Determination of acid surface pH *in vivo* in rat proximal jejunum

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SUMMARY Surface pH of rat intestine was measured *in vivo* in exteriorised loops using pH-electrodes. Surface pH was approximately 6·1 in Krebs-phosphate buffer in proximal jejunum and was significantly more acid (p<0·01) than the bulk medium pH of 7·2. Values for the midgut were approximately 6·5, yet the distal ileum gave values of 7·3, which was marginally more alkaline than the buffer. These results agree with the known acidification phenomenon in the jejunum and the alkalinisation process in the ileum. No difference in surface pH was detected when bicarbonate buffer was used, nor when glucose was included in the buffer. The acid surface pH was almost completely inhibited when jejunal loops were made anoxic by completely occluding the blood supply, showing that the low surface pH is not itself a consequence of preparative anoxia. If glucose was included in the buffer, allowing glucose access from the luminal surface, surface pH was not significantly altered after occlusion. This indicates that previously reported *in vitro* results in the presence of glucose are similar to the present *in vivo* findings. The present experiments confirm the existence *in vivo* of the 'acid-microclimate' proposed to alter the absorption profiles of some dissociable drugs.

Previous experiments with surface pH electrodes have shown *in vitro* that in rat proximal jejunum[1] and in human biopsy samples[2] the pH recorded at the surface of these tissues is considerably more acid than the bulk solution bathing them. This discrepancy between bulk and surface pH has often been proposed[3] but *in vivo* evidence for this difference has been lacking. Attention has focused on this point because a low pH in the jejunum would provide a site for optimal absorption of weak-acids and a possible site for derangement of absorption in disease states as was shown *in vitro* with coeliac tissue. Additionally, an area of low pH would mean tht digestive enzymes might function in an environment completely different from that supposed on the basis of bulk solution measurements. To date, two studies have failed to confirm the existence of a low mucosal pH[4 5] although one study did confirm the existence of an acid surface pH *in vitro*.[4] The experiments described here were designed to supplement the available *in vitro* data by showing

the presence of an acid surface pH *in vivo* in rat proximal jejunum.

Methods

Male Wistar rats or female guinea pigs were anaesthetised (15 mg/kg body weight of urethane) and their body temperature was maintained at 37°C by a rectal probe thermistor coupled to a heating element under the body. A midline incision was made and the proximal jejunum identified. A loop of about 10 cms was brought through the abdominal wall which was then closed by suturing through the mesentery, taking care to maintain blood flow to the loop. The animal was then placed on its side to avoid any adverse vascular pressure gradient which might have occurred had the loop been positioned directly above the abdomen. The exposed loop was maintained at 37°C in a perspex chamber with cork base and held in place by pins through the mesentery. After longitudinal incision to one side of the anti-mesenteric border, the reflected intestinal wall was pinned onto the chamber base, allowing an exposed section of mucosa available for surface pH measurement. For anoxic experiments, the

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mesenteric blood vessels were ligatured and then cut, and in addition the loop was ligated above and below the site of measurement to occlude any collateral circulation in the mucosa. The exteriorised loop was then completely without blood supply and resembled previous *in vitro* experiments.

In a second series of experiments, a different protocol was carried out for use with catheter rather than surface pH electrodes. This was to enable a catheter mounted pH electrode to measure surface pH in a loop in areas not immediately adjacent to the incision. In these experiments, a loop was exteriorised in the usual way but was left on the surface of the closed abdomen and kept moist with saline. An incision was made to allow the catheter pH assembly to be introduced into the loop, together with reference electrode and flushing cannula, some five or more centimetres distant from the incision. A second incision was made beyond where the catheter electrode had been placed to allow drainage of buffer, 5 cm beyond the site of measurement. In effect, the catheter electrode was introduced into a sleeve of jejunum which could be periodically flushed with buffer.

ELECTRODE CONSTRUCTION AND RECORDING DETAILS

Electrodes, constructed as described[1][6] had slope values of at least 55mV/decade as tested in buffer solutions over the pH 4–9 range. They were connected by chlorided silver wire to an Ancom 15-A pre-amplifier of sufficiently high input impedance whose non-inverted output went directly to a pH meter (Pye-Unicam 9409). The reference electrode was a calomel half-cell connected *via* agar bridging to the chamber. Where catheter pH electrodes were used, as a check on pH at other sites inaccessible to the surface pH electrode, these were connected directly to the Pye 9409. The reference electrode here was a section of portex tubing sealed with an asbestos plug, filled with 3M KC1 and connected *via* chlorided silver wire. The pH sensor and reference electrode were fastened together with a flushing cannula so that the complete assembly could be inserted through an incision down the lumen of the jejunum in order to measure surface pH in an area distant from the site of incision, itself a source of possible edge damage.

