Determinants of oesophageal 'alkaline' pH environment in controls and patients with gastro-oesophageal reflux disease

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Abstract

The determinants of the oesophageal alkaline pH environment are poorly understood. Saliva (pH 6.4–7.8) may be a major contributor, although some argue the importance of refluxed alkaline duodenal contents. Acid and alkaline reflux parameters were studied over 2 days in 30 subjects (controls, oesophagitis and Barrett's patients; 10 each) using glass pH electrodes. In phase 1, one pH electrode was placed 1 cm below the upper oesophageal sphincter to assess the influence of saliva and the other 5 cm above the lower oesophageal sphincter. Phase 2 was identical except that one pH probe was 5 cm below the lower oesophageal sphincter to record duodenogastric reflux. Patient groups spent, on average, 50 fold more time during the upright and supine periods at acidic pH than controls. Saliva was responsible for the percentage of time the pH > 7 and contributed significantly to the percentage of time the pH > 6 in both the proximal and distal oesophagus of control subjects, as shown by an absence of pH > 7 and a significant ($p < 0.001$) fourfold decrease in pH > 6 during sleep. A similar pattern was seen in the proximal oesophagus of both reflux groups. The reflux and Barrett's patients, however did not show a significant decrease in the percentage of time the pH > 6 at night in the distal oesophagus suggesting a relative increase in 'alkaline' exposure from another source. This was not because of duodenogastric reflux as the corresponding pH rises in the fundus of the stomach were non-existent. Although this was not studied specifically, it is believed to be a protective mechanism, the result of alkaline secretion produced by submucosal oesophageal glands.

(Gut 1993; 34: 309–316)

Gastroesophageal reflux disease is a common disorder caused by excessive reflux of gastro-duodenal contents in the oesophagus. While the role of acid and pepsin in the production of symptoms and mucosal injury is well documented,^{1–7} the importance of refluxed alkaline duodenal contents, especially in patients with an intact pylorus, is controversial. There is compelling evidence from animal studies that unconjugated bile acids are injurious to the oesophageal mucosa.^{1,2,8–11} Supporting human observations include the following: (1) bile reflux can cause heartburn¹² and may cause more symptoms than acid alone¹³; (2) oesophagitis can occur in the presence of atrophic gastritis associated with pernicious anaemia¹⁴ and after total gastrectomy^{15–17}; (3) increased amounts of duodenogastric reflux have been reported in patients with oesophagitis compared with those without oesophagitis^{18,19}; and (4) Barrett's oesophagus had followed total gastrectomy.²⁰ However, aspiration studies attempting to identify bile acids in refluxed material have been conflicting. Two groups^{21,22} reported no or very low concentrations of bile acids in refluxed material, while a third study²³ found that 87% of patients with gastro-oesophageal reflux had measurable bile acids, especially at night. Nevertheless, these aspiration methods are too cumbersome for routine testing and may dilute out the bile acids by stimulating saliva production.²¹

Unconjugated bile acids and pancreatic enzymes (especially trypsin) damage the oesophageal mucosa, primarily when the oesophageal pH is > 7.⁹ Therefore, Pelligrini *et al*²⁴ suggested that the percentage of time spent at a pH > 7 during 24 hours oesophageal pH monitoring would be a good indicator of alkaline reflux and a simple indirect measure of the

TABLE I Demographic data

Group	No of subjects/ sex	Age (y) (mean (range))	Entry criteria	Endoscopy
Control	10 (4M, 6F)	42.5 (35–48)	<2 Episodes of heartburn/month; no regurgitation, no dysphagia; normal 24 h pH parameters	Not done
Reflux	10 (4M, 6F)	53.0 (39–65)	Symptoms of heartburn/regurgitation; abnormal 24 h pH parameters*	All had prior histories of erosive oesophagitis; 2 had strictures; 1 had oesophageal ring; 1 had oesophageal ulcer
Barrett's oesophagus	10 (8M, 2F)	54.7 (38–68)	Biopsy proved columnar lined epithelium extending >3 cm above the gastric folds	Three had strictures and oesophageal ulcers; 1 had oesophageal ulcer; none had dysplasia or malignancy

