

PAPERS

Effect of topical oesophageal acidification on human salivary and oesophageal alkali secretion

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Abstract

Recent human studies suggest that oesophageal HCO_3^- secretion, in conjunction with salivary HCO_3^- secretion and secondary oesophageal peristalsis, is important for the protection of oesophageal mucosa from refluxed gastric contents. This study evaluated simultaneously the responsiveness of oesophageal and salivary HCO_3^- secretion to oesophageal acidification in eight healthy subjects. A 10 cm segment of oesophagus was perfused at a constant rate of 5 ml/min with a specially designed tube assembly. Saline was used initially, and then 10 mM and 100 mM HCl. The perfusates contained ^3H -polyethylene glycol (PEG) as a concentration marker to determine volumes. Corrections were applied for a small degree of contamination by swallowed saliva and refluxed gastric alkali. Oesophageal perfusion with 10 mM HCl did not cause symptoms (nausea and heartburn), but tripled the oesophageal HCO_3^- output from a baseline of 51 $\mu\text{mol}/10\text{ cm}/10\text{ min}$ ($p=0.021$), while doubling the rate of salivary HCO_3^- secretion from a median basal value of 140 $\mu\text{mol}/10\text{ min}$ ($p=0.021$). Oesophageal perfusion with 100 mM HCl was associated with symptoms of nausea and heartburn in all subjects. The median oesophageal HCO_3^- output increased 32 fold to 1659 $\mu\text{mol}/10\text{ cm}/10\text{ min}$ (interquartile range 569 to 3373; $p=0.036$), and salivary HCO_3^- secretion approximately tripled from basal values ($p=0.036$). In conclusion, oesophageal acidification stimulates both salivary and oesophageal HCO_3^- secretion, responses which may be protective to the oesophageal epithelium.

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Keywords: oesophageal secretion, saliva, bicarbonate, acid.

The stomach of vertebrates and mammals secretes a highly corrosive mixture of acid and pepsin, which is a potential threat to the integrity of the upper gastrointestinal mucosa. Reflux of gastric contents into the lower oesophagus is associated with either transient

relaxation of the lower oesophageal sphincter or prolonged absence of sphincter pressure.¹ Oesophageal cells are susceptible to acid attack which results in decreased ionic transport, cell swelling and death, and a local inflammatory response.^{2–4} Acid reflux initiates secondary peristaltic waves,^{5,6} which serve to sweep acid back into the stomach, thereby reducing its volume and the length of time it is in contact with oesophageal mucosa. The oesophageal juxtamucosal pH remains low, however, until neutralisation of acid is achieved by alkali from either swallowed saliva,^{7–9} or oesophageal secretion.^{10–12}

The human stomach and duodenum respond to topical acid by an increased rate of epithelial HCO_3^- transport,^{13,14} and such an 'autoregulatory mechanism' may be protective to gastroduodenal mucosa. Several groups have already reported increased salivary secretion in response to oesophageal acidification, which in some instances is associated with symptoms such as heartburn and nausea.^{7,8,12,15} The present experiments were designed to examine the oesophageal and salivary alkali responses to oesophageal acidification.

Methods

Basal secretion of alkali from salivary glands and the normal human oesophagus was quantified using a previously described and validated perfusion technique.¹² In brief, a 10 cm segment of oesophagus was perfused at its proximal end with saline containing a non-absorbable marker (^3H -polyethylene glycol (PEG), 12.5 $\mu\text{Ci}/\text{l}$) at a rate of 5 ml/min. Samples were continually aspirated from the distal end of the segment (35 cm from the incisors) and the alkali concentration was determined by back titration. Salivary contamination was minimised by aspirating the buccal cavity continuously and requesting subjects not to swallow during experiments. Inadvertently swallowed saliva was recognised by the appearance of amylase in oesophageal aspirates and corrections were applied for swallowed aliquots of salivary HCO_3^- . The stomach was perfused with a second marker solution (^{14}C -PEG 12.5 $\mu\text{Ci}/\text{l}$, 5 ml/min) and corrections were applied for any oesophageal contamination by refluxed gastric fluid, from the appearance of the gastric marker in oesophageal aspirates.

