Mucosal surface pH of the large intestine of the rat and of normal and inflamed large intestine in man

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SUMMARY The surface pH of rat distal colonic mucosa and human rectal mucosa was measured in vitro using first a small pH electrode with a flattened tip. In buffer with pH 7·56 the mean rat colonic surface pH was 6·72. Lowering the buffer pH in steps resulted in a small fall in surface pH, the values being buffer pH 7·06 surface pH 6·64, buffer pH 6·58 surface pH 6·61 and finally buffer pH 6·09 surface pH 6·39. Similar results were obtained with a buffer where butyrate, 30 mmol/l replaced chloride and when a CO₂/bicarbonate buffer was used. During the time taken for the study transmural potential difference only changed by 1–2 mV. Serosal surface pH changed with buffer pH, suggesting that the maintained surface pH is a property of the mucosal surface only. The surface pH of human rectal mucosa was similar to that of rat distal colonic mucosa. As buffer pH fell from pH 7·51 to 5·96 mucosal surface pH only fell from pH 6·80 to 6·26. The values obtained in ulcerative proctitis did not differ from normal mucosa. Secondly pH microelectrodes were used to measure the juxta mucosal pH and the pH-microclimate thickness when luminal pH was controlled. The microclimate had a pH 6·63 adjacent to the mucosa with a thickness of 840 μm. The importance of mucus in maintaining the microclimate was shown by n-acetyl cysteine thinning and prostaglandin E2 thickening the layer. These results describe a surface microclimate in the large intestine of appreciable thickness and a constant juxta mucosal pH. Luminal pH changes produce only a small change in microclimate pH.

It has been proposed that adjacent to the mucosa of the gastrointestinal tract there is a thin layer whose composition changes little despite large changes in luminal composition. In the stomach the mucus bicarbonate layer 1 provides a pH gradient such that the juxta mucosal surface is around pH 7. In the small intestine the thin layer has been called the unstimred layer and its thickness determined using absorption studies of lipids1 and glucose.2 Studies of drug absorption in the small bowel1 have concluded that there is a mucosal region of low pH, and this has been measured as a microclimate using small pH electrodes at pH 5·5 adjacent to the mucosal surface.3 The microclimate pH was higher in intestinal disease, an increase related to reduced absorption of folic acid.3

Similar studies in the large intestine have used drug absorption to propose that there is a region of pH 6·5 between lumen and blood through which absorption occurs.4 This concept of a region close to the mucosa where pH changes little has not been fully studied in the colon and may be of consequence for the absorption of weak electrolytes, particularly ammonia5 and short chain fatty acids.6

We have studied this potential juxta mucosal surface layer, first with small flattened tip glass pH electrodes and second with pH-microelectrodes to assess the pH and thickness of the layer under various conditions in vitro.

Methods

GLASS ELECTRODES

A flat tipped glass electrode, 2 mm in diameter and produced by the method of Lucas et al,7 was used to measure the pH adjacent to the mucosa when luminal

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pH was varied. The response of the electrode was linear over the range pH 5 to 8, 50.50±0.2 mV/pH unit, and within 1 minute reached 93% of the maximum response. The electrode, with a calomel reference electrode was connected to a Radiometer model PHM25 pH meter, input impedance over 10¹⁰Ω, and the response recorded on a Servoscribe 2S recorder. The electrode was calibrated by measuring its response to buffers of known pH at 37°C before each study. Pressing the electrode onto a cork surface in buffer produced the same results as the pH of the buffer. In order to reduce interference all studies were done within an earthed wire cage surrounding the specimens and electrode.

**Studies on rats**

For these studies the distal colon of freshly killed female Wistar rats weighing 150–200 g was used. After cleansing the intestine with saline, a 2 cm length was opened, pinned onto a cork sheet with the mucosal surface exposed and immersed in 250 ml oxygenated buffer at 37°C.

After an equilibration period of five minutes in the buffer the pH electrode was lowered onto the mucosal surface. The response was rapid and steady values were obtained when the electrode had been on the mucosal surface for about three and a half minutes. Buffer pH was simultaneously measured with a standard glass pH electrode and this value used. The buffer was then drained out of the study bath and replaced immediately by a buffer of similar composition but different pH. This process was repeated four times with each colon studied. The order in which the buffers of differing pH were used did not affect the results obtained.