* Abnormal parameters defined as present when any one of the three parameters for upright, supine, or total acid exposure times exceeded the 95th centile values (time upright pH < 4 > 8.2%, time supine pH < 4 > 3.5% and total time pH < 4 > 5.8%) obtained from studies in 110 healthy controls.²⁰

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Accepted for publication
10 August 1992

potential for bile reflux. This group subsequently reported²⁵ an increased prevalence of alkaline reflux in patients with oesophagitis and especially those with complicated Barrett's oesophagus (that is, stricture, ulcer, or dysplasia). Mattioli *et al*,²⁶ however, studied 82 subjects with upper gastrointestinal symptoms by placing 3 pH probes in series, one above the lower oesophageal sphincter and the others in the fundus and antrum of the stomach, and found that duodeno-gastric reflux was very rarely (0.8% of all reflux episodes) associated with an increase in oesophageal pH > 7. Instead, they suggested that an oesophageal pH rise above 7 was more probably the result of saliva or food. Saliva seems particularly important as it has a pH between 6.4 and 7.8 predominantly because of its bicarbonate content.²⁷ Furthermore, both saliva and bicarbonate production are stimulated by acid reflux via a vagally mediated reflex arc from the distal oesophagus to the salivary glands.²⁸

To clarify these conflicting data, we performed a study assessing the contributions of saliva and gastric contents to the relative alkaline milieu (pH > 6–7) of the oesophagus in healthy volunteers and in patients with oesophagitis and Barrett's oesophagus.

Methods

The study protocol was approved by the Institutional Review Board for Human Use of the University of Alabama at Birmingham on December 12, 1990.

STUDY POPULATION

The population consisted of 30 subjects in three groups of 10 each. The demographic data, entry criteria, and endoscopic findings for each group are summarised in Table I. None of the patients or volunteers had had prior oesophageal or gastric surgery nor did they have any major medical illness. None of the subjects had sicca syndrome and there was no evidence of periodontal oral infection or untreated dental caries. All peptic strictures were dilated to at least a 48 French gauge diameter before the study.

GENERAL STUDY DESIGN

Before beginning these studies, all subjects stopped taking any medication known to effect gastrointestinal motility or acid secretion for at least 48 hours. Patients taking omeprazole stopped the drug a week before the study; however, all patients could take antacids up to 6 hours before the study. All three groups were studied twice using two combined glass pH electrodes. In phase 1 of the study, both pH electrodes were placed in the oesophagus. The proximal electrode was positioned 1 cm below the lower border of the upper oesophageal sphincter to assess saliva secretion and the distal electrode was placed 5 cm above the upper border of the lower oesophageal sphincter as determined by previous manometric studies. One week later, the second phase was performed in an identical manner except that one pH electrode was placed across the lower oesophageal sphincter into the stomach to record duodeno-gastric reflux. In this phase, the proximal electrode was located 5 cm above the upper border of the lower oesophageal sphincter and the distal electrode 5 cm below the lower border of the lower oesophageal sphincter (Fig 1).

SALIVA TESTING

Basal saliva secretion was tested in each subject before starting phase 1 of the study. Saliva was collected in a glass beaker in 10 minute aliquots for 30 minutes. The first 10 minute sample was discarded. The volume and pH of the two remaining aliquots were determined immediately. Stimulated salivary volume and pH were calculated in the similar fashion before phase 2 of the study. A neutral chewing gum was used as a salivary stimulant. The pH of the saliva was calculated immediately after collection at 37°C using radiometer autoburette (Radiometer, Copenhagen, Denmark).

