Luminal alkalinization by guinea-pig cecum in vitro, an electro-neutral process

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Summary. Guinea-pig cecum was found to alkalinize its mucosal media in vitro at a chemical equivalents rate greater than the short circuit current (Isc). Alkalinization was inhibited by conditions which did not affect Isc and by low mucosal Na suggesting an electro-neutral process, dependent on Na.

Microbial activity in the herbivore cecum results in the generation of fatty acids which require buffering to prevent excessive acidity. Powell has demonstrated net secretion (Isc) of cecum in vitro, relating it to the net sodium ion of bicarbonate into the guinea-pig cecal lumen in vivo, and generation of fatty acids which require buffering to prevent excessive acidity.

Materials and methods. Female Hartley guinea-pigs weighing 350-650 g, fed ad libitum, were sacrificed by cervical transection. The cecum was opened, rinsed free of its contents with warm 0.9% NaCl, and mounted as a flat sheet between 2 lucite hemichambers whose aperture was 1.5 cm². Each hemichamber contained 8 ml of incubation fluid maintained at 37°C and stirred by a continuous stream of 95% O₂/5% CO₂. The low bicarbonate and pH solution, always used as the serosal bathing fluid had the composition, in mM: Na⁺, 145; K⁺, 5; Ca²⁺, 1.2; Mg²⁺, 1.2; HCO₃⁻, 25; Cl⁻, 125; and phosphate, 3; glucose, 10; pH 7.4 at 37°C when equilibrated with 95% O₂/5% CO₂. The high bicarbonate and pH solution used as the mucosal fluid, had the composition: HCO₃⁻, 5.7; Cl⁻, 144; and pH 6.8, but was otherwise identical to the high bicarbonate solution. In 1 series of experiments, pH values were maintained constant at 7.4.

Comparison of mucosal fluid alkalinization and electrical parameters of guinea-pig cecum at 4 initial mucosal pH values. Bicarbonate concentration of mucosal fluid was manipulated to achieve 4 mucosal pH values with serosal pH constant at 7.4. Alkalinization was inhibited by low PD and Isc at pH 6, and opposite relationship at pH 7.4. Intermediate mucosal pH 6.8 was selected for other experiments. Cecal contents found to be pH 6.7±0.2 in situ. Bars show mean ±SD for 6 experiments.
experiments the effect of mucosal pH at 4 different levels, pH 6, 6.4, 6.8, and 7.4 was studied by varying the mucosal bicarbonate concentration while holding the serosal pH constant at 7.4. Low-Na solutions with 2, 10, or 50 mM Na were prepared by equimolar substitutions of choline chloride for a portion of the NaCl. Chloride-free solutions were also used in which NaCl had been completely replaced by Na isethionate and the divalent cations were added in the form of sulfates. Measurements of bathing fluid pH were made by direct immersion of a glass pH electrode and an external reference electrode into the incubation media. No consistent changes in the serosal fluid pH were observed. However the mucosal fluid pH increased with time, hence the term alkalinization. The results are presented as the change in mucosal fluid pH, i.e., the final pH at the end of the 40-min incubation period minus the initial value. Since the changes were always in the direction of increasing mucosal pH, it was termed luminal alkalinization. The buffering capacity of the pH 6.8 solution was measured empirically under the experimental conditions by titration with 0.02 N HCl and found to require 0.77 μEq to change the 8 ml volume by 0.01 pH units. Alkalinization results are expressed as the mean value of the change±SD for the 40-min incubation periods.

Results and discussion. Immediately after sacrifice the cecal contents of guinea-pigs were found to have a pH 6.7±0.2 (n = 5) in situ. The in vitro technique mimicked this environment by utilizing a mucosal incubation fluid pH 6.8 and a serosal fluid pH 7.4, as described above. Under these conditions the transmural PD was 6.5±1.4 mV, serosa positive, and \( I_{SC} \) was 113±28 μA/cm², calculated as explained previously. In the control condition the mucosal fluid pH increased (alkalinization) about 0.07-0.08 pH units during the 40-min period (table 1). No consistent changes in the serosal fluid pH were observed, probably due to the greater buffering of this high-bicarbonate solution. Lack of equilibration of the solutions with the CO₂ in the gas mixture as a source of the observed alkalinization was ruled out by finding that in the absence of tissue no variation in pH occurred. While the luminal alkalinization appears miniscule, when the buffering capacity of the mucosal bathing fluid is taken into account, the alkalinization rate was \(-5.8 \mu\text{Eq} \text{H}^+/\text{h cm}^2\) tissue, compared to the \( I_{SC} \) on an equivalent basis of 4.5 μEq/cm². The magnitudes of these parameters suggest that the alkalinization phenomenon is no less significant than the \( I_{SC} \). The effect of mucosal fluid pH on the magnitude of luminal alkalinization and the electrical parameters was studied by changing the mucosal bicarbonate concentration to obtain 4 different pH values while holding the serosal fluid constant at pH 7.4 (figure). At mucosal pH 6.0 the observed pH change was very high, possibly due to the low buffering power of the fluid at a low bicarbonate concentration. However, the PD and \( I_{SC} \) were low, possibly due to the removal of a bicarbonate enhancement of the PD or to an oppositely oriented diffusion potential for chloride. Since alkalinization could not be reliably measured at mucosal pH 7.4, and PD and \( I_{SC} \) were depressed at pH 6.0, the intermediate value of pH 6.8 was chosen for further studies, a value close to that observed in situ.