EXPERIMENTAL PROTOCOLS

Surface pH electrodes were calibrated immediately before use. On exposure of the mucosal surface, the electrodes were introduced into the buffer to measure bulk pH and then placed onto the surface with the aid of a Prior micromanipulator. Between two or three minutes elapsed after exposure of the

mucosa and measurement. After measurement, bulk solution pH was measured and the electrode recalibrated. Where drift occurred (never more than 0·1 pH units per experimental period of two hours), readings were corrected by assuming constant linear drift. Experiments were carried out in an earthed Faraday cage.

With the catheter electrodes, much the same procedures were carried out. On incision of the loop, rather than a large longitudinal incision a small incision was made sufficient to allow insertion of the electrode assembly. Buffer was flushed through the cannula while the assembly was present in the loop. When surface pH was measured, flushing was discontinued and the electrode was additionally moved against the surface of the mucosa. Although this could not be seen directly, the electrode assembly could be seen to distend the loop; in fact the natural flexibility of the assembly ensured that it was against the mucosa at all times and flushing with buffer was needed to record anything other than surface pH. Occasionally, motility in the loop precluded stable surface pH values. In these few instances, the lowest recorded pH was taken as a measurement. Change in pH was not because of any interruption of the fluid path between sensor and reference electrode as where this did occur, as would be expected, an infinite resistance developed and no reading was possible.

Results

When surface pH electrodes are placed on the mucosa of the rat proximal jejunum a significant fall in measured pH was recorded. When the electrode resistance is measured by the resistance in parallel method,[7] no measurable change is recorded by contact with the gut mucosa and consequently no iR change which would affect the measurements. Placing the electrode reference electrode either on the mucosa close to or far from the pH electrode had no effect on measured signal. It is therefore unlikely that the change in measured signal was an electrical or electrode artefact. Representative values (Table a) indicate that surface pH in rat proximal jejunum is the same whether the tissue is bathed with Krebs bicarbonate buffer or phosphate buffer. In these circumstances the presence or absence of buffer glucose is immaterial.

The low surface pH can only be measured with facility in bicarbonate buffer when the measurement is made only at relatively short times after exposure of the mucosa. If a recording is made within the first two minutes (Fig. 1a) in bicarbonate buffer, a low pH can be recorded as seen in a typical trace. The bulk pH is clearly slightly above pH 7 but when an

Table *Surface pH in rat and guinea pig intestine measured in vivo under various experimental conditions. (a) Representative values in rat proximal jejunum; (b) values in rat proximal jejunum under various circumstances and in other tissues*

(a) *Values for rat proximal jejunum*

	(i) *Bicarbonate buffer*	(ii) *Phosphate buffer*
Zero glucose	6·25±0·25(4)	6·11±0·08(10)
10 mmol glucose	6·34±0·12(4)	6·06±0·10(7)

(b) *Values in phosphate buffer without glucose under various circumstances*

	Surface electrode	*Catheter electrode*
Rat proximal jejunum	6·10±0·10(7)	6·43±0·02(6)*
Ditto anoxic	7·03±0·06(7)†	6·73±0·07(6)*§
Rat distal ileum	7·23±0·06(6)†	
Guinea pig proximal jejunum	7·22±0·11(4)†	
Ditto anoxic	6·86±0·12(4)†‡	

Results are given as mean SEM with number of experiments in parentheses.
Statistical significance:
* = $p<0·02$ compared with rat proximal jejunum;
† = $p<0·01$ compared with rat proximal jejunum;
‡ compared with guinea pig ($p<0·05$);
§ compared with rat proximal jejunum by catheter electrode ($p<0·02$).
Bulk pH = 7.05±0·05.

electrode is placed on the surface, there is an immediate change in pH to a value around pH 5·9. When the electrode is taken off the preparation the pH rises to buffer pH values. The same manouevre done 19 minutes after exposure of the mucosa shows
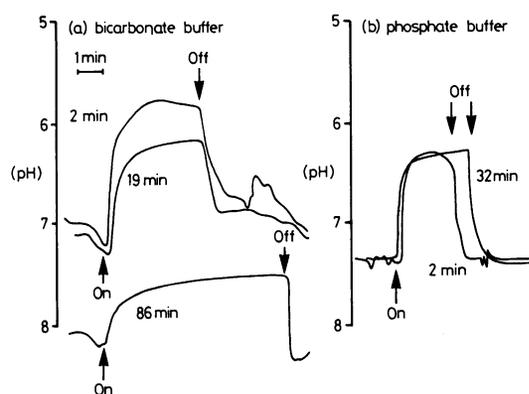