OE SOPHAGEAL MANOMETRY

All subjects underwent oesophageal manometry after an overnight fast to locate their upper and lower oesophageal sphincters. This was done using a round polyvinyl catheter (diameter 4.5 mm; Arndorfer Specialties, Inc, Greendale, WI, USA) continuously perfused with distilled water at a rate of 0.5 ml/minute by a low compliance pneumohydraulic capillary infusion system (Arndorfer). The station pull through technique was used to determine the location and length of both sphincters, as described in detail elsewhere.²⁹

AMBULATORY 24 HOUR OE SOPHAGEAL PH MONITORING

The 24 hour pH studies were performed using two separate glass electrodes (Model Lot 440 M3, Mui Scientific, Missauga, Canada) with a combined diameter of approximately 4.0–4.5 mm and a built in reference electrode (Ag/AgCl) near the end of each tip. The electrodes were calibrated at 37°C in pH 7 and pH 1, using a buffer solution (Fischer Scientific, Fairlawn, NJ, USA) before and after completing each

Figure 1: Location of the glass pH electrodes during the two phases of our study.

study. This buffer has very little pH change (<0.02 pH units) over a wide range of temperatures in both acid and alkaline pH environments. The two electrodes were passed separately through the nose and placed at different positions depending on the phase of the study (Fig 1). Small dental rubber bands held the two electrodes together as they exited the nose. Both electrodes were connected to a portable digital data recorder (Mark II Gold, Synectics, Irving, TX, USA) which stored pH data every 4 seconds for 24 hours.

After placing the pH electrodes, all subjects were given three precooked standardised meals specially prepared by the General Clinical Research Center. The meals consisted of a total of 2200 calories, with 50% of the calories coming from fat; 34% from carbohydrates, and the remainder from proteins. All foods had a pH of between 5 and 7. Patients were advised to eat these three meals during fixed time periods and not to eat or drink anything between meals except for room temperature water. Patients returned home with instructions to keep a diary recording symptoms, meal time, time of lying down for sleep, and the time of rising in the morning. All participants were also encouraged to perform and keep a record of their normal daily activities during the study.

ANALYSIS OF THE 24 HOUR PH DATA

After the 24 hour pH study, the data recorded on the two channel digitrapper were downloaded on to a compatible IBM computer for analysis using a Gastrosoft (Gastrosoft Inc, Irving, TX, USA) computer program. The pH data were analysed separately for total, upright, and supine periods for all parameters studied.

Acid pH data (percentage of time the $\text{pH}>4$) were compared between the proximal and distal oesophagus in all three groups of subjects.

Alkaline pH data (percentage of time the $\text{pH}>7$) and time the percentage of the $\text{pH}>6$ were calculated separately. To identify the role of saliva, the data from the proximal oesophagus were compared with those from the distal oesophagus during phase 1 of the study in all three groups. Similarly, the distal oesophageal pH data were compared with the gastric pH data during phase 2 of the study to assess the contribution of duodenogastric reflux. The individual pH tracings were also reviewed for evidence of duodenogastric alkaline reflux – defined as a pH rise >7 appearing first in the stomach followed by the oesophagus in phase 2 of the study. Secretion of saliva is present only in the day and virtually stops during sleep, even with a pH electrode in place (personal communications –

James Helm).³¹ Therefore, we assessed the contributions made by saliva to the alkaline pH (percentage of time $\text{pH}>7$) and the percentage of time $\text{pH}>6$, by comparing the upright pH data to the supine data at both the proximal and distal oesophageal sites. Meal periods were excluded when the percentage of time the $\text{pH}>6$ was calculated since the standardised meals had a pH between 5 and 7.

Gastric pH data from phase 2 of the study were analysed separately for duodenogastric reflux according to previously used criteria^{26,32} in the three groups of subjects. Total, upright, and supine time spent at $\text{pH}>4$ were compared separately among the groups. Meal times (both plateau and decline) were excluded for the analysis. A discriminate score³³ was also calculated for each subject from the 24 hour gastric pH data. A score of more than $+2.2$ indicated that the individual had a high probability of having abnormal duodenogastric reflux.

STATISTICAL ANALYSIS

Saliva data are presented as mean (SEM). Paired *t* tests were used to compare the basal and stimulated saliva volumes and pH values within the three groups of subjects. One way analysis of variance tests were used to compare the salivary pH and volume in the three groups at basal and stimulated states.

