

PAPERS

Measurement of bicarbonate output from the intact human oesophagus

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Abstract

Injury of the oesophageal mucosa can result from exposure to refluxed gastric acid and pepsin. Competence of the lower oesophageal sphincter and peristaltic activity serve to reduce contract time between luminal acid and oesophageal mucosa, but intraluminal neutralisation of residual acid by bicarbonate may also be important in preventing oesophageal mucosal injury. Whereas swallowed saliva contains bicarbonate, recent experiments have also demonstrated alkali secretion from the mammalian oesophagus. Bicarbonate secretion from the human oesophagus was therefore examined with an intubation technique and perfusion of the oesophagus with a non-absorbable marker. Saliva, gastric, and oesophageal aspirates were collected and bicarbonate concentrations determined by measurements of pH and pCO₂ or by back titration. In 32 normal subjects (17 women, 15 men) median basal oesophageal bicarbonate secretion determined by a pH/pCO₂ method was 416 (range 139-1050) $\mu\text{mol}/\text{hour}/10\text{ cm}$. In a subgroup of 15 experiments median oesophageal bicarbonate output was 489 (range 157-1033) $\mu\text{mol}/\text{hour}/10\text{ cm}$ (pH/pCO₂ method) compared with a median alkali output of 563 (range 135-799) $\mu\text{mol}/\text{hour}/10\text{ cm}$ as determined by back titration. The difference was not significant. Salivary contamination of the oesophagus accounted for 25% of all bicarbonate measured within the oesophagus and refluxed gastric bicarbonate accounted for 2.5%. Bicarbonate secretion from the normal human oesophagus may, in combination with swallowed salivary bicarbonate, play a part in preventing oesophageal mucosal damage due to refluxed gastric acid and pepsin.

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of gastric contents into the oesophagus. Intra-abdominal pressure upon the abdominal portion of the oesophagus also minimises reflux after meals and with changes in posture, and the diaphragm may also contribute a little to oesophageal continence. Absence of lower oesophageal sphincter pressure facilitates acid reflux into the oesophagus either by transient relaxations of the sphincter in 82% of reflux episodes or else by more prolonged absence of sphincter pressure in 13% of reflux episodes.²

Once acid has entered the lower oesophagus peristaltic waves initiated within the body of the oesophagus sweep the bolus away into the stomach,³ but may still leave a juxtamucosal acidic environment in the lower oesophagus. Intraluminal neutralisation of acid occurs in part by swallowed saliva and previous studies have shown enhancement of salivary flow in response to dyspeptic symptoms caused by acid entering the lower oesophagus.⁴ Intrinsic bicarbonate secretion from the oesophageal mucosa may also contribute to intraluminal acid neutralisation, and such secretion has been shown in vivo in the opossum oesophagus, which is known to contain submucous glands.⁵ Rabbit oesophagus is devoid of these glands and does not secrete significant quantities of alkali, which suggests the importance of these glands for oesophageal bicarbonate secretion. Similar glands are present throughout the length of the oesophagus in humans⁶ and bicarbonate secretion has previously been found in human stomach and duodenum in vivo.^{7,8} Experiments were therefore designed to try to show the existence of bicarbonate secretion by the normal human oesophagus. Such alkaline secretion may be important in preventing mucosal injury in some patients with gastro-oesophageal reflux disease.

Methods

The experimental technique involved continuous perfusion of an open segment of oesophagus and the stomach with saline containing non-absorbable markers. This was by means of a multilumen catheter. Samples were continually aspirated from the oesophagus and stomach, pooled at 15 minute intervals, and analysed for bicarbonate and alkali content. Refluxed gastric alkali was determined by appearance of gastric marker in oesophageal aspirates. Saliva was continually aspirated from the mouth and salivary bicarbonate contamination of the oeso-

Gastro-oesophageal reflux disease affects over 10% of the population in the United Kingdom, and symptoms are caused by the presence of excess acid entering the lower oesophagus from the stomach and damaging the surface epithelium. Gastro-oesophageal reflux disease is influenced by the magnitude and duration of acid reflux episodes,¹ and a number of mechanical factors serve to prevent acid reflux in health. The lower oesophageal sphincter acts as a valve allowing passage of swallowed liquid and food into the stomach, while preventing regurgitation

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phagus determined by appearance of amylase in oesophageal aspirates. The oesophageal output of bicarbonate or alkali could then be determined.

