Jejunal surface pH measurements in tropical sprue

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Abstract
The jejunal surface pH of jejunal biopsy samples incubated in vitro in Krebs-phosphate buffer was measured. Biopsies from 7 healthy individuals or tropical sprue subjects in complete remission had a mean surface pH of 5.8 ± 0.09, similar to values for normal Caucasian subjects living in the UK. The mean surface pH of biopsies from 20 sprue patients, 6.0 ± 0.08, was significantly higher (P<0.05) than that of the control subjects. Sprue patients could be sub-divided into those with 2 or more abnormalities in 3 intestinal function tests, and those with one or no abnormal test. The 9 low scorers showed a mean surface pH of 5.7 ± 0.06 resembling the control mean, whereas the 11 high scorers had a higher (P<0.01) mean surface pH of 6.17 ± 0.08. Mucosal surface pH correlated directly with 3 mean faecal fat excretion and inversely with xylose and vitamin B₁₂ absorption values but not with the nutritional indicators serum albumen, folate or blood haemoglobin levels. As in coeliac disease, mucosal surface pH in the jejunum is elevated above normal in tropical sprue and may reflect the extent to which normal ion transport processes are affected.

Introduction
The presence of an acid ‘microclimate’ at the mucosal surface has been proposed (Hugtenburg et al., 1959) to facilitate absorption of weak acids in the jejunum, despite disadvantageously high intraluminal pH values. The existence of such an area of low pH has been confirmed both in vivo (Lucas et al., 1975; Reichkeimer et al., 1979; Daniel et al., 1985) and in vitro (Shah & Fernandez, 1981; Lucas, 1983; Hering & Winne, 1983) in rat proximal jejunum and in human jejunal biopsy tissue (Lucas et al., 1978) incubated in vitro in a neutral bathing medium. Mucosal surface pH was distinctly lower than neutral bathing media in biopsies from normal subjects. In contrast, it was close to neutrality in biopsies from untreated adult coeliacs. This elevation in surface pH correlated with enterocyte damage (Lucas et al., 1978) and with in vivo alterations in absorption of weak electrolytes (Kitt et al., 1982). Surface pH probably affects weak electrolyte absorption by determining the amount of undissociated species at the enterocyte luminal membrane. Consequently, the alteration in surface pH in coeliac disease may contribute to disturbed intestinal function. In tropical sprue, a malabsorption syndrome widely prevalent in southern India, enterocyte damage occurs (Mathan et al., 1973) as in coeliac disease. For this reason, in vitro surface pH investigations were undertaken at Vellore on biopsy samples from patients with tropical sprue to see whether similar values could be obtained.

Methods
Patients
A control and a patient group with tropical sprue were studied. All subjects were admitted to a metabolic ward for intensive investigation, for which informed consent was obtained. The diagnosis of tropical sprue was established by investigational criteria (Baker & Mathan, 1971) in current use in Vellore. Tropical sprue is defined as a primary (idiopathic) malabsorption syndrome occurring in residents and visitors to the tropics (Baker & Mathan, 1968; Kleistpen & Baker, 1970). In southern India, sprue occurs as a temporally and spatially definable epidemic (Baker & Mathan, 1971) which clinically and biochemically is indistinguishable from the endemic form (Mathan & Baker, 1971). The sprue group consisted of 15 epidemic sprue patients who were admitted from a village 42 km south of Vellore, where an epidemic had begun in January, 3 months before the start of this study, and 5 endemic sprue patients, living in widely different parts of India, with a history of chronic diarrhoea ranging from 3 months to 5 years. Control subjects consisted of 3 healthy volunteers with no history of gastrointestinal complaints and 4 subjects with endemic sprue who had been in complete clinical and biochemical remission for more than 6 months.

Clinical tests
All subjects were given a controlled hospital diet containing 50 g of fat per day. Stools were collected for an assay of daily stool weight and mean 3 of faecal fat excretion (Kramer et al., 1949). The extent of xylose absorption after a 2 g oral dose was assessed by collecting the urine for 8 h in 3 fractions (Baker et al., 1971). The extent of vitamin B₁₂ absorption was tested by measuring the 6 h blood level after administration of 1 mg vitamin B₁₂ labelled with ¹³C as standardised and validated in this laboratory (Mathan et al., 1973). Nutritional state was assessed by measuring haemoglobin and serum albumin levels by standard methods, with fasting serum folate measured using the Lactobacillus casei microbiological assay (Baker et al., 1959). Jejunal biopsies were taken from all subjects at the same time of day with a Crosby capsule, positioned fluoroscopically in the first loop of the jejunum just distal to the ligament of Treitz. A portion of the biopsy was processed for routine histological examination, the remainder being taken for surface pH measurements. Biopsies were graded ‘blind’ as having no or mild morphological change as distinct from moderate to severe morphological change.