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TABLE I Baseline experimental data in eight subjects, before acid perfusion of the oesophagus

Subject			% marker recovery	Salivary HCO_3^- contamination ($\mu\text{mol}/10 \text{ min}$)	Gastric reflux contamination ($\mu\text{mol}/10 \text{ min}$)	Net oesophageal HCO_3^- secretion ($\mu\text{mol}/10 \text{ cm}/10 \text{ min}$)	Total salivary HCO_3^- secretion ($\mu\text{mol}/10 \text{ min}$)
No	Sex	Periods of analysis					
1	M	4	73.0	0.1	0.0	17.5	17.9
2	F	3	47.2	16.8	0.1	84.5	1079.9
3	M	1	45.5	27.3	0.1	54.2	101.8
4	F	4	63.3	75.7	0.0	47.9	178.4
5	F	3	70.7	14.3	0.0	39.4	478.6
6	F	4	53.6	0.7	0.0	15.7	62.9
7	M	2	64.0	0.9	0.1	54.5	221.8
8	F	2	24.1	0.3	0.1	56.6	27.0
Median			58.4	7.6	0.1	51.0	140.1

SUBJECTS

Experiments were performed in eight healthy subjects (three men and five women aged between 20 and 27 years) with no evidence of current or previous gastrointestinal disease. Subjects specifically denied dyspeptic symptoms such as heartburn or nausea, had no gastrointestinal investigations, and had not taken any medication for dyspeptic symptoms in the past. Informed written consent was obtained and the experimental procedures were approved by the Salford Health Authority Ethics Committee.

EXPERIMENTAL DESIGN

Salivary and oesophageal alkali secretions were measured during a 60 minute basal period by perfusing a 10 cm segment of oesophagus with saline containing ^3H -PEG. The perfusate was then changed to an identical solution containing 10 mM HCl and perfused at the same rate of 5 ml/min. To minimise contamination by any residual saline from the original perfusate, the test segment was flushed with a 50 ml bolus of the 10 mM HCl solution during the first five minutes of acid perfusion. Aspirates were discarded during this five minute period. Salivary, oesophageal, and gastric aspirates were then collected during a further 10 minute period of acid perfusion. Alkali secretion into the oesophageal segment was calculated from direct titrations of the acid perfused and oesophageal aspirate. After a 30 minute 'washout period' when the acid perfusate was replaced by the saline perfusate, the same procedure was repeated using 100 mM HCl in six of the eight subjects.

Total oesophageal HCO_3^- content during the 10 minutes of acid perfusion could then be

calculated from the oesophageal volume (determined by marker dilution) and reduction in titratable acidity of the acid perfusate caused by oesophageal alkali. In order to derive oesophageal HCO_3^- secretion, corrections were applied for swallowed saliva and refluxed gastric content as previously described.¹²

ASSUMPTIONS AND VALIDATION

(1) During acidification, amylase was denatured and enzymatic activity could not be detected within the oesophageal aspirates. It was therefore assumed that the proportion of saliva swallowed into the test segment remained constant during the entire experiment.

(2) In order to assess the efficiency of acid flushing in reducing residual saline within the test segment, experiments were conducted to measure the residual volume in four subjects. After a basal period of perfusion with labelled saline, the perfusate was changed to unlabelled saline for 15 minutes. During the initial five minutes of this 15 minute period, the segment was flushed with a 50 ml bolus of unlabelled saline. From measurements of the concentration of ^3H -PEG found in the final 10 minutes of saline perfusion, the residual volume of the initial perfusate could be calculated and its potential impact on oesophageal HCO_3^- output determined.

STATISTICS

The results are expressed as medians and interquartile ranges (Q1 to Q3). The significance of change in paired data was assessed using the Wilcoxon signed rank test, and p values of less than 0.05 were assumed to be significant. NS denotes non-significant changes.