All 24 hour pH data are presented as median values with interquartile ranges. For each subject group, paired non-parametric (Wilcoxon) tests were used to compare the pH values (both alkaline and acid) between the proximal and distal oesophagus (phase 1) and distal oesophagus and stomach (phase 2). Non-parametric unpaired (Mann-Whitney) tests were used to compare the pH data between the various groups. Non-parametric analyses were used for the 24 hour pH data because the distributions were highly skewed. A *p* value ≤ 0.05 was considered significant for all data analyses.

Results

SALIVA

The mean basal salivary production for all three subject groups was approximately 0.4 ml/minute, with mean pH values between 7.0–7.2. After stimulation with gum, there was a significant ($p<0.01$) increase in the saliva volume and pH in each of the three groups. However, there were no significant differences between the groups in basal or stimulated saliva pH or volume (Table II).

24 HOUR PH MEASUREMENTS

(a) Acidic pH

Both distal and proximal oesophageal acid exposure times were significantly ($p<0.01$) greater in reflux disease and Barrett's oesophagus patients than in control subjects. The patient groups, however did not differ from each other in terms of these variables (Table III). The reflux disease and Barrett's patients also had

TABLE II Saliva volume and pH in the basal and stimulated states (values, mean (SEM))

Group	Basal saliva ml/min	pH	Stimulated saliva ml/min	pH
Control	0.38 (0.06)	7.09 (0.12)	1.64 (0.19)*	7.71 (0.06)*
Reflux	0.43 (0.07)	7.07 (0.10)	1.50 (0.27)*	7.57 (0.11)*
Barrett's oesophagus	0.41 (0.09)	7.18 (0.11)	1.15 (0.15)*	7.66 (0.07)

* $p<0.01$ v corresponding basal state

TABLE III Percentage of time at an acidic pH (pH<4) in the proximal and distal oesophagus (values median (interquartile range))

	Control Total	Upright	Supine	Reflux Total	Upright	Supine	Barrett's Total	Upright	Supine
Proximal oesophagus	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	1.9† (1.2-2.0)	2.0† (0.9-2.7)	0.7† (0.0-3.9)	1.1† (0.4-1.6)	1.0† (0.5-1.8)	0.3 (0.0-1.6)
Distal oesophagus	0.7 (0.1-1.0)	0.2 (0.1-1.0)	0.1 (0.0-1.5)	9.8*† (6.3-10.6)	11.7*† (5.5-12.7)	5.3*† (2.8-8.9)	12.4*† (6.6-17.1)	14.0*† (9.0-18.3)	8.1*† (1.5-19.9)

*p<0.01 v proximal oesophagus; †p<0.01 v controls

TABLE IV Percentage of time at an alkaline pH>7 (A) and pH>6 (B) in the proximal and distal oesophagus (values, mean (interquartile range))

	Control Total	Upright	Supine	Reflux Total	Upright	Supine	Barrett's Total	Upright	Supine
(A)									
Proximal oesophagus	5.5 (1.0-11.5)	8.8 (1.5-19.7)	0.0 (0.0-0.1)	1.6 (0.4-2.9)	2.8 (0.0-4.8)	0.0 (0.0-0.1)	0.7 (0.1-3.5)	1.0 (0.1-6.2)	0.0 (0.0-0.0)
Distal oesophagus	6.7 (0.5-9.8)	10.7 (0.8-15.9)	0.0 (0.0-0.0)	3.5* (0.5-5.4)	3.5* (0.6-8.7)	0.0 (0.0-0.2)	2.6† (1.0-3.8)	3.9 (0.1-6.2)	0.1 (0.0-0.8)
(B)									
Proximal oesophagus	69.6 (51.4-79.4)	96.7 (82.2-98.5)	31.4 (15.0-47.2)	51.9 (27.1-68.3)	68.5‡ (44.5-79.0)	20.1 (5.4-45.4)	47.4‡ (42.6-56.4)	68.2‡ (57.3-70.1)	20.9 (11.6-31.5)
Distal oesophagus	63.5 (46.9-70.5)	91.8 (75.6-95.4)	20.9† (5.3-27.2)	41.4 (31.4-53.2)	44.4‡ (35.2-62.3)	37.0† (21.7-51.6)	44.1 (32.0-53.7)	52.5‡ (33.8-55.5)	35.7* (14.8-53.7)

†p<0.05 v proximal oesophagus; *p<0.01 v proximal oesophagus; ‡p<0.05 v controls.

significantly (p<0.01) more acid reflux into the distal oesophagus than the proximal oesophagus.