Jejunal surface pH measurements
These measurements were carried out using minia-
tute pH electrodes (PORTNOY, 1967) of 1.5 mm outside diameter to an approximate sensing depth of 30 μm (LUCAS & BLAIR, 1978). Whilst the spatial discrimination obtained with microelectrodes is not possible with pH electrodes, there was no difference between electrode tips when placed on to biopsy surfaces. One batch of electrodes was made in the UK and was used throughout the study. There was no difference between electrode batches regarding operating characteristics. Electrodes had a resistance at 10 kHz of 60 MΩ and a sensitivity of 59 ± 1 (n=9) nV per pH unit over the pH range of interest (4-9) and a response time (solution change method of approximately 2 sec. The jejunal surface pH measuring protocol was exactly as described by LUCAS et al. (1978) for studies on coeliac biopsy tissues.

Before human biopsy investigations, validation studies were made on rat proximal jejunum to ensure that measurements made in the UK laboratory could be successfully duplicated at Vellore. Rat proximal jejunum in the presence of 10 mm glucose gave surface pH values of 5.72 ± 0.09 before and 5.43 ± 0.08 after incubation (n=9), comparable to values from the UK laboratory of 5.62 ± 0.10 and 5.84 ± 0.15 (n=6) for the same conditions. Consequently, the reassembled measuring system operated in Vellore exactly as it had been done in the UK.

Statistical analysis

Absorption was a values, nutritional measurements, jejunal surface pH, age, sex and duration of symp- toms were collated for each subject. In addition, an index of severity was computed based on the results from the 3 absorption function tests. A score of one point was awarded for each abnormal function test, giving a subdivision of the combined score and non-irritable data into high scores (2 or more abnormal test) and low scores (one or no abnormal tests). Data were then analysed with reference to diagnosis and to severity of malabsorption using the BMDP statistical software package (JENNIH & RALSTON, 1979). Unless indicated otherwise, significance levels are based on normal statistics. The logarithmic conver- sion of pH to hydrogen ion before analysis is uncorrected, both on theoretical and empirical grounds. The errors from pH electrodes are normally distributed (BATES, 1964) and logarithmic conversion introduces non-normality (LUCAS, 1977).

Results

Comparing nutritional indicators, there were no differences (Table 1) between the 2 groups. The sprue patients had haemoglobin, serum folate and albumin levels resembling those of the control group. In contrast, marked differences were apparent in absorptive function. In the endemic sprue group, vitamin B12 absorption was more halved (P<0.025), mean 3d fecal fat excretion was over 3 times the control level (P<0.002), stool weight increased and urinary xylose excretion was less than half the control values both at 5 h (P<0.005) and 8 h (P<0.005). Similar differences in xylose absorption (P<0.005) and fecal fat excretion (P<0.001) existed in the epidemic sprue group, although vitamin B12 absorp- tion was not significantly affected in this relatively small group. These changes in absorption are ex- pected for this syndrome and indicate that a repre- sentative group of sprue patients was under investiga- tion.

When jejunal biopsy surface pH was measured in vivo, the pH values in the control and sprue groups were found to be higher at the start of incubation. In contrast, a higher mean surface pH of 6.03 ± 0.08 (n=20, P<0.05) was found in the sprue group (Figure, A). About half this population was significantly more than one standard deviation above the normal mean despite overlap between the jejunal and the sprue groups (P<0.01, χ²). Inspection of the data showed the likelihood of 2 sprue populations being present, one with a mean surface pH of 5.97 ± 0.06 (n=9), the other with a mean of 6.29 ± 0.05 (n=11).

An assessment of correlation between variables established that surface pH correlated with the absorption measurements, although all possible cor- relations were calculated. There were no significant associations among the jejunal and the absorp- tive indicators. As expected, the function tests correlated amongst themselves (Table 2). Fecal fat excretion correlated significantly with xylose and with vitamin B12 absorption, although there was no cor- relation between the latter pair. Jejunal surface pH correlated significantly with xylose absorption (P<0.05), fecal fat excretion, and also with vitamin B12 absorption (P<0.025). Though the correlation between surface pH and the absorption test values was relatively modest, it was of similar magnitude to those between the absorption indicators themselves. When the biopsies were graded on the basis of histological appearance, the mean surface pH of 5.83 ± 0.05 for those with morphological change was significantly from the value of 6.13 ± 0.11 for the group with moderate to severe morphological change (P<0.05).