An adjacent length of large intestine was excised and incubated simultaneously in the same oxygenated buffer. The transmural potential difference of the length, which had one end tied off, was measured with chloride silver wire electrodes at both the beginning and end of the study period. Studies were completed within 40 minutes of the death of the rats.

On three occasions the gut was mounted in the buffer with the mucosal surface against the cork and the serosal surface pH was measured using the same buffers under identical conditions. This was to see whether the results were generated by anaerobic metabolism of the tissue which might lower pH by producing lactic and other organic acids.

The first buffer system used was a Krebs-Henseleit buffer which contained: sodium 162 mmol/l, potassium 5 mmol/l, chloride 142 mmol/l, bicarbonate 24 mmol/l, and glucose 11.1 mmol/l. This was brought to approximately pH 7.5, 7.0, 6.5, and 6.0 using 1 mol/l hydrochloric acid ('chloride buffer'). These pH values are within the ranges described in the large intestine *in vivo*. The second buffer was prepared by replacing 30 mmol/l chloride with butyrate 30 mmol/l, using hydrochloric acid to correct pH ('butyrate buffer'). We used butyrate as it has been considered a better energy source for the large intestinal mucosa than glucose, and so might alter the behaviour of the mucosa. The third buffer used bicarbonate/CO₂ to control pH.

Specimens were examined histologically and showed no damage from these studies.

**Human studies**

The surface pH of human large intestinal mucosa was measured on rectal biopsy specimens. The specimens were taken from eight women and three men, aged 22–79 years, for clinical diagnostic purposes and studied before routine histological examination. Six biopsies were taken from a rectum that appeared normal when examined through the sigmoidoscope and was also normal when examined histologically. Five were taken from patients with ulcerative proctitis. The proctitis was graded using the criteria of Baron *et al*. into active disease in four and inactive disease in one. These studies were carried out in a similar manner to that used for rat distal colon with the exception that 125 ml chloride buffer only was used because of the small size of the biopsies. Transmural potential difference was not obtained on these biopsies.

**Microelectrode studies**

The thickness and pH gradient of the juxta mucosal layer were measured using glass pH microelectrodes. These were prepared by drawing glass tubing, internal diameter 200 μm, to a tip diameter of 0.5 μm. After silanizing, the tip was broken back to 5–10 μm. The pH sensitive solution of 10% tridodecyamine with 0.7% sodium tetraphenyl boron in o-nitrophenyl octyl ether was introduced by capillary into the electrode tip. The electrode, was back-filled with pH 5 citrate buffer. The reference electrode contained 4 mol/l KCl with an agar plug in the tip. The electrodes were connected through silver-silver chloride wires to millivoltmeter and chart recorder.

The electrode system was robust, needing minimal screening and produced a response of approximately 60 mV/pH unit. Electrode pairs were calibrated in buffers of known pH at 37°C.

Studies were undertaken on rat distal colon. Male Wistar rats, mean weight 350 g, were killed and the distal colon exposed. This was divided distally and a 2 cm length flushed clean with 50 ml of one of three flushing solutions over two minutes. The colon was then excised, opened and mounted in oxygenated Krebs-Henseleit buffer, mean pH 7.70 at 37°C. The colonic specimen was pinned firmly over a cork
support. The microelectrodes were lowered through the buffer and juxta mucosal layer using a micro-manipulator, the study being complete within 15–20 minutes of exposing the distal colon. Measurements were taken from the electrode position where pH started to change from the buffer pH and both thickness and final pH taken at the step prior to entering the mucosa. The rate of fall in pH per millimetre was used as a measure of the mucus protective properties.

Fig. 1  Surface pH of rat colonic mucosa incubated in 'chloride' buffer with mean points linked.

Fig. 2  Surface pH of rat colonic mucosa incubated in 'butyrate' buffer. The results for individual colonic samples are linked.
Three flushing solutions were used. One hundred and fifty millimoles/litre sodium chloride solution was used as a control. To ascertain the role of mucus in the maintenance of the juxta mucosal layer a second flushing solution contained 20 mmol/l n-acetyl cysteine in saline to thin the mucus layer. The third flushing solution contained 0.28 mmol/l prostaglandin E2 in saline which was chosen because it stimulates mucus production and secretion at this concentration.17

Student's t-test was used for statistical comparison and the results expressed as mean±standard error.