TABLE II Oesophageal volume increased in only three of six subjects (3, 4, and 5) during perfusion with 100 mM HCl. In these subjects the maximum possible contribution of swallowed saliva to measured oesophageal alkali content is estimated from the increase in oesophageal volume multiplied by the salivary alkali concentration. Since the oesophageal volume did not increase in subjects 2, 6, and 8, the possible increase in swallowed salivary alkali was not calculated and has been marked by * in the table

Subject no	Oesophageal volume ($\text{ml}/10 \text{ min}$)		Oesophageal alkali concentration (mM)		Salivary alkali concentration during acid perfusion (mM)	Increased oesophageal HCO_3^- with 100 mM HCl ($\mu\text{mol}/10 \text{ min}$)	Possible contribution from \uparrow salivary swallowing ($\mu\text{mol}/10 \text{ min}$)
	Basal	Acid	Basal	Acid			
2	61.2	56.0	0.36	22.88	22.18	1136	*
3	56.9	70.9	1.41	49.14	6.76	3303	95
4	57.8	65.4	2.13	36.46	11.82	2049	90
5	52.6	57.4	1.02	16.01	14.46	533	69
6	52.3	53.7	0.30	10.42	9.05	542	*
8	62.7	55.3	0.95	62.02	7.22	3361	*

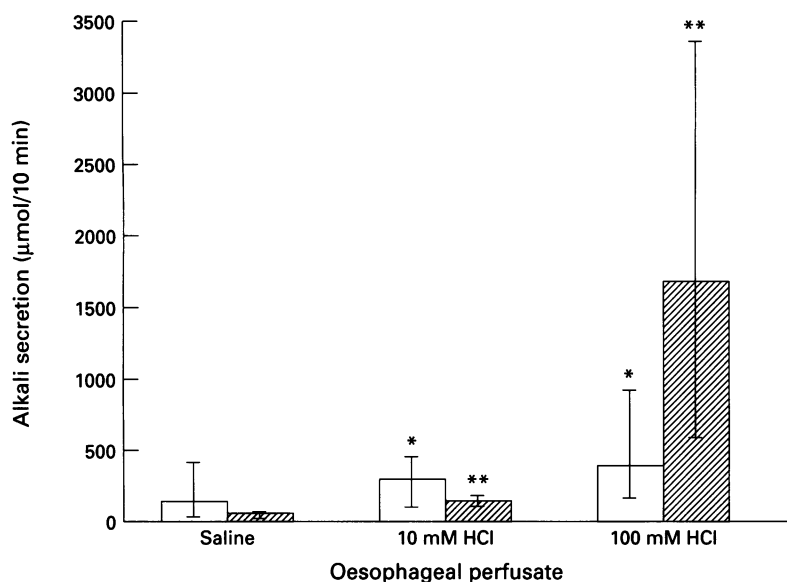


Figure 1: Salivary (open bars) and oesophageal (hatched bars) alkali outputs in response to oesophageal acidification. Medians and interquartile ranges are shown with significant changes from baseline indicated (* and **, $p \leq 0.036$).

Results

BASELINE

Basal experimental data on the eight subjects undergoing acid perfusion are outlined in Table I and net oesophageal alkali output includes correction for swallowed saliva and gastric reflux.

DURING ACID PERFUSION

Validation data

Calculation of swallowed saliva during acid perfusion. Further support for the validity of the assumption that the proportion of swallowed saliva remains unchanged during these experiments, was obtained from the calculated oesophageal volumes. If excess (unrecognised) saliva was swallowed during acid perfusion periods, an increase in the calculated

