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Luminal alkalinization by guinea-pig cecum in vitro, an electro-neutral process[1]

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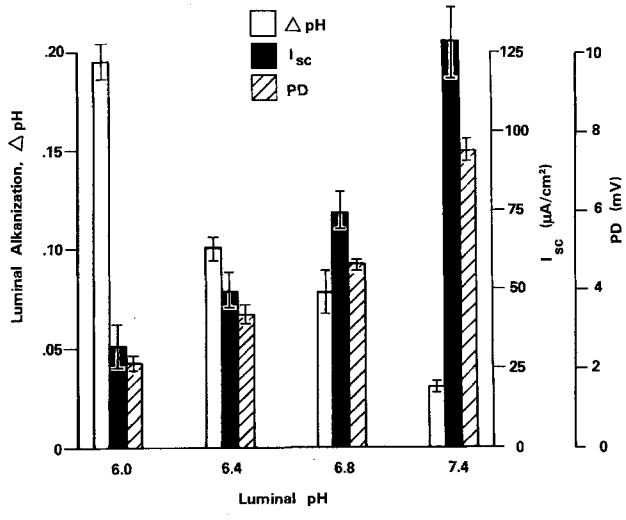
Summary. Guinea-pig cecum was found to alkalinize its mucosal media in vitro at a chemical equivalents rate greater than the short circuit current (I_{SC}). Alkalinization was inhibited by conditions which did not affect I_{SC} and by low mucosal Na suggesting an electro-neutral process, dependent on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

Microbial activity in the herbivore cecum results in the generation of fatty acids[2] which require buffering to prevent excessive acidity. Powell[3] has demonstrated net secretion of bicarbonate into the guinea-pig cecal lumen in vivo, a process which could perform the required buffering, and a simultaneous net absorption of Na and Cl. Ussing and Andersen[4] had previously measured the short circuit current (I_{SC}) of cecum in vitro, relating it to the net sodium absorption. However the relationship between the ability of the cecum to secrete bicarbonate, and thus to modify the pH of its luminal contents, and the I_{SC} or the net movement of sodium has not been reported. Acid-base metabolism of hamster and rat ileum in vitro[5,6] is known to alkalinize the fluid in contact with the mucosal surface. However this phenomenon has not been recorded for the cecum. In this study the guinea-pig cecum is shown to be capable of alkalinizing its mucosal bathing fluid in vitro, a process which may reflect the secretion of bicarbonate. This alkalinization process reported here also appears to depend upon the presence of sodium in the mucosal fluid, but to be electrically neutral or separate from the I_{SC}, in contrast to the findings for amphibian tissues[7].

Materials and methods. Female Hartley guinea-pigs weighing 500–650 g, fed ad libitum, were sacrificed by cervical separation. 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 cm^2 Each hemichamber contained 8 ml of incubation fluid maintained at 37 °C and stirred by a continuous stream of 95% O_2/5% CO_2. The transepithelial potential difference (PD) was measured by an electrometer connected to the chamber by a pair of calomel half-cell electrodes and 3 M KCl, 3% agar salt bridges. External current was passed through the tissue for resistance and short circuit current (I_{SC}) determination via a 2nd pair of electrodes. The open circuit PD was continuously recorded except for brief periods of current flow at 10-min intervals to determine I_{SC}. Since a linear current-voltage plot was obtained for this tissue at values of current between ±150 μA/cm^2, a modification of Clarkson's[8] method was applicable such that a single current-voltage measurement allowed calculation of I_{SC} as follows:

$$I_{SC} = (I_0 \times PD) / (PD - I_0 \times R_{so}).$$

PD, the open circuit potential difference; and I_0, the current required to reduce the observed potential to zero were measured each 10 min; while R_{so}, the solution resistance was measured for each solution in the absence of tissue. The tissue was maintained at open circuit except for the periodic current-voltage measurement to calculate I_{SC} and during voltage clamping at 25 mV, a special procedure described later. 2 solutions of different pH and bicarbonate concentration were utilized on opposite sides of the tissue. The high-bicarbonate and high-pH solution, always used as the serosal bathing fluid had the composition, in mM: Na^+, 145; K^+, 5; Ca^{2+}, 1.2; Mg^{2+}, 1.2; HCO_3^-, 25; Cl^-, 125; and phosphate, 3; glucose, 10; pH 7.4 at 37 °C when equilibrated with 95% O_2/5% CO_2. The low bicarbonate and pH solution used as the mucosal fluid, had the composition: HCO_3^-, 5.7; Cl^-, 144; and pH 6.8; but was otherwise identical to the high bicarbonate solution. In 1 series of



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. ΔpH is alkalinization during 40 min incubation in vitro. Note the high alkalinization but low PD and I_{SC} 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/cm^2, 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 CO_2 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 μEq H^+/h cm^2 tissue, compared to the I_{SC} on an equivalents basis of 4.5 μEq/h cm^2. The magnitudes of these parameters suggest that the alkalinization phenomenon is no less significant than the I_{SC}.