(b) 'Alkaline' pH at proximal and distal oesophageal sites (phase I)

(i) Percentage of time pH>7. In the distal oesophagus, the percentage of time pH>7 in both the upright and supine positions was similar in the three groups (Table IVA). In contrast, the values for the percentage of time pH>7 were numerically less but not significant in the proximal oesophagus for reflux (p=0.16) and Barrett's (p=0.06) patients in the upright position compared with controls.

There was no difference in alkaline exposure (the percentage of time pH>7) in the proximal and distal oesophagus of controls. Both reflux disease and Barrett's patients, however had significantly more time with a pH>7 in the distal oesophagus than in the proximal oesophagus (Table IVA). These differences occurred only during the awake (upright) period as the pH was virtually never >7 during sleep in any group.

(ii) The percentage of time at pH>6. At a lower threshold (percentage of time at pH>6), there were some significant and surprising differences between the three groups (Table IVB). In the distal oesophagus, the percentage time the pH was >6 in the upright position was significantly greater in controls than in reflux disease and Barrett's oesophagus patients in the upright position because of the excessive amount of acid reflux in the patient groups. Surprisingly, the opposite relationship was noted in the supine position. Here, the reflux patients had significantly (p<0.05) more time when the pH was >6 than the controls and the Barrett's patients had a numerically greater but not significant (p=0.08) percentage of time with the pH>6 than the controls subjects. These observations were particularly surprising since both patient groups spent on average 50 fold more time at an acid pH than the control groups (Table III). This would underestimate the time spent at a more neutral pH since acid and alkaline reflux would occur

together but the pH electrode will only read an acid pH.

In the proximal oesophagus, the percentage of time with pH>6 in the upright position was significantly greater in controls than in reflux disease and Barrett's patients. However, in contrast to the distal oesophagus, in the supine position the percentage of time at pH>6 was similar among all groups. In fact, the patient groups tended to have less time at this pH than the control subjects (Table IVB)

(iii) Effect of saliva on the alkaline pH (the percentage of time spent at pH>7) and on the percentage of time pH>6 in the proximal and distal oesophagus. The percentage of time the pH>7 was noted in all groups only during the upright (awake) time. During the supine (sleep) time, the pH was virtually never >7 in any group (median value 0.0 Table IVA) suggesting that this intraoesophageal alkaline environment was the result of saliva production during the day.

A lower threshold of pH>6 permits a better assessment of the influence of saliva on the relative intraoesophageal 'alkaline' environment. In the proximal oesophagus, all three groups showed a significant (p<0.001) fourfold decrease in the percentage of time spent above pH 6 during sleep, probably because of considerably decreased salivary secretions (Fig 2). As would be anticipated, the controls had a similar significant (p<0.0001) fourfold fall in the time spent at a pH>6 during sleep in their distal oesophagus. In contrast, patients with reflux disease and Barrett's oesophagus did not show a significant decrease in this variable. This suggested that they experienced a relative increase in 'alkaline' exposure (Fig 2), resulting in a significantly (p=0.04 in reflux and p=0.0007 in Barrett's) higher percentage of time spent at pH>6 during sleep in the distal compared with the proximal oesophagus (Table IVB). Conversely, the control subjects spent significantly (p<0.05) more time during sleep with their oesophageal pH above 6 in the proximal than the distal oesophagus.

(C) 'Alkaline' pH: oesophageal v gastric sites (phase 2). Table V shows the results for the percentage

of time the $\text{pH} > 7$ and $\text{pH} > 6$ during the second phase of the study with simultaneous pH monitoring in the distal oesophagus and proximal stomach. The discriminant score suggested that two controls, four patients with reflux, and three with Barrett's oesophagus had abnormal duodenogastric reflux.