Coefficients for correlations between surface pH and individual tests of absorptive function may have been moderate, although significant because of differ- ing site specificity of the function tests, while surface pH is exclusively a jejunal measurement. For this reason, the results of the 3 absorption tests were combined into a single index of the severity of malabsorption. A severity index ranging from zero to a maximum of 3 correlated significantly with severity of symp- toms with surface pH (P<0.01; r=0.68) and also with stool weight. Low scores (index of one or less) had a mean surface pH of 5.72 ± 0.06 (n=0), not different from that of controls, whilst high scores (index of 2 or more) had a significantly higher mean surface pH, 6.17 ± 0.08 (n=11, P<0.01). The only other variable to show a difference was stool weight, which was elevated in the high score group as would be expected.

Overlap of control and sprue patient surface pH values could indicate partial recovery in those patients whose symptoms began 3 months before the present investigations. This was checked by correlating sur- face pH with duration of symptoms. A linear model failed to account for variation in surface pH (r=0.94), whereas a sinusoidal function gave a significant fit if patients whose onset was more than 3 months before the study were excluded (r=0.73; P<0.01). An alternative approach (Figure, B) was to restrict linear regression to sprue patients of 10 weeks duration or less, which gave a significant fit to the data (r=0.68; P<0.01). Patients who had sprue for a longer
Table 1. Clinical function and biochemical test values in control and sprue subjects, expressed as means and standard errors of mean with number of subjects in parentheses*

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Epidemic sprue</th>
<th>Endemic sprue</th>
<th>All sprue</th>
<th>Severe sprue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean faecal fat (g), 3 d average</td>
<td>3-6 ± 1:34(7)</td>
<td>10:1 ± 1:19 (15)</td>
<td>13:6 ± 1:86(5)</td>
<td>10:9 ± 1:04 (20)</td>
<td>12:5 ± 1:5 (11)</td>
</tr>
<tr>
<td>Vitamin B12 absorption (IU/litre plasma)</td>
<td>0:9 ± 0:17(6)</td>
<td>0:7 ± 0:18 (10)</td>
<td>0:4 ± 0:14(5)</td>
<td>0:6 ± 0:14(15)</td>
<td>0:5 ± 0:16(11)</td>
</tr>
<tr>
<td>5 hour xylose (g) (% excetration)</td>
<td>25:1 ± 3:9 (7)</td>
<td>10:8 ± 1:3 (15)</td>
<td>7:7 ± 1:6 (5)</td>
<td>10:1 ± 1:0 (20)</td>
<td>7:8 ± 1:0 (11)</td>
</tr>
<tr>
<td>8 hour xylose (g) (% excetration)</td>
<td>30:3 ± 4:4 (7)</td>
<td>15:8 ± 1:4 (15)</td>
<td>11:3 ± 2:4 (5)</td>
<td>14:7 ± 1:3 (20)</td>
<td>11:7 ± 1:5 (11)</td>
</tr>
<tr>
<td>Haemoglobin (g/100ml)</td>
<td>11:7 ± 0:5 (7)</td>
<td>11:1 ± 0:5 (15)</td>
<td>9:6 ± 0:7 (5)</td>
<td>10:7 ± 0:4 (20)</td>
<td>10:6 ± 0:5 (11)</td>
</tr>
<tr>
<td>Albumin (g/100ml)</td>
<td>3:6 ± 0:11(7)</td>
<td>3:5 ± 0:16(14)</td>
<td>3:5 ± 0:2 (5)</td>
<td>3:5 ± 0:12(20)</td>
<td>3:6 ± 0:1 (11)</td>
</tr>
<tr>
<td>Surface pH</td>
<td>5:77 ± 0:90(7)</td>
<td>6:00 ± 0:90(15)</td>
<td>6:12 ± 0:13(5)</td>
<td>6:03 ± 0:08(20)</td>
<td>6:17 ± 0:08(11)</td>
</tr>
</tbody>
</table>

*Significance refers to comparison with control group.

Figure A. Jejunal mucosal surface pH in jejunum biopsy samples from normal subjects and sprue patients, incubated in vitro in Krebs-phosphate buffer containing 10 mEq glucose. Results are expressed as scatter plots and as means with standard errors of mean, with the number of patients in parentheses. Significance levels refer to t and U tests. B. Sprue mucosal surface pH values plotted against time in months after onset of symptoms, showing correlation (r=0.71) if regression is restricted to 2 months or less after onset.