SUBJECTS

Experiments were performed on 32 healthy volunteers with no evidence of current or past gastrointestinal disease. Seventeen subjects were women and 15 were men. The median age was 22 (range 18–32) years. Informed written consent was obtained and the experimental techniques were approved by the Salford Health Authority Ethics Committee.

MULTILUMEN CATHETER

A multilumen polyvinyl chloride tube was constructed from a French size 16 nasogastric tube (internal diameter 3.5 mm, external diameter 5 mm) with an infusion port cemented 10 cm above the aspiration holes of the nasogastric tube by means of tetrahydrofuran (Fisons, Loughborough). The infusion lines were 1 mm internal diameter and 1.6 mm external diameter (Portex, Hythe). A size 8 nasogastric tube (2 mm internal diameter, 2.5 mm external diameter) was cemented on to the assembly with the aspiration holes 10 cm above the lower infusion port. A second infusion port was cemented on to the assembly 10 cm above the aspiration holes of the size 8 nasogastric tube, and for *in vivo* validation studies a third infusion port was cemented on to the assembly at the same point to allow introduction of known amounts of sodium bicarbonate into the upper oesophagus. The intact catheter was 7.5 mm × 5 mm in cross sectional external diameter. After the first eight experiments, a second size 8 nasogastric tube was cemented on

to the assembly with aspiration holes at the same position as the other size 8 nasogastric tube, and both were connected externally by a Y connector. This was to facilitate more efficient aspiration of fluid from the lower oesophagus. Cross sectional diameters of this ovoid tube were 10 mm × 6 mm.

EXPERIMENTAL DESIGN

Subjects were studied after a nine hour fast and the multilumen tube was passed orally and positioned with the oesophageal aspiration ports (size 8 nasogastric tubes) 35 cm from the incisors. Positioning was validated fluoroscopically in six subjects, with no adjustment of tube required, and fluoroscopy was therefore not considered essential in subsequent subjects.

Experiments were performed with subjects in a semireclined position and the oesophagus was perfused with [³H] polyethylene glycol ([³H]PEG; 12.5 µCi/litre in 0.9% sodium chloride adjusted to pH 7.0 with 0.2 M sodium hydroxide) at a rate of 5 ml/minute. The stomach was similarly perfused with [¹⁴C]PEG (12.5 µCi/litre in 0.9% sodium chloride at pH 7.0). Oesophageal and gastric contents were continuously aspirated (–10 mm Hg), collected, and pooled at 15 minute intervals for analysis while fresh 1 ml samples of oesophageal and gastric fluid were collected from the aspiration tubes midway through each 15 minute collection for immediate measurement of pH and pCO₂. Collections were commenced after an initial 45 minute equilibration period during which the oesophagus and stomach were perfused and aspirates discarded. Throughout each experiment subjects were instructed to avoid swallowing saliva, which was collected in 15 minute aliquots by continuous buccal aspiration. Samples (1 ml) were taken for measurement of salivary pH and pCO₂ and the remainder was retained for further analysis.

One hour before intubation subjects were given an oral dose of ranitidine (300 mg), and acid suppression was maintained throughout the experiment by intravenous ranitidine (50 mg/hour) administered by a cannula (Venflon size 17G) sited in a forearm vein (Fig 1).

ANALYSIS AND CALCULATIONS

Bicarbonate concentrations

The pH and pCO₂ of fresh aspirates were immediately measured with a Corning 170 pH/blood gas analyser (Ciba Corning, Essex). When pH was outside the calibrated range (6.838 to 7.382) it was recorded manually by a glass electrode attached to a standard pH meter (PHM 82, Radiometer, Copenhagen) and calibrated by standard buffer solutions at pH 4, 7, and 10. With these values the oesophageal and gastric HCO₃⁻ concentrations (mmol/litre) were calculated from the equations:

$$\text{HCO}_3^- \text{ as CO}_2 = \text{pCO}_2 \times \text{solubility constant} \quad (1)$$

$$\text{Free HCO}_3^- \text{ from pH} = \text{pKa} + \log \frac{\text{free bicarbonate}}{\text{pCO}_2 \times \text{solubility constant}} \quad (2)$$