As can be seen by comparing Tables IV and V, placing a pH probe across the lower oesophageal sphincter caused a nearly twofold reduction in the percentage of time the $\text{pH} > 7$ and $\text{pH} > 6$ in the distal oesophagus among all groups. We believe that this phenomena was secondary to poor clearance of refluxed acid when a probe was

Figure 2: Influence of circadian cycle on the intraluminal alkaline pH (percentage of time the $\text{pH} > 6$) in the proximal and distal oesophagus of the three groups of subjects. (A) In the proximal oesophagus, all groups had a similar significant fourfold decrease in the percentage of time the $\text{pH} > 6$ in the supine position primarily during sleep. This suggests that saliva ($\text{pH} 6.4\text{--}7.8$) is the major contributor to the intraluminal alkaline pH during the upright (day) period but this falls at night because saliva secretion nearly stops with sleep. (B) In the distal oesophagus, the controls had a similar significant fourfold decrease in the percentage of time $\text{pH} > 6$ in the supine position. However, neither group of reflux patients showed a significant decrease in this variable. This suggests that these patients experienced a relative increase in alkaline exposure from an unknown source resulting in a significantly higher percentage of time spent at $\text{pH} > 6$ during sleep in the distal compared with proximal oesophagus. These changes were particularly remarkable since the patient groups simultaneously had nearly 50 fold more distal acid reflux during both the upright and supine periods compared with controls.

TABLE V Percentage of time at alkaline pH>7 (A) and pH>6 (B) in the distal oesophagus and proximal stomach (values, median (interquartile range))

	Control Total	Upright	Supine	Reflux Total	Upright	Supine	Barrett's Total	Upright	Supine
(A)									
Distal oesophagus	1.8 (0.6–2.4)	3.0 (1.0–3.8)	0.0 (0.0–0.0)	0.7 (0.2–2.0)	0.7 (0.3–1.9)	0.0 (0.0–0.0)	1.0 (0.0–2.0)	1.6 (0.0–3.2)	0.0 (0.0–0.0)
Proximal stomach	0.0* (0.0–0.0)	0.0* (0.0–0.0)	0.0 (0.0–0.0)	0.0* (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0† (0.0–0.0)	0.0† (0.0–0.0)	0.0 (0.0–0.0)
(B)									
Distal oesophagus	56.4 (41.6–60.6)	88.3 (70.0–93.6)	10.1 (0.1–11.4)	33.5‡ (26.1–42.4)	53.0‡ (40.4–57.6)	9.6 (0.6–23.4)	27.7‡ (25.4–36.6)	39.4‡ (29.9–50.3)	11.4 (3.3–31.7)
Proximal stomach	2.2* (0.6–4.2)	1.7* (0.2–3.6)	1.2† (0.0–6.9)	0.9* (0.2–3.1)	0.8* (0.4–2.1)	0.0 (0.0–1.9)	4.5* (1.1–7.2)	3.4* (1.0–7.3)	0.4 (0.0–5.2)

†p<0.05 v distal oesophagus; *p<0.01 v distal oesophagus; ‡p<0.05 v controls.

Figure 3: Example of a dual pH study during phase 2 in a patient with Barrett's oesophagus. The bolder line shows an episode of alkaline duodenogastric reflux to the level of the proximal stomach during the early hours of the morning (4 am–6 am) while the subject was asleep. However, no corresponding rise in the oesophageal pH (approximately pH 4.5) shown by the lighter line was recorded by the pH electrode positioned 5 cm above the lower oesophageal sphincter. Overall, only three (1 control and 2 Barrett's) of 30 patients had a gastric pH rise>7 and none of these events was associated with a corresponding rise in oesophageal pH>7.

across the lower oesophageal sphincter.³⁴ Nevertheless, most of the oesophageal pH values were still significantly higher than the simultaneously obtained values from the fundus of the stomach. If the changes in oesophageal alkaline pH (pH>7) and pH>6 were the result of duodenogastric reflux, then the anticipated values obtained from the stomach should have been significantly higher, rather than the converse. Therefore, the percentage of time the pH>7 and pH>6 in the distal oesophagus is *not* the result of duodenogastric reflux. In fact, only three (one control and two Barrett's) of 30 patients had a gastric pH rise above 7. None of these events was associated with a corresponding rise in the oesophageal pH>7 that would be interpreted as an example of true alkaline reflux arising from the stomach and duodenum (Fig 3). Even using the more liberal criteria for duodenogastric reflux of the percentage of time spent at gastric pH>4^{26,32} during the non-meal periods, there were no differences noted among the three groups of subjects during any period (Table VI).