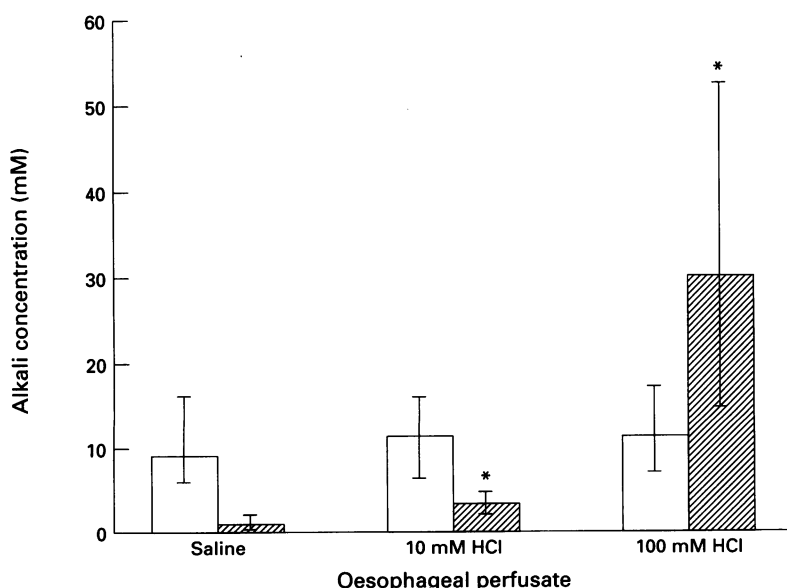


Figure 2: Salivary (open bars) and oesophageal (hatched bars) alkali concentrations at baseline (saline perfusion), and in response to oesophageal perfusion with 10 mM and 100 mM HCl. Medians and interquartile ranges are shown with significant changes from basal values indicated (*, where $p \leq 0.036$).

oesophageal volume would be expected. No volume increase was observed in subjects 2, 6, and 8, and it is unlikely that the quantity of swallowed saliva changed between basal and acid perfusion periods in these. Subjects 3, 4, and 5, did exhibit a slight increase in oesophageal volume during 100 mM HCl perfusion, which could be explained either by an increase in undetected swallowed saliva or enhanced oesophageal secretion (Table II).

Dilutional error. Validation 'flushing' experiments in four subjects showed a median residual volume of 2.5 ml (range 0.7 to 5.0) which could cause a 5% dilution of acid infused into the oesophagus over 10 minutes. Net oesophageal alkali output would therefore be overcalculated by approximately 5% but correction for this possibility did not alter the overall significance of the acidification response (Table III).

Acidification response. Median net oesophageal alkali output trebled from a basal median of 51 $\mu\text{mol}/10 \text{ cm}/10 \text{ min}$ (Q1 to Q3=23 to 56) to 140 $\mu\text{mol}/10 \text{ cm}/10 \text{ min}$ during perfusion with 10 mM HCl (Q1 to Q3=91 to 174, $p=0.021$). With 100 mM HCl, oesophageal HCO_3^- secretion showed a 32 fold increase from basal levels to 1659 $\mu\text{mol}/10 \text{ cm}/10 \text{ min}$ (Q1 to Q3=569 to 3373, $p=0.036$; Table III and Fig 1). The proportionate increase in oesophageal HCO_3^- output with 100 mM HCl, compared with basal, was much greater than the salivary response. Correction for residual saline in the oesophagus did not significantly influence these findings (Table III). The increased oesophageal alkali output was due to a threefold increase in oesophageal HCO_3^- concentrations with 10 mM HCl ($p=0.021$), and a 30 fold increase in oesophageal HCO_3^- concentrations with 100 mM HCl ($p=0.036$; Fig 2). Oesophageal volumes did not significantly change from basal values during acid perfusion periods (Fig 3).

Salivary HCO_3^- secretion doubled from a median basal level of 140 $\mu\text{mol}/10 \text{ min}$ (Q1 to Q3=36 to 414), to 291 $\mu\text{mol}/10 \text{ min}$ (Q1 to Q3=86 to 450) during oesophageal perfusion with 10 mM HCl ($p=0.021$), and nearly tripled to 373 $\mu\text{mol}/10 \text{ min}$ (Q1 to Q3=150 to 907) during oesophageal perfusion with 100 mM HCl ($p=0.036$; Fig 1). The increase in salivary HCO_3^- output occurred because of a 23% increase in median salivary volume during oesophageal perfusion with 10 mM HCl, and a 100% increase during oesophageal perfusion with 100 mM HCl (Fig 3). Salivary alkali concentrations did not significantly change during acidification (Fig 2).

Symptoms of nausea and heartburn were absent during perfusion with 10 mM HCl, but were present in all subjects during perfusion with 100 mM HCl.