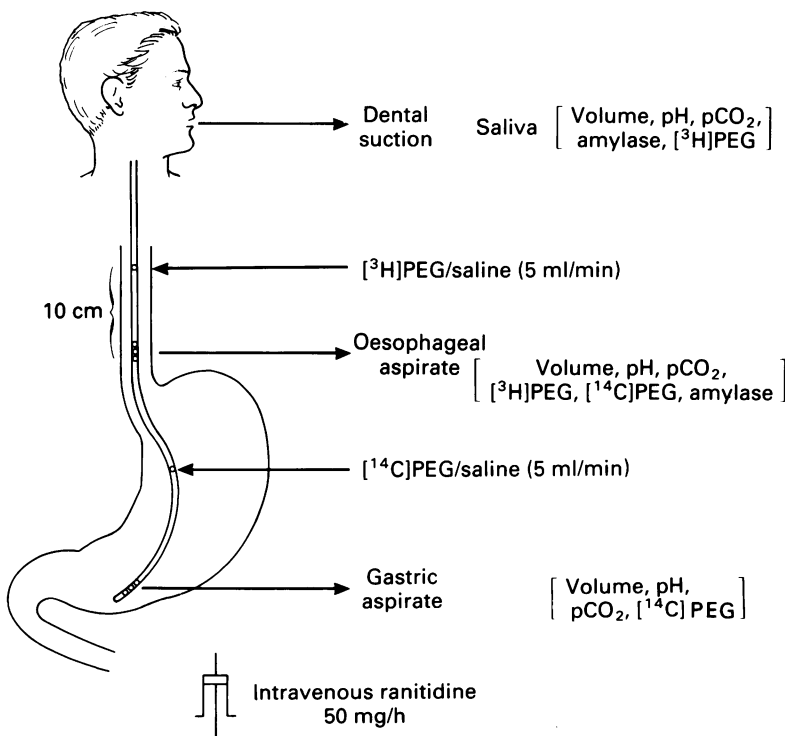


Figure 1: Diagram of method used to measure total oesophageal bicarbonate content, swallowed salivary bicarbonate, and refluxed gastric bicarbonate in humans.

$$\begin{aligned} \text{Total HCO}_3^- \text{ concentration} &= (1) + (2) \\ &= p\text{CO}_2 \times \text{solubility constant} (1 + 10^{\text{pH} - \text{pKa}}) \\ \text{where solubility constant} &= 0.031; \text{pKa} = 6.1 \end{aligned}$$

Oesophageal volume and volume of gastric refluxate

The oesophageal volume represents that volume available within which bicarbonate of oesophageal origin can be distributed. It includes volume of oesophageal perfusate (75 ml/15 minutes), volume of oesophageal secretion, volume of swallowed saliva, and volume of gastric refluxate.

In a steady state perfusion system:

$$C_1 \times V_1 = C_2 \times V_2$$

where C_1 = concentration of non-absorbable marker [^3H]PEG in infusate; V_1 = volume of infusate (75 ml/15 minutes); C_2 = concentration of non-absorbable marker [^3H]PEG in oesophageal aspirates.

Hence the oesophageal volume V_2 can be determined:

$$\begin{aligned} \text{Oesophageal volume} &= \text{volume infused (75 ml/15 min)} \times \\ &\frac{[\text{^3H}] \text{ PEG] infusate}}{[\text{^3H}] \text{ PEG] oesophageal aspirate}} \end{aligned}$$

The volume of gastric refluxate can be determined by appearance of the gastric non-absorbable marker, [^{14}C]PEG, in oesophageal aspirates:

$$\begin{aligned} \text{Volume of gastric refluxate} &= \text{Oesophageal volume} \times \\ &\frac{[\text{^{14}C}] \text{ PEG] oesophageal aspirate}}{[\text{^{14}C}] \text{ PEG] gastric aspirate}} \end{aligned}$$

The [^3H] and [^{14}C]PEG concentrations in oesophageal and gastric samples were determined by dual isotope counting with an LKB β scintillation counter (LKB Wallac 81000, Sweden). Determinations of oesophageal volume and volume of gastric refluxate were by adaptations of previously described methods.^{7,9}

The oesophageal HCO_3^- content and HCO_3^- refluxed from the stomach could then be calculated:

$$\begin{aligned} \text{Oesophageal HCO}_3^- \text{ content (}\mu\text{mol/15 min)} &= \\ &\text{oesophageal volume (ml/15 min)} \times \\ &\text{oesophageal HCO}_3^- \text{ concentration (mmol/l)} \\ \text{Refluxed gastric HCO}_3^- &= \\ \text{volume refluxed} \times \text{gastric HCO}_3^- \text{ concentration} \end{aligned}$$