Table 1. Alkalinization of mucosal fluid and electrical parameters of cecum exposed to 5 conditions. Note the reduced alkalinization with acetazolamide and chloride-free solution, and the reduced alkalinization PD and I_{SC} with DNP

Condition	n	Δ pH (pH units)	PD (mV)	I_{SC} (μA/cm^2)
Control	6	0.075 ± 0.017	6.8 ± 0.8	120 ± 10
DNP, 1 mM	6	0.017 ± 0.017*	0.6 ± 0.3*	7 ± 2*
Acetazolamide, 1 mM	5	0.036 ± 0.003**	6.9 ± 0.2	114 ± 3
Hyperpolarized	4	0.075 ± 0.032	25	-
Chloride-free	6	0.039 ± 0.014**	11.2 ± 1.0*	105 ± 10

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

Luminal Na concentration	n	Δ pH (pH units)	PD (mV)	I_{SC} (μA/cm^2)
Control, 145 mM	11	0.071 ± 0.014	5.3 ± 0.9	113 ± 35
50 mM	6	0.043 ± 0.007	4.6 ± 1.1	87 ± 36
10 mM	6	0.039 ± 0.007	3.3 ± 0.8	49 ± 25
2 mM	11	0.003 ± 0.010	1.0 ± 0.3	11 ± 6

Values are means ± SD.

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 alkalinization 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[9] 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 colon[10,11] and ileum[12] or with a secretion of sodium bicarbonate[13,14] for guinea-pig ileum. Guinea-pig cecum thus

appears capable of alkalinizing its contents, which may serve a protective function in vivo. The alkalinization process appears to be electrically neutral and distinct from that responsible for I_{SC}, and to function at a faster rate on the basis of chemical equivalents.

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Stimulus-response function at several levels of background luminance, in the cat visual areas 17 and 18

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Summary. Stimulus-response curves of simple cells of the visual cortex were obtained by using 500-msec stationary stimuli. Background influence on single unit responses was studied. The contrast sensitivity of simple cells increases as a function of background luminance. The resolution power of these cortical cells for detecting differences in stimulus contrast decreases at background levels above 0.09 cd/m^2.

In a previous paper[2], we described the stimulus-response function of single neurons in visual areas 17 and 18 of the cat. Discrete stimuli were positioned on the neuron receptive field and series of 500-msec flashes of increasing intensities were presented on a background of constant luminance. Curves were constructed by plotting the response magnitude versus the logarithm of stimulus intensity. For all cortical neurons studied under these conditions, the stimulus-response relation was described by a S-shaped curve. We also observed that if the level of background illumination was changed, similar stimulus-response functions were obtained but the curves were shifted along the abscissa (log luminance) by an amount equal to the amount of change in background luminance; therefore, the plotting of the above responses versus the contrast between stimulus and background illumination eliminates the abscissa shift and superimposition of curves occurs. In this investigation we describe results concerning the influence of background illumination on the stimulus-response relation of cortical neurons. A similar study was conducted by Sakmann and Creutzfeldt[3] on the ganglion cells of the retina and by Virsu et al.[4] on the neurons of the lateral geniculate nucleus in the cat; they described changes in response sensitivity at different background levels.

Material and methods. Experiments were carried out on locally anaesthetized cats, paralyzed with Flaxedil and artificially ventilated. The closed chamber technique[5,6] was used to record impulse activity from an extracellular position by means of tungsten microelectrodes. Sequences of impulses were recorded on magnetic tape and subsequently analyzed with a digital computer (Honeywell, mod. 316). The cat eyes were covered by contact lenses with an artificial pupil 5 mm in diameter. Focal visual stimuli, appropriate in shape and size according to the receptive field, were projected on a tangent milky screen which was 1 m in front of the animal; the luminance of the screen was between 2×10^{-3} and 2×10^{0} cd/m^2 or, expressed as retinal illumination, between 3×10^{-2} and 3×10^{1} effective trolands. The light intensity of the stimuli was randomly changed by using neutral density filters in order to test neurons from threshold to saturation responses; 10 repetitions of each stimulus intensity were carried out in a random order.

The conditions of stimulations were defined in terms of contrast (that is I-B/B, where I is the intensity of the focal stimulus and B the background luminance). When the level of background luminance was changed, some 40–50 min were allowed for retinal adaptation[7]. Anatomical controls were routinely carried out; as reported elsewhere[8] we found, in area 18, neurons of the simple type according to Hubel and Wiesel's definition[9]. This finding was also reported by other authors[10,11].

Results and discussion. Results were obtained from 41 neurons, 31 of which histologically identified in area 17 and 10 in area 18; all were simple cells. Stimulus-response curves were constructed by plotting the response magnitude (mean frequency of discharge) versus the logarithm of stimulus contrast. The mean frequency of discharge was calculated by dividing the total number of spikes for each response by the duration of the response; when present, the spontaneous activity was subtracted. Each stimulus-response curve shows a minimal (threshold) and a maximal value at the extremes of a nearly straight portion. The plots in the figure (A and B) examplify our results; changes in background illumination were accompanied by several changes in the stimulus-response function of cortical neurons. In fact, a) an increase in background luminance caused a decrease in contrast threshold which was dependent on the amount of background increase (note in the figure A, the shift to the left of the curve obtained with background luminance of 2 cd/m^2, and in B a similar shift for the curve obtained with 0.09 cd/m^2); b) at all levels of background luminance explored, maximal responses were always obtained with log contrast +1.3, +1.4; c) as a