Discussion

Jejunal mucosal surface pH, measured in vitro using biopsy tissue, was distinctly more acid than the neutral bathing medium that constituted the bulk phase. The mean pH of 5.8 resembles previous in vitro findings for normal subjects drawn from a largely

duration divided into a high and a low surface pH group for whom xylose absorption also significantly differed (P<0.01). A reasonable model may be that surface pH increases with duration of sprue. After 12 weeks, sprue persists or resolves, causing the surface pH to fall to normal values.
Table 2. Correlation coefficients for the linear regression of pairs of variables using corrected data from normal and spray subjects.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylitol</td>
<td>Fat</td>
<td>0.44*</td>
</tr>
<tr>
<td>Fat</td>
<td>Vitamin B12</td>
<td>-0.51*</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>Stearic</td>
<td>-0.44**</td>
</tr>
<tr>
<td>Stearic</td>
<td>Surface pH</td>
<td>0.25</td>
</tr>
<tr>
<td>Surface pH</td>
<td>pH</td>
<td>0.40***</td>
</tr>
</tbody>
</table>

Statistical significance: *P<0.05; **P<0.01; ***P<0.001

Caucasian UK population (LUCAS et al., 1978). Mean surface pH was higher than normal in patients with tropical sprue. Eleven of 20 spray patients had values that were significantly different from the normal, resembling findings in coeliac patients (LUCAS et al., 1978). Unlike the coeliac findings, some spray pa-
tients had values within the normal range. This may be because tropical sprue can occur as an acute episode while, in contrast, untreated adult coeliac disease is likely to be a long standing illness. Some of the spray patients may have been spontaneously recovering or responding to treatment at the time of biopsy. Supporting this view is the observation that the epidemic spray group subdivided into a severely affected and less affected group, separated by the severity of symptoms. In addition, there were indications of time-dependence in the severity of symptoms. This also accords with the clinical experience of spray in southern India, where 65% of patients can have remission 3 months after the onset of symptoms. As high pH was associated with severity of symptoms, it seems likely that the natural progression of events in the epidemic spray group was a rise and then a fall in mucosal pH, which would explain the observed distribution of results.

The elevation in surface pH in the spray group may indicate altered jejunal ion transport processes. Bicar-
bonate absorption in the normal jejunum is detectable as luminal hydrogen ion appearance (TURNBERG et al., 1970; WRIGHT et al., 1979; KRESS, 1983). Eleva-
tion of surface pH may result from some factor produced by the spray agent similar to the bacterial enterotoxins which alter bicarbonate transport (STROMBECK, 1972; LEPOT & BANWELL, 1976). Alternatively, spray may allow the production of enterotoxin from opportunistic bacterial growth. However, bacterial growth was not associated with the presented cases. If bacterial cultures were posi-
tive, they were identified and the biopsy data ex-
cluded from the study. An indirect action of spray by way of prostaglandin-mediated inflammatory re-
sponses is also possible and elucidation of the mechanism must await more detailed knowledge of the factors affecting ion transport. Regardless of mechanism, the elevation of surface pH is not unique to spray. Similar results have been obtained in coeliac patients (LUCAS et al., 1978) and in half of a group of patients with diarrhoea of unknown aetiology (unpub-
lished observations). In the present study, one patient with Sjögren's syndrome had a pH of 6.4, amongst the highest recorded, but was excluded from the study.

Elevated surface pH seems to be feature of upper jejunal enterocyte dysfunction such as steatorrhoea, xyllose and vitamin B12 malabsorption. In particular, the greater the number of abnormal tests, the higher the surface pH. It is unlikely that the change in surface pH was caused although this might be an aspect of the fat malabsorption (SHIAU & LEVINE, 1980). Far more likely is a common depend-
ence on the extent of underlying damage. All 3 absorption tests correlate with the degree of mucosal damage (MATHAN et al., 1975) and the changes in surface pH may simply, reflect a similar dependence. In contrast, no correlation was found with measure-
ments associated with nutritional deficiency. This confirms the impression that these measurements are related more to the duration of illness and that changes in them are not directly proportional to persistent damage. In conclusion, elevation in the jejunal surface pH occurs in tropical sprue, particularly in those patients with severe malabsorption and moderate to severe mucosal morphological abnormality. Elevated mucosal surface pH is another indicator of enterocyte damage, the basic lesion in tropical sprue. These observations resemble similar findings in coeliac patients and may be common to diseases which alter the normal jejunal ion movements. The significance of an elevated surface pH lies in the changed microcli-
mate next to the brush border which may affect digested and absorbed processes, thereby differenti-
ating to the malabsorption that occurs in upper gastrointestinal disease.

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