Salivary HCO_3^- contamination

The pH and $p\text{CO}_2$ of saliva were measured at 15 minute intervals to determine HCO_3^- concentration. Amylase concentrations were measured in paired salivary and oesophageal fluid samples by an automated enzymatic method.^{10,11} Contamination of oesophageal fluid by swallowed salivary HCO_3^- was determined from the equations:

$$\text{Contaminating salivary HCO}_3^- = \text{volume of contaminating saliva} \times \text{salivary HCO}_3^- \text{ concentration}$$

$$\text{Salivary HCO}_3^- \text{ concentration} = p\text{CO}_2 \times 0.031 (1 + 10^{\text{pH} - \text{pKa}})$$

$$\text{Volume of contaminating saliva} = \text{oesophageal volume} \times$$

$$\frac{[\text{amylase}] \text{ oesophageal aspirate}}{[\text{amylase}] \text{ saliva}}$$

Hence net oesophageal bicarbonate secretion can be determined:

$$\begin{aligned} = \text{total bicarbonate within oesophagus} &- (\text{refluxed gastric HCO}_3^- \\ &+ \text{swallowed salivary HCO}_3^-) \end{aligned}$$

Also, total salivary bicarbonate secretion could be calculated:

$$\begin{aligned} = (\text{volume of swallowed saliva} + \text{volume of buccally aspirated saliva}) \\ \times [\text{HCO}_3^-] \text{ saliva} \end{aligned}$$

VALIDATION STUDIES

In vivo infusion of sodium bicarbonate

Experiments were performed on seven subjects to determine the accuracy of the method for measuring oesophageal bicarbonate content. After a 45 minute equilibration period, salivary oesophageal and gastric aspirates were collected and pooled at 15 minute intervals for one hour, and pH and $p\text{CO}_2$ were measured in fresh samples to determine basal oesophageal bicarbonate secretion as already described. During the next 15 minute period a 5 ml bolus containing a known amount of sodium bicarbonate (100, 200, 500, or 1000 μmol) was infused slowly and the total amount of bicarbonate determined by dilution of [^3H]PEG and pH and $p\text{CO}_2$ of oesophageal aspirates. In most subjects a second bolus containing a different quantity of bicarbonate to the first was infused 30 minutes after the first bolus. The 15 minute basal rate of oesophageal bicarbonate secretion was subtracted from the calculated bicarbonate outputs during periods when exogenous bicarbonate was infused, to calculate the quantity of infused bicarbonate.

Measurements of amylase in oesophageal fluid

Because saliva contains high concentrations of bicarbonate, even small volumes of swallowed saliva may significantly contaminate the oesophageal segment. Confidence in the amylase measurements in oesophageal fluid as a marker of salivary contamination is therefore essential. The major potential confounder in this analysis is pH, because pH values of oesophageal fluid are about 1.0 log point lower than pH values of saliva. Studies were therefore required to exclude a major effect of pH on measurements of amylase.

Saliva and oesophageal fluid were taken from two subjects and pooled. pH of the saliva pool was 8.20 whereas pH of the oesophageal pool was 7.20. Each pool was divided into two subgroups and one saliva subpool was titrated down to pH 7.20 with 0.1 N HCl and one oesophageal fluid subpool was titrated up to pH 8.20 with 0.2 N NaOH. In each of the four subpools, 20 or 21 estimations of amylase activity were performed. Oesophageal fluid was undiluted but salivary samples were diluted to 1 in 30 to bring amylase values into the known linear range for the assay. Amylase concentrations in saliva and oesophageal fluid were compared at both pH 7.20 and 8.20.

Bicarbonate and alkali output: comparison of the pH/ $p\text{CO}_2$ method and the back-titration method

In 15 subjects oesophageal bicarbonate secretion was determined by the pH/ $p\text{CO}_2$ technique described earlier. Also, alkali concentrations were determined in salivary, oesophageal, and