PD and \( I_{SC} \) were inhibited 90% by application of 2,4-dinitrophenol (1 mM), but were unaffected by acetazolamide (1 mM) (table 1). However, introduction of either inhibitor into both mucosal and serosal fluids significantly reduced the alkalinization. Near abolition of the alkalinization with DNP, and significant reduction with acetazolamide removed the suspicion that the luminal alkalinization was due to passive diffusion of bicarbonate from the serosal solution. This notion was confirmed when the transmural PD was increased to 25 mV by a hyperpolarizing current and the alkalinization was not affected. These observations support the idea that the alkalinization is related to a cellular metabolic process rather than leakage or simple diffusion of an ionized species between the bulk phases along a favorable concentration gradient. Bilateral replacement of chloride with isethionate diminished the magnitude of alkalinization by 50% (table 1) while the PD was increased. Since the \( I_{SC} \) did not change the increase in PD appears related to an increase in resistance of the tissue. Constancy of the \( I_{SC} \) accompanied by a substantial reduction in alkalinization, in the presence of acetazolamide and chloride-free solutions, indicates that these 2 processes are separable. Considered along with the hyperpolarization result these observations strongly suggest the presence of an alkalinization process which is electrically neutral, and therefore which does not contribute to the \( I_{SC} \).

Replacement of luminal fluid Na with choline, shown in table 2, diminished both the luminal fluid and electrical parameters of guinea-pig cecum. Either 50 mM or 10 mM luminal Na decreased the alkalinization to 50% of the control value. At 2 mM Na, luminal alkalinization was completely abolished. PD and \( I_{SC} \) were incrementally reduced with replacement of greater portions of luminal Na. Parallel inhibition of alkalinization, PD and \( I_{SC} \) in low-Na media and with DNP suggests that cecal alkalinization depends upon the presence of luminal Na and may depend upon Na absorption. The acetazolamide-susceptible carbonic anhydrase present in guinea-pig cecum may be involved in the alkalinization process since we observed a decrease in its magnitude in the presence of this inhibitor. Enhancement of alkalinization in the presence of chloride was also indicated by the 50% reduction seen in chloride-free media. Reductions of alkalinization by either acetazolamide or chloride-free media with an unaffected \( I_{SC} \) indicates that the luminal alkalinization mechanism is electrically neutral. All of these observations are consistent with a neutral chloride-bicarbonate exchange at the mucosal membrane, as proposed for colon13,14 and ileum15 or with a secretion of sodium bicarbonate16,17, for guinea-pig ileum. Guinea-pig cecum thus

### Table 1. Alkalinization of mucosal fluid and electrical parameters of cecum exposed to 5 conditions. Note the reduced alkalinization with DNP

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>ΔpH (pH units)</th>
<th>PD (mV)</th>
<th>( I_{SC} ) (μA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.075±0.017</td>
<td>6.8±0.8</td>
<td>120±10</td>
</tr>
<tr>
<td>DNP, 1 mM</td>
<td>6</td>
<td>0.017±0.017*</td>
<td>0.6±0.3</td>
<td>72*</td>
</tr>
<tr>
<td>Acetazolamide, 1 mM</td>
<td>5</td>
<td>0.036±0.003**</td>
<td>6.9±0.2</td>
<td>114±2.3</td>
</tr>
<tr>
<td>Hyperpolarized</td>
<td>4</td>
<td>0.075±0.032</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Chloride-free</td>
<td>6</td>
<td>0.029±0.014**</td>
<td>11.2±1.0</td>
<td>105±10</td>
</tr>
</tbody>
</table>

Values are means±SD. * p < 0.001 (t-test); ** p < 0.005.

### Table 2. Effect of mucosal Na on alkalinization, PD and \( I_{SC} \)

At reduced mucosal Na with constant serosal Na, decreased alkalinization, PD and \( I_{SC} \) show dependence of all three parameters on the presence of mucosal Na

<table>
<thead>
<tr>
<th>Luminal Na concentration</th>
<th>n</th>
<th>ΔpH (pH units)</th>
<th>PD (mV)</th>
<th>( I_{SC} ) (μA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 145 mM</td>
<td>11</td>
<td>0.071±0.014</td>
<td>5.3±0.9</td>
<td>113±35</td>
</tr>
<tr>
<td>50 mM</td>
<td>6</td>
<td>0.043±0.007</td>
<td>4.6±1.1</td>
<td>87±36</td>
</tr>
<tr>
<td>10 mM</td>
<td>6</td>
<td>0.039±0.007</td>
<td>3.3±0.8</td>
<td>49±25</td>
</tr>
<tr>
<td>2 mM</td>
<td>11</td>
<td>0.003±0.010</td>
<td>1.0±0.3</td>
<td>11±6</td>
</tr>
</tbody>
</table>

Values are means±SD.