrated in the equine colon. The results indicate that the mechanisms of HCO\textsubscript{3}− secretion are different between two different parts of the equine colon (RDC vs RVC) and that HCO\textsubscript{3}− secretion in the equine RDC is inhibited by PBZ. Inhibition of HCO\textsubscript{3}− secretion by PBZ could play a role in pathogenesis of right dorsal colitis through failure to buffer luminal acid, but further studies are required to determine how the prostaglandin-driven mechanism for secretion of this anion is controlled under normal conditions.

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