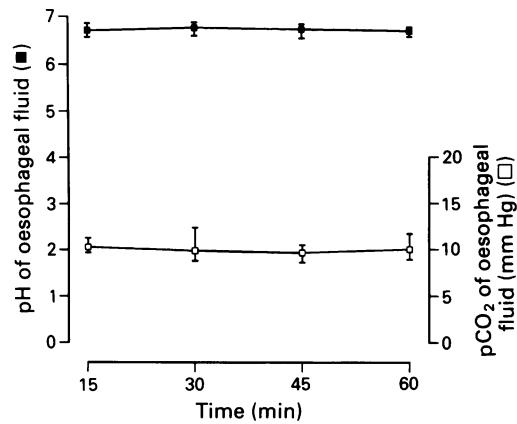


Figure 2: Basal oesophageal fluid pH and pCO₂ in 32 subjects over 60 minutes. Median values and 95% confidence intervals are given.

gastric samples by a previously described sensitive back titration technique.⁹ Briefly, a known excess of H⁺ ions (250 µl of 0.1 M H₂SO₄ = 50 µEq H⁺) was added to 2 ml of sample and 5 ml of deionised H₂O. This was gassed with CO₂ free nitrogen (washed through saturated barium hydroxide solution) for five minutes to remove dissolved CO₂ from the sample, which was then titrated up to the reaction endpoint at pH 8.4 (pH meter PHM 82 linked to an autoburette ABU 80 and controlled by an autotitrator TTT 80 all units Radiometer, Copenhagen) with 15 mM NaOH. The alkali content of each sample was determined from the volume of titrant required to reach the reaction endpoint.

Net oesophageal alkali output was calculated from the previously described volume measurements.

In vitro alkali studies

To determine the accuracy of back titration in determining alkali concentrations, triplicate measurements of alkali concentrations were performed on standard bicarbonate solutions (1, 2, 2.5, 5, 10, 15, 20, and 25 mM sodium bicarbonate), deionised water, and standard oesophageal perfusate containing [³H]PEG and titrated to pH 7.0 with 0.2 M NaOH.

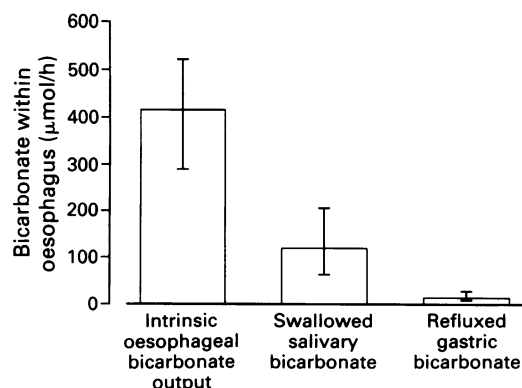


Figure 3: Basal bicarbonate secretion from a 10 cm segment of oesophagus, and salivary and gastric bicarbonate contamination of the oesophagus in 32 subjects. Median values and 95% confidence intervals are given.

ACID PERFUSION STUDIES

After a 45 minute equilibration period and a further 60 minutes during which time basal salivary bicarbonate secretion was determined, the oesophageal perfusion solution was changed from saline (containing 12.5 µCi/litre [³H]PEG) to 0.1 N hydrochloric acid (containing 12.5 µCi/litre [³H]PEG). Acid was perfused at a rate of 5 ml/minute for five minutes in eight subjects and for 30 minutes in 12 subjects. During this time it was not possible to determine oesophageal bicarbonate outputs by the pH/pCO₂ method as the pH was below 6.1. The salivary secretory response to oesophageal acidification was, however, examined.

STATISTICAL ANALYSIS OF DATA

The data were analysed on the computer with Minitab 7.1. As most were not normally distributed, results are expressed as median values with 95% confidence intervals (95% CIs). Significance levels for comparative data were determined by the Wilcoxon signed rank test. Independent data populations such as the percentage marker recovery from single and twin lumen oesophageal aspiration assemblies were examined for significant differences with the Mann-Whitney test.

Results

OESOPHAGEAL MARKER RECOVERY

Percentage recovery of the oesophageal marker was determined from the volume of the 15 minute oesophageal aspirates and their [³H]PEG concentrations. In the first eight subjects median recovery of the oesophageal marker from the single oesophageal lumen aspiration assembly was 26.4% (95% CI 15.5–37.0). Efficiency of recovery increased in the subsequent 24 subjects with the twin lumen oesophageal aspiration assembly, with a median marker recovery of 48.2% (95% CI 42.7–56.9).