Discussion

The present perfusion studies were designed to measure both salivary and oesophageal HCO_3^- secretion, and the response to oesophageal acidification. They represent the first attempt to explore possible 'acid

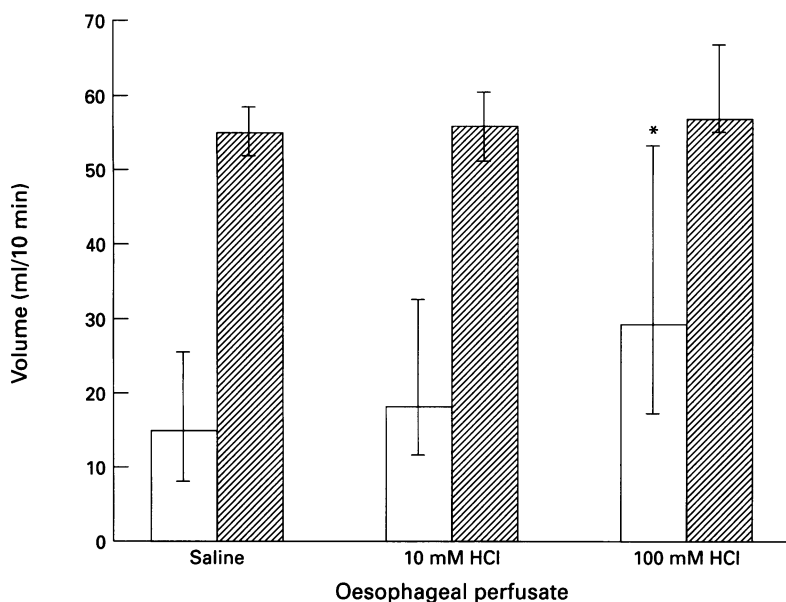


Figure 3: Salivary (open bars) and oesophageal (hatched bars) volumes at baseline (saline perfusion), and in response to oesophageal perfusion with 10 mM and 100 mM HCl. Medians and interquartile ranges are shown with significant changes from baseline indicated (*, where $p=0.036$).

autoregulation' of human oesophageal alkali secretion.

Salivary HCO_3^- secretion doubled in response to oesophageal perfusion with 10 mM HCl, in the absence of perceived symptoms such as nausea and heartburn. When 100 mM HCl were infused into the oesophagus, all subjects perceived symptoms and the rate of salivary alkali secretion approximately tripled. This response was similar to previous experimental results, where HCO_3^- concentrations were determined from measurements of pH and pCO_2 .¹² There are conflicting data on the importance of symptom perception in mediating the salivary response to oesophageal acid, but such discrepancies can be reconciled if the degree of oesophageal exposure to acid is taken into consideration. It seems likely that the oesophageal acid threshold required to increase salivary secretion, is below that required for the generation of symptoms in each subject. Activated chemoreceptors in the oesophagus may stimulate salivary glands via neural reflex arcs, before the cerebral cortex is stimulated. Helm *et al* found that salivary secretion increased only in subjects who

developed symptoms as a consequence of oesophageal acid perfusion.⁸ They suggested that symptoms were important in mediating this response, the so called 'oesophago-salivary reflex'. Previous studies by Sonnenberg had, however, shown that oesophageal acidification could enhance salivary flow in the absence of symptoms.¹⁵ In these experiments the human oesophagus was perfused with 10 mM HCl at a low rate (16.7 ml/h) and salivary secretion increased in asymptomatic subjects after an hour of acid perfusion. In the present studies, a higher rate of perfusion with 10 mM HCl (5 ml/min) increased salivary HCO_3^- output within 15 minutes in the absence of symptoms. In our own previously reported studies, oesophageal perfusion with 100 mM HCl at a rate of 5 ml/min for five minutes, produced no symptoms or change in salivary secretion, but when perfusion was continued for 30 minutes, 10 of 12 subjects noticed symptoms and exhibited an increased salivary alkali output.¹² Interestingly, the two asymptomatic subjects had a very high basal rate of salivary HCO_3^- secretion. In the present studies, all subjects experienced symptoms between five and 10 minutes after the beginning of acid perfusion.