Results

Glass Electrode Studies

Rat colonic mucosa

The surface pH of rat distal large intestine when measured in the chloride buffer at four initial pH values is shown in Figure 1, for colonic samples from 11 rats. The mucosal pH was 6.72±0.08 when buffer pH was 7.56±0.02 (t=9.53, p<0.001). When the more acid buffer pH of 7.06±0.03 was used mucosal pH was 6.64±0.05 (t=9.10, p<0.001). The third buffer whose pH was 6.58±0.02, proved to be almost identical to mucosal surface pH, 6.61±0.05, however, the mucosal surface pH was significantly higher, 6.39±0.04, than the most acid buffer, pH 6.09±0.04, (t=8.79, p<0.001).

Nine rat colonic samples were studied in butyrate buffer, to see if the presence of butyrate enhanced or altered the response when compared with the chloride buffer. Very similar results were obtained (Fig. 2): the four paired values were buffer pH 7.69±0.04 mucosal pH 6.78±0.04 (t=13.42, p<0.001), buffer pH 7.15±0.05 mucosal pH 6.74±0.06 (t=7.19, p<0.001), buffer pH 6.61±0.04 mucosal pH 6.61±0.04 and finally buffer pH 6.08±0.05 mucosal pH 6.32±0.06 (t=3.62, p<0.001). When a CO₂/bicarbonate buffer system was used the results obtained were similar to those obtained with the previous buffers: buffer pH 7.45±0.07, mucosal pH 6.87±0.05; buffer pH 6.99±0.04, mucosal pH 6.79±0.04; buffer pH 6.65, mucosal pH 6.77±0.02 and buffer pH 6.16±0.05, mucosal pH 6.28±0.02 (n=4).

Transmural potential differences measured on adjacent lengths of intestine were −11.3±0.7 mV at the end of the study with only a slight rise, 1–2 mV, during the study.

When the serosal surface was studied a different response was obtained (Fig. 3). The surface pH fell at the same rate as the buffer pH without being maintained within a narrow pH range as occurred on the mucosal surface.

Human mucosa

The results of the studies done on normal rectal biopsies were similar to those found in the studies of rat distal colon mucosa (Fig. 4). The pHs of the buffer and of the mucosal surface were found to be: buffer pH 7.51±0.03, mucosal pH 6.80±0.08 (n=6) (t=10.17, p<0.001); buffer pH 6.98±0.06, mucosal pH 6.77±0.06 (t=3.71, p<0.02); buffer pH 6.56±0.06, mucosal pH 6.71±0.06 (N.S.) and finally buffer pH 5.96±0.07, mucosal pH 6.26±0.09 (t=6.38, p<0.001).

When the surface pH of rectal biopsy specimens taken from patients with ulcerative proctitis was studied in the chloride buffer, results were similar to those obtained on the normal rectal biopsies without differences between varying degrees of inflammatory activity (Fig. 4).

Ph Microelectrode Studies

A typical recording obtained during a study is shown (Fig. 5). The values below the trace show the depth of the juxtamucosal region in this example (μm). At (a) the electrodes were being lowered in large, 500 μm, steps through the buffer till the juxta mucosal region was reached, (b). As soon as this response was detected the electrodes were withdrawn to their previous position and time allowed for equilibration, (c). From (c) the electrodes were cautiously lowered in progressively smaller steps until a plateau of pH was reached at which time the electrodes were being advanced very slowly, (d). The mucosa was breached at (e), thereafter the electrodes were withdrawn in large steps, (f) and (g), until the buffer pH was again obtained. The thickness was 750 μm in the recording depicted here. Thickness measurements in the with-
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Fig. 4 Surface pH of human large intestinal mucosa. ● normal mucosa; △ inactive proctitis;
△ active proctitis.

drawal phase proved unreliable, probably due to mucus that could be seen adhering to the electrode tips.

Duplicate studies were done on the same tissue sample using either the same electrodes or, more usually, a second pair mounted alongside which were used because mucus tended to adhere to the first electrode pair. Close agreement between the duplicate results was found for both thickness and the ultimate pH measured.