BASAL BICARBONATE SECRETION IN HUMAN OESOPHAGUS

After a 45 minute equilibration period, bicarbonate content of the oesophagus and contamination by swallowed salivary bicarbonate and refluxed gastric bicarbonate were measured over 60 minutes. Intragastric pH was maintained between pH 6 and 7 with ranitidine. Oesophageal fluid pH was consistent with median pH values of 6.71 to 6.66 over 60 minutes. More variability in corresponding pCO₂ values were found with medians of 10.3 to 10.0 mm Hg over 60 minutes (Fig 2). Net oesophageal bicarbonate secretion in 32 subjects, after deductions for contaminating salivary and gastric bicarbonate, ranged from 139.2 µmol/hour/10 cm to 1050 µmol/hour/10 cm with a median of 416.3 µmol/hour/10 cm (95% CI 290.4–521.8) (Fig 3).

SALIVARY CONTAMINATION OF OESOPHAGEAL CONTENTS

All oesophageal samples in all subjects contained

TABLE I Individual data in 32 subjects indicating number of analysable periods, percentage oesophageal marker recovery, net oesophageal bicarbonate output, and contamination by both swallowed saliva and refluxed gastric fluid

Subject		Periods of analysis	% Marker recovery	Net oesophageal HCO_3^- secretion ($\mu\text{mol/h}/10\text{ cm}$)	Salivary HCO_3^- contamination ($\mu\text{mol/h}$)	Gastric HCO_3^- contamination ($\mu\text{mol/h}$)
No	Sex					
1	M	4	36.6	460.9	216.2	3.0
2	M	4	24.4	377.1	71.4	16.6
3	F	4	24.7	779.5	211.1	42.0
4	F	4	14.3	1050.0	528.7	255.5
5	M	4	28.2	244.0	394.9	6.0
6	M	4	15.6	198.0	7.1	3.4
7	M	4	28.9	238.5	63.5	28.0
8	F	4	43.5	139.2	11.9	38.9
9	F	3	43.2	256.0	119.4	14.3
10	M	3	86.0	431.3	6.4	66.7
11	F	4	21.6	450.0	69.8	72.2
12	F	3	53.5	561.2	3.5	199.6
13	F	3	59.5	603.7	106.0	14.3
14	M	4	48.4	222.8	15.1	308.3
15	F	4	55.0	371.8	46.4	11.6
16	F	4	45.2	290.5	2.9	141.0
17	M	4	58.6	305.0	15.9	0
18	F	4	33.0	251.2	117.8	11.3
19	F	4	40.4	521.7	11.6	12.9
20	M	4	73.0	393.2	201.7	19.7
21	M	4	81.2	157.0	3.2	3.7
22	M	4	43.1	417.9	250.9	54.3
23	M	4	59.9	567.4	150.5	10.8
24	M	3	47.2	414.7	206.2	9.1
25	M	3	61.7	243.1	168.3	21.4
26	F	4	29.6	489.5	350.0	1.2
27	F	3	26.7	838.6	77.9	4.4
28	F	4	56.5	206.3	419.0	1.4
29	F	4	40.6	1033.6	165.9	3.6
30	M	4	53.1	640.5	311.5	12.1
31	F	4	35.5	828.1	288.6	66.1
32	F	6	48.0	665.6	276.0	2.9

varying amylase concentrations indicative of swallowed saliva, and the amount of bicarbonate in the oesophagus derived from saliva was calculated. Continuous buccal aspiration was efficient, collecting a median 97% of saliva, although 3% of saliva was swallowed (95% CI 1.0–4.7).

A median of 118.6 $\mu\text{mol}/\text{hour}$ of salivary bicarbonate was swallowed (95% CI 63.5–206.2), accounting for about 25% of all bicarbonate within the oesophageal segment (Fig 3).

GASTRIC REFLUX OF BICARBONATE INTO THE OESOPHAGUS

Reflux of gastric bicarbonate into the oesoph-

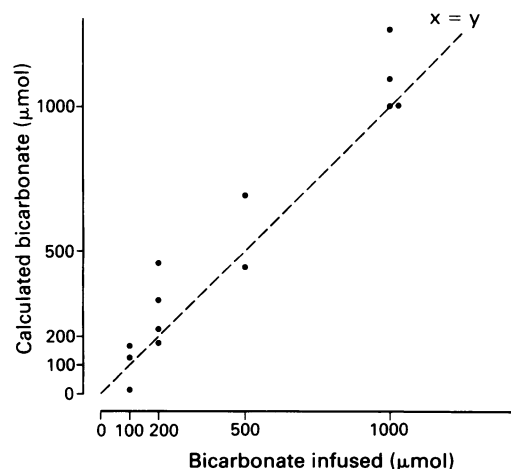


Figure 4: Relation between infused and calculated exogenous bicarbonate in seven subjects (correlation coefficient $r=0.965$, $p=0.0001$, average difference = +69 μmol).