Discussion

During 24 hour pH monitoring with a glass electrode, the intraluminal oesophageal pH of healthy volunteers is between 4 and 7 for 94% of the time.³⁵ While there is no dispute that any fall in pH below 4, not associated with food or drink, is the result of acid gastro-oesophageal reflux, the determinants of the alkaline oesophageal pH environment have never before been systematically studied.

Our study clearly shows that the oesophageal

alkaline pH environment (percentage of time pH>7) in patients with an intact pylorus was *not* the result of reflux of duodenogastric contents across the lower oesophageal sphincter (Table V), despite nearly one third of subjects in each group having evidence of abnormal duodenogastric reflux into the fundus of the stomach based on a positive discriminant score developed by Fuchs *et al*.³³ Firstly the oesophageal alkalisation was observed only during the day and was negligible at night, suggesting an effect of saliva, which stops during sleep. On the other hand, the reflux of duodenogastric contents should have produced the opposite effect as this physiological process occurs predominantly at night.²⁶ Secondly, the percentage of time pH>7 should have been higher in the fundus of the stomach than in distal oesophagus, rather than the opposite, since not everything in the fundus refluxes into the oesophagus. Finally, not only was the gastric pH rise above 7 in the stomach infrequent, but there was no corresponding rise in oesophageal pH noted during the three episodes recorded in our patients (Fig 3). These findings agree with other reports²⁶ of very infrequent rises in oesophageal pH>7 because of duodenogastric reflux in patients with upper gastrointestinal symptoms. This suggests that the pH rise above 7 in the oesophagus is the result of local factors in the oesophagus and not of alkaline gastro-oesophagus reflux as suggested by DeMeester *et al*.^{24,25} who did not monitor intragastric pH simultaneously with their oesophageal pH recordings.

In our control subjects, saliva seems to be the prime contributor to the 'alkaline' pH environment observed in the proximal and distal oesophagus. At both of these sites the percentage of time the intraluminal pH was greater than 7 was only apparent during the upright (awake) period, with virtually no pH>7 during sleep. This is consistent with our findings and previous observations that saliva has a pH between 6.4 and 7.8^{27,28} and its secretion virtually stops during sleep.³¹ Saliva was also a major contributor to a pH>6 in both the proximal and distal

TABLE VI Gastric pH monitoring: percentage of time pH>4 in the stomach during different periods in three groups of subjects (values medians with interquartile ranges)

	Control	Reflux	Barrett's
% Total time pH>4	5.1 (1.0–15.6)	1.9 (0.2–4.6)	6.7 (1.2–17.0)
% Upright time pH>4	2.7 (1.0–6.2)	0.9 (0.1–3.3)	5.0 (1.0–13.7)
% Supine time pH>4	8.3 (0.2–25.6)	1.0 (0.0–3.4)	4.7 (0.0–10.8)

oesophagus as the controls had a fourfold decrease in the time spent above pH 6 during the supine compared with the upright period at both these sites (Fig 2).

The determinants of the 'alkaline' oesophageal pH environment are more complex in our two groups of reflux patients. Saliva undoubtedly plays a role since the percentage of time the pH > 7 was virtually nil during the supine periods at both oesophageal sites. Similar to controls, both reflux groups showed a fourfold decrease in the percentage of time spent at pH > 6 in the proximal oesophagus during the supine period as a result of the loss of saliva. However, unlike controls, both patients with reflux and Barrett's oesophagus had an insignificant decrease in the percentage of time the pH was > 6 in the distal oesophagus while asleep (Fig 2). This increased relative distal alkalisation in the two reflux groups compared with controls (Table IVB) must have been caused by factors other than increased saliva production occurring as a protective reflex mechanism to excessive acid reflux, since concomitant rises were not seen in the proximal oesophagus. The distal alkalisation was also translated into a significantly longer percentage of time the pH was > 6 in the distal oesophageal site compared with the proximal site in both groups of reflux patients (Table IV). Since alkaline and acidic materials tend to move pH values in the opposite directions, the percentage of time at pH > 6 or 7 measured by the electrodes in both patient groups is a gross underestimation of alkaline secretions since they had nearly 50 fold more time at an acidic pH in their distal oesophagus than controls (Table III).