With the control flushing solution the thickness of the juxta mucosal layer was 840 μm (Table). The juxta mucosal pH was 6.63 and pH fell at 1.16 units/mm. A two minute wash with 20 mmol/l n-acetyl cysteine significantly thinned the microclimate to 430 μm and raised the juxta mucosal pH to 6.79. The rate of fall of pH was twice as great at 2.33 units/mm. On the other hand, prostaglandin E2 resulted in a microclimate thickness 1340 μm but lowered juxta mucosal pH insignificantly. The rate of fall of pH/mm was unchanged from control values.

Discussion

These results show that the immediate surface of the distal colon in rat and man has a region where pH is maintained over a narrow range. The theory of a large intestinal microclimate put forward by Schanker has been confirmed by a physical method. The microelectrode studies have confirmed these findings with a similar juxta mucosal pH but have added a dimension, thickness, to the microclimate. They have placed the region of pH 6.5 just above the epithelial cell, an identical position to studies in the small intestine.
There was a tendency for pH to fall with luminal pH in study 1 and several possible explanations exist for this. Firstly, the tissue was of small size compared with 250 ml buffer and may have been unable to neutralise adequately the increasing H⁺ ion flux at the lowest buffer pH. A second explanation is that the tip was imperfectly flat and in part exposed to the buffer: thus a composite reading – mostly microclimate, with some buffer – was obtained. Alternatively the tip occluded an area of mucosa trapping some buffer and altering the final pH.

Using other buffers such as CO₂ bicarbonate to control pH did not effect the results. Butyrate was used to replace some chloride as butyrate is well absorbed and considered to be an energy source, used in preference to glucose, for the colon. No difference in effect was found and this suggests that the glucose in the chloride and butyrate buffers or intrinsic energy sources were adequate under the conditions used.

Previous studies of colonic mucosal function conducted in vitro have reported continuing viability from one to two and a half hours. The present studies were completed well within these limits and potential difference was well maintained. The transmural potential difference was within the range found by Edmonds for rat distal colon, -8-18 mV. The big difference between mucosal and serosal surfaces suggest that the phenomenon is a function of the mucosa.

The relationship of the microclimate to mucus is emphasised by the alterations produced by N-acetyl cysteine and prostaglandin E₂. The thickness of the pH microclimate is much greater than the thickness of mucus measured optically. An unstirred layer is likely to contribute in part to the pH gradient but our finding matches the stomach where the effective thickness of the mucus bicarbonate layer is considerably thicker than the visible mucus layer. In the small intestine Shiau et al found that the microclimate could be removed by stirring, and replaced by incubation with glucose and they considered the mucus layer, the unstirred layer and the microclimate to represent different views of the same phenomenon. The importance of mucus to the microclimate and vice versa is shown when one considers that the integrity of the mucus is maintained when pH is kept constant, and the constant pH of mucus when released by goblet cells will help maintain the microclimate. The ability of mucus to retard diffusion has been shown for both the hydrogen ion and for butyrate, one of the three major short chain fatty acids that are absorbed by the colon.

There would appear to be a species difference in the microclimate with guinea pig distal colon having a pH of 6.9 in vitro and in vivo, independent of luminal pH. A difference between proximal and distal colon was also found in the guinea pig which may exist in rat colon.

The concept of a microclimate with a constant pH at its depth has important implications for absorption (and secretion) which must occur through it. Though drug absorption will be modified by the pH barrier, these findings may help explain anomalies in the absorption of weak acids, short chain fatty acids, and weak bases. Ammonia. Short chain fatty acids are thought to be absorbed principally in the unionised form yet, in man, increasing the chain length and hence the lipid solubility of the unionised form does not increase absorption. If short chain fatty acids have to pass through a region of constant pH, 6-5, where they will be nearly all ionised however, then the effects of chain length and lipid solubility would be slight. Similarly, ammonia is thought to be absorbed five times more rapidly unionised than ionised yet increasing the proportion of unionised ammonia 10 000-fold by changing perfusate pH from 4-2 to 9-0 resulted in only a three-fold increase in absorption. If the microclimate pH changes little even under such extremes of luminal pH then the observed increased in absorption would be expected.

In summary, these studies have defined a juxta mucosal microclimate in the large intestine. The pH of the microclimate is maintained within a narrow range and the thickness and effectiveness of the pH microclimate seems to depend upon the integrity of the mucus layer. As absorption and secretion will take place through the microclimate its importance remains to be established.

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