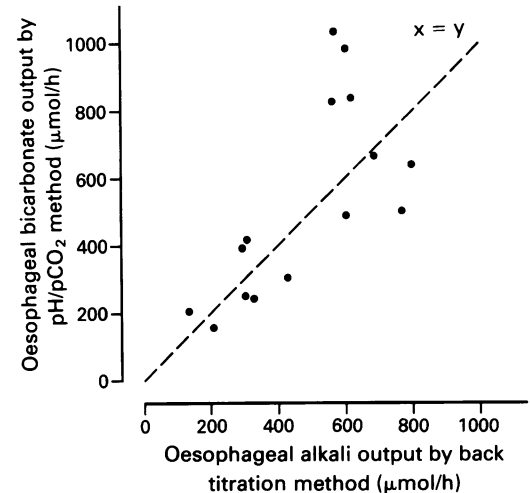


Figure 5: Comparison of bicarbonate output from a 10 cm segment of oesophagus determined by the pH/pCO₂ method, with oesophageal alkali output determined by the back titration method in 15 experiments ($r=0.69$, $p<0.02$, no significant bias $p=0.38$).

agus was determined from the appearance of gastric marker [¹⁴C]PEG in oesophageal aspirates and bicarbonate concentration of gastric fluid. A median of 13.6 $\mu\text{mol}/\text{hour}$ of gastric bicarbonate refluxed into the oesophageal segment (95% CI 9.1–28.0) accounted for about 2.5% of total bicarbonate within the oesophagus (Fig 3).

Table I presents data for individual subjects.

VALIDATION STUDIES

In vivo infusion of sodium bicarbonate

The results show an excellent correlation between the quantity of bicarbonate infused into the oesophagus and that measured and calculated from the aspirates (correlation coefficient $r=0.965$, $p=0.0001$, Fig 4). These results reflect accuracy, not only of bicarbonate concentration determination, but also of volume measurements including contaminating saliva and gastric fluid.

Measurement of amylase in oesophageal fluid

Because saliva contains relatively high concentrations of alkali, accounting for about 25% of bicarbonate within the oesophagus, it is important that amylase concentrations measured in oesophageal fluid accurately reflect salivary contamination.

Median amylase concentration in saliva at pH 7.20 was 4229 U/l ($n=20$), coefficient of variation 2%, and at pH 8.20 median amylase concentration was 4321 U/l ($n=20$, coefficient of variation = 2%). Thus reduction in pH of saliva from pH 8.2 to 7.2 accounted for a 2.2% reduction in amylase detection ($p=0.004$). Median amylase activity in oesophageal fluid at pH 7.20 was 2825 U/l ($n=21$, coefficient of variation = 2.5%) whereas at pH 8.20 median amylase activity was 2859 U/l ($n=21$, coefficient of variation = 2.7%); the difference was not significant ($p=0.55$).

TABLE II Oesophageal bicarbonate and alkali output, swallowed saliva, and refluxed gastric contamination in 13 subjects (15 experiments): individual data comparing the two methods

Subject No	Sex	pH pCO ₂ method			Back titration		
		Oesophageal HCO ₃ ⁻ output (μmol/h/10 cm)	Salivary contamination (μmol/h)	Gastric contamination (μmol/h)	Oesophageal alkali output (μmol/h/10 cm)	Salivary contamination (μmol/h)	Gastric contamination (μmol/h)
17	M	305.0	15.9	0	429.0	20.0	0
18	F	251.2	117.8	11.3	304.1	122.2	11.3
20	M	393.2	201.7	19.7	295.2	145.2	8.7
21	M	157.0	3.2	3.7	209.5	2.0	2.8
22	M	417.9	250.9	54.3	309.1	97.6	15.0
25	M	243.1	168.3	21.4	329.1	313.5	2.6
26	F	489.5	350.0	1.2	603.9	66.5	1.4
27	F(a)	838.6	77.9	4.4	619.1	71.9	2.8
	(b)	983.8	125.1	5.6	603.3	28.1	3.4
28	F	206.3	419.0	1.4	135.3	110.5	1.0
29	F	1033.6	165.9	3.6	569.5	47.7	2.6
30	M	640.5	311.5	12.1	799.2	146.8	4.6
31	F	828.1	288.6	66.1	563.4	114.2	20.8
32	F(a)	665.6	276.0	2.9	687.9	113.8	1.6
	(b)	502.0	14.4	1.3	770.0	10.0	2.6