Fig. 1 *Surface pH measurements in rat* in vivo *proximal jejunum in (a) Krebs-Henseleit bicarbonate and (b) Krebs-Ringer phosphate buffer. Electrode placed on surface (↑) and after a short interval taken off (↓), with the experiment repeated at times stated after onset of experiment. Further explanation, see text.*

similar but attenuated values. After 86 minutes, the buffer pH has become alkaline owing to CO_2 loss and the recorded surface pH though still lower is not acid and is only 0·5 units below the buffer pH. In contrast, the same experiments done in phosphate buffer (Fig. 1b) at 2 and 32 minutes after exposure of the mucosa show very similar patterns. The measured surface pH remained stable and was found in this instance to be about 6·3. On balance, values in phosphate buffer tended (Table) to be slightly lower than those in bicarbonate buffer.

In contrast in the distal ileum, low pH values were not found, the surface pH being slightly higher than bulk solution values in phosphate buffer (Table b). Similarly, when proximal jejunum from the guinea pig was used a marginally alkaline surface pH was detected. When the tissues were made deliberately anoxic, the rat proximal jejunum could not maintain a low surface pH as the measured values were not significantly different from the buffer pH. In contrast, in the guinea pig jejunum, the marginally alkaline pH values became slightly more acid but did not equal the low values seen in rat jejunum. Broadly similar values were found for the rat proximal jejunum when the catheter pH assembly was used. Here a low pH was again recorded under normal conditions although this was not as acid as with the surface pH values. Under anoxic conditions there was a significant rise in surface pH, as previously.

The effect of anoxia was investigated in detail in both buffers in the presence and absence of glucose, in rat proximal jejunum. In phosphate buffer, surface pH was measured in the intact loop immediately and after thirty minutes (Fig. 2). Surface pH remained low and stable for thirty minutes in both buffer containing glucose and in that without glucose.. When the blood supply was interrupted, the surface pH became almost neutral in buffer without glucose. Where glucose was present, the surface pH remained acid as substrate was available from the luminal surface, overcoming the interruption of normal supply from the vasculature. Much the same occurred (Fig. 2) in bicarbonate buffer where because of volatility the check on stability was dispensed with. Again when blood supply was occluded, the surface pH tended towards neutral values in the absence of glucose whereas in the presence of glucose over shorter periods the pH remained acid.

Discussion

The present experiments show by direct measurement that the measured pH at the surface of rat proximal jejunum *in vivo* is at least 1 pH unit more
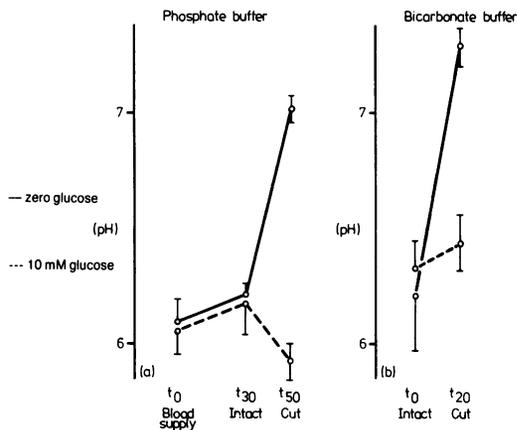
Fig. 2 *Effect of interruption of blood flow on measured surface pH in rat proximal jejunum* in vivo *in (a) phosphate and (b) bicarbonate buffer. In phosphate buffer, surface pH was measured at onset (t_0) and after 30 minutes (t_{30}) to check for stability, then blood flow was occluded and surface pH measured after 20 minutes of anoxia (t_{50}). In bicarbonate buffer, surface pH was measured at the onset of experiment (t_0), blood supply was occluded with surface pH measured again after 20 minutes anoxia (t_{20}).*

acid than bulk bathing solutions of neutral pH. This is broadly in agreement with previously reported values from *in vitro* preparations where a somewhat lower pH can be measured[1][4] because of the ease of access and less intestinal motility. Several features point to this not being an electrical artefact; it seems also that this is not simply caused by contact of the electrode with the mucosa as the reported pH in guinea pig jejunum is not acid nor is it in rat ileum. Both these tissues are associated with luminal alkalinisation[8][9] whereas in contrast the rat proximal jejunum is associated with luminal acidification both *in vitro* and *in vivo*[10–13] even in the presence of buffered solutions.[14–16]

It is also clear from the experiments where blood supply was occluded that interruption with consequent anoxia was not the cause of the low pH measured at the surface. When blood supply was interrupted, in both phosphate and bicarbonate buffer, the surface pH reached almost neutral values. Yet if anoxia were the cause, one might expect no detectable surface pH with intact vasculature and a low surface pH to become evident on occlusion. Although a trend to neutral values might be evident in bicarbonate buffer simply because of the volatility of CO_2, the phenomenon was seen in both buffers and could also be shown in bicarbonate buffer over short experimental periods.