Median oesophageal HCO_3^- secretion nearly tripled in response to perfusion with 10 mM HCl; a response which, although significant, was of a lesser magnitude than the increase in salivary alkali secretion. When the oesophagus was perfused with 100 mM HCl, there was a very large rise in local oesophageal alkali output (a 32 fold increase), which far exceeded the salivary response. These effects are almost certainly due to local stimulation by H^+ ions rather than a volume effect, since the perfusion rates were identical for basal and acid perfusion periods, and there were no significant changes in oesophageal volume (Fig 2). This study shows for the first time that the human oesophagus is capable of responding to an acid load by significantly increasing its output of alkali. Such an 'autoregulatory' response may be important in mucosal defence, and assumes relatively greater significance in the presence of prolonged oesophageal exposure to acid, or in the absence of swallowed saliva during the nocturnal period. Similar protective responses have previously been shown in the human stomach¹³ and duodenum.¹⁴

It was not possible in these studies to examine an isolated segment of oesophagus, since in pilot studies subjects were intolerant of attempts to isolate and perfuse a segment between two occluding balloons. We were also unable to obtain 100% recovery of the infused marker, and therefore the methodology is quite complex. The accuracy of measurements of oesophageal volume, contamination by swallowed saliva and refluxed gastric fluid, and calculation of alkali content of recovered samples, have all been previously validated.¹²

Two further problems, which could potentially confound the results, were encountered with these studies. Firstly, amylase could not be measured in the acidic oesophageal aspirates, presumably due to inactivation of its enzymatic activity. The proportion of saliva which

TABLE III Net oesophageal alkali output in eight subjects, when the oesophagus is perfused with saline, 10 mM HCl, or 100 mM HCl. The data are presented with and without correction for dilutional error. Subjects 1 and 7 did not proceed to the stage of oesophageal perfusion with 100 mM HCl, and the missing data are shown by * in the table

Subject	Net oesophageal alkali secretion ($\mu\text{mol}/10 \text{ cm}/10 \text{ min}$)				
	Saline	10 mM HCl		100 mM HCl	
		Uncorrected	Corrected	Uncorrected	Corrected
1	17.9	128.1	121.7	*	*
2	84.5	177.0	166.0	1220.9	1156.7
3	54.2	165.0	137.1	3357.0	3182.8
4	47.9	116.3	101.8	2097.1	1977.8
5	39.4	0.0	0.0	573.3	527.3
6	15.7	82.4	78.2	557.7	529.7
7	54.5	217.4	206.4	*	*
8	56.6	151.1	143.3	3417.9	3246.5
Median	51.0	139.6	129.4	1659.0	1567.5
p Value		0.021	0.021	0.036	0.036

was swallowed was therefore measured for each subject during basal periods, and assumed to remain constant during the course of each experiment. Supporting evidence for the validity of this assumption came from previous experiments when a cholinergic stimulus increased median salivary secretion by 35%, but the proportion of swallowed saliva did not change significantly, with a median of 6% during basal periods and 1.2% during cholinergic stimulation. Furthermore, if this assumption were incorrect, any unrecognised swallowed saliva would increase the oesophageal volume, and such an increase was observed in only three of six subjects during perfusion with 100 mM HCl. Even if all the increase in oesophageal volume were assumed to be due to swallowed saliva, its contribution to the oesophageal alkali content would be inadequate to explain the significant increase observed after acidification (Table II). This implies that oesophageal, rather than undetected salivary HCO_3^- was responsible for the oesophageal HCO_3^- response to infused acid.

The second potential confounding problem in these experiments, is whether any residual saline remained in the oesophageal test segment after the flushing procedure, thereby leading to simple dilution of infused acid, and an overestimation of oesophageal alkali content. Validation experiments in four subjects calculated that this could lead to a 5% over calculation of oesophageal alkali secretion, which was insufficient to influence the significance of values (Table III).

In summary, while these experiments lack technical perfection, the validation data provide support for the accuracy of both basal and acid stimulated oesophageal alkali output. These studies show that salivary HCO_3^- secretion is enhanced by acid perfusion of the oesophagus, and that this may occur independent of symptoms. They show, for the first time, that human oesophageal HCO_3^-

secretion is responsive to topical acid, which implies a protective autoregulatory mechanism. Both secretory responses to oesophageal acid occur in dose-dependent manner, and may well be relevant to the protection of the oesophageal mucosa against acid mediated injury.

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