If this distal oesophageal alkalisation in reflux patients is neither caused by reflux of alkaline duodenogastric content nor an effect of saliva, what is its cause? One important source may be alkaline secretions produced by submucosal oesophageal glands. Submucosal glands are present throughout the human oesophagus³⁶⁻⁴⁰ but their function, until recently, has not been studied in health or disease. A recent study in the opossum oesophagus showed that these submucosal glands secrete bicarbonate in the basal state which increases fourfold in response to intraluminal acid infusion.⁴¹ This bicarbonate secretion can clear acid from the oesophageal lumen and is not inhibited by atropine. Similar changes were not seen in the rabbit oesophagus, which lacks submucosal glands. In a preliminary report,⁴² the same group also found that the human oesophagus in healthy controls was capable of secreting bicarbonate which could raise the pH of a residual volume of refluxate from 2.5 to 6-7. Other possible sources could be sloughed surface cells or the transudation of plasma across the injured oesophageal mucosa. The latter seems unlikely since the oesophagitis in both groups of reflux patients had been adequately controlled by H₂ receptor antagonist or omeprazole before these studies.

The origin of intraluminal oesophageal pH changes has important clinical relevance to the reflux constituents known to damage oesophageal mucosa in experimental animals. Unconjugated bile acids only increase mucosal permeability at pH > 7 while pancreatic enzymes can produce

erosive oesophagitis at pH 5-8.⁹ However, our study and others²⁶ shows that an oesophageal pH > 7 rarely occurs in the patient with an intact stomach. Furthermore, the origin of this alkaline pH seems to be saliva rather than reflux or duodenogastric contents. Even when bile acids are present in the refluxed material, they would rarely be at an alkaline pH sufficient to damage the oesophagus. This would not be the case for trypsin, but a recent study⁴² found that this pancreatic enzyme is rarely present in the oesophageal aspirate from patients with oesophagitis and Barrett's oesophagus. Pepsin, conjugated bile acids, and H⁺ ions cause damage in an intraluminal pH < 4.⁹ Conjugated bile acids can be detected in 75% of reflux patients, mainly at night, but only 2% of aspirates contain concentrations likely to increase mucosal permeability.⁴³ Thus, H⁺ ions and pepsin, probably acting synergistically, are the most important components of the refluxate causing clinically relevant oesophageal damage. These conclusions may not apply to reflux patients after surgery, in whom the pylorus has been compromised or removed. However, studies using simultaneous oesophageal and duodenogastric pH monitoring, possibly combined with new spectrophotometric measurement of bilirubin as a marker of duodenogastric reflux,⁴⁴ are required to address this issue appropriately.

In conclusion, ours is the first study showing that human oesophageal mucosa responds with luminal alkalisation to abnormal amounts of acid reflux. Although our evidence is inferred, we have shown that neither saliva nor duodenogastric contents are the source of this 'alkalinisation' at night. This form of mucosal protection may be extremely important when other methods (gravity, peristalsis, and saliva) of acid clearance are absent. Further studies are needed to prove that this alkalisation indeed arises from secretions of the oesophageal submucosal glands and its mechanisms. It is possible that destruction of these glands by severe oesophagitis or Barrett's oesophagus could be another factor for perpetuating these more severe forms of gastro-oesophageal reflux disease.

We thank Debbie Beam and Linda Pugh for excellent secretarial assistance in the preparation of this manuscript. We also thank Dr Edwin Bradley for his valuable consultation regarding the statistical analyses. Diets were prepared by the General Clinic Research Centre of The University of Alabama at Birmingham through grant number M01 RR00032.

The preliminary results of this study were presented at the annual meeting of the American Gastroenterological Association held in San Francisco, California on May 10-14, 1992.

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