The low surface pH was not dependent on the presence of glucose as the surface pH did not significantly differ when glucose was present in the luminal fluid. This is to be expected in the *in vivo* preparations, in contrast with the *in vitro* preparation, as glucose would reach the tissues from the vasculature. When the blood supply was cut and glucose was present in the luminal fluid, in contrast with the previous experiments, the surface pH remained low. Surface pH was slightly more acidic in phosphate buffer because of the volatility of pH in the bicarbonate buffer. This showed that lack of substrate rather than anoxia is the critical factor in the maintenance of a low surface pH. This accords with *in vitro* data[17] as surface pH change when the tissue is made anoxic, though significant, is never as great as when glucose is omitted from the buffer.

Given that any experimental procedure may perturb the normal conditions in the bowel, it is prudent to ask whether the low surface pH is because of local damage caused by a nearby incision. This question can to some extent be answered by the experiments involving the catheter pH electrode which measured surface pH in an area distant from the site of incision. As before, a low pH was detected at the surface, in the absence of luminal glucose. This was not evident when the blood supply was interrupted but was maintained in the presence of glucose. The measured surface pH in this instance was higher than that recorded by the glass surface pH electrodes. As some of the sensing surface, however, was unavoidably exposed to the bulk solution, this was not unexpected.

While low surface pH has been found by others *in vitro*,[4] their *in vivo* procedures failed to detect the low surface pH, except when the blood supply was severed. It is likely that this previous failure to detect a low surface pH is because bicarbonate buffer was exclusively used for the *in vivo* studies and that the preparative procedure may have been a lengthy one. The interdigestive intraluminal pCO_2 in the rat is relatively high (50 mm Hg) and remains higher than the mesenteric venous blood pCO_2 even when the animal is made hypercapnic.[18] The interdigestive lumen also contains appreciable quantities of bicarbonate.[19] Therefore after an incision and exposure of the loop to the air, the volatile component of the naturally occurring buffer can evaporate, causing a rapid alkalinisation. Consequently if the preparative procedure is a long one, or a measurement of suface pH is undertaken long after the initial exposure, the measured surface pH would be very close to the initial buffer values. This could explain the previous reports as bicarbonate cannot buffer fluctuations in the volatile carbon dioxide component. It is only when a non-volatile buffer such as phosphate was used that the surface

pH became stable enough to be measured over any length of time as was seen in the present experiments.

The present experiments shed no light on the mechanism of the process that causes a low surface pH. Some evidence exists for hydrogen ion secretion *per se* either as part of a sodium:hydrogen ion exchange process[20] or by surface hydrolysis of ATP[21] or possibly by carbonic acid secretion.[22] To some extent mucosally directed carbon dioxide from the mucosa is probably involved as the hydrogen ion that would have to be secreted to account for typical pH changes seems large. Carbon dioxide, however, is also produced metabolically by the ileum which did not show a low pH. Whatever the mechanism by which hydrogen ions are produced, the low pH *in vivo* represents an accumulation either in the mucus or in the unstirred layers at the cell surface and can only be superficial, although the absence of definitive indicator studies has been claimed[23] to show otherwise. It must also be remembered that the present experiments produce minimum estimates of the surface pH at the enterocyte brush border and that smaller electrodes may show considerably lower values.

The finding of a low pH *in vivo* in this species lends support to the original premise[3] that a low 'virtual' pH leads to apparent deviations from the pH partition hypothesis when drug absorption occurs. Frequently, absorption of weak acids occurs at a luminal pH at which there should be almost no absorption. Conversely, it might be expected that in diseased tissue, as low pH maintenance is dependent on adequate tissue metabolism a shift to the alkaline region might occur at the surface favouring the absorption of bases as has been proposed for propranolol absorption in coeliac disease.[24] There are without doubt exceptions to this general rule and it is probable that there could be paracellular malabsorption as well as failure of solutes to permeate the mucosa by the transcellular route. The fact that the pH is lower than might be expected does not exclude the likelihood of ionised permeation for small molecules or the involvement of unstirred layers. It seems, however, appropriate to take low surface pH into consideration as a determinant of weak electrolyte permeation in the jejunum of certain species.

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