In vivo human oesophageal bicarbonate and alkali outputs: comparison of pH/pCO₂ and back titration methods

In 15 experiments median oesophageal bicarbonate concentration was 2.06 mmol/l (95% CI 1.57–2.64) derived from pH and pCO₂ whereas median oesophageal alkali concentration was 1.65 mmol/l (95% CI 1.32–2.08), determined by back titration. This difference achieved significance ($p=0.04$).

Although the pH/pCO₂ method overcalculates the alkali content of oesophageal fluid by about 25%, there is a greater error in salivary alkali determination by the pH/pCO₂ method probably attributable to the higher pH. Median bicarbonate concentration in saliva determined by the former method was 28.1 mmol/l (95% CI 19.79–38.74) compared with 14.74 mmol/l alkali in saliva determined by back titration (95% CI, 12.95–16.69) – that is, a 90% overestimation ($p=0.003$).

Overall when bicarbonate or alkali outputs from the oesophagus were determined after deductions for contaminating saliva and gastric fluid, there was a good correlation between the two methods. Median oesophageal bicarbonate secretion was 489.5 μmol/hour/10 cm (95% CI 271–767), compared with median alkali output of 563.4 μmol/hour/10 cm (95% CI 306–613). There was a significant correlation between the two methods ($r=0.69$; $p<0.02$), with no significant bias ($p=0.38$) (Fig 5).

Due to the differences in salivary bicarbonate

and salivary alkali concentrations determined by the pH/pCO₂ or back titration method, there are pronounced differences in total salivary outputs in 15 experiments (13 subjects). Median basal salivary bicarbonate output (pH/pCO₂ method) was 4473 μmol/hour (95% CI 2595–7999), whereas median basal salivary alkali output was 2777 μmol/hour (95% CI, 2098–3540). The difference is significant ($p=0.003$).

Table II shows individual data comparing the two methods of bicarbonate and alkali determination.

In vitro alkali studies

There is an excellent correlation between actual and measured concentration of alkali in standard bicarbonate solutions (0, 1, 2, 2.5, 5, 10, 15, 20, and 25 mM of NaHCO₃) (Fig 6). This validates the accuracy of the back titration technique in determining the alkali content of solutions ($r=1.0$, $p=0.0001$). The average percentage difference between actual and measured alkali content was 0.2%. Oesophageal perfusing solutions (0.9% sodium chloride containing 12.5 μCi/l of [³H]PEG), were titrated to pH 7.0 with 0.2 M NaOH and were found to contain no titratable alkali.

INTRASUBJECT VARIATION

Six subjects were examined on two separate occasions and mean basal oesophageal bicarbonate outputs, and salivary, and gastric bicarbonate contamination of the oesophagus were quantified by the pH/pCO₂ method (Table III). Oesophageal alkali outputs were also determined in two subjects on separate occasions by back titration (Table III). There are reasonable approximations of bicarbonate and alkali secretion for each subject on separate occasions. Subjects were more tolerant of the procedure on the second occasion with a tendency towards reduced salivary and gastric contamination within the oesophagus.

ACID PERFUSION STUDIES: EFFECT ON SALIVARY SECRETION

During the period in which the oesophagus was perfused with 25 ml of 0.1 M HCl over five

TABLE III Data illustrating the intrasubject variation in oesophageal bicarbonate secretion, swallowed salivary bicarbonate, and refluxed gastric bicarbonate in six subjects when examined on two separate occasions by the pH/pCO₂ method, and in two subjects by back titration

Subject No	Oesophageal bicarbonate secretion (μmol/h/10 cm)		Salivary contamination (μmol/h)		Refluxed gastric contamination (μmol/h)	
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2
<i>pH/pCO₂ method</i>						
7	238	158	63	44	28	4
8	139	301	12	5	39	10
9	256	255	119	17	14	13
13	604	301	106	112	14	53
27	839	984	78	125	4	6
32	666	502	276	14	3	1.3
<i>Back titration</i>						
27	619	603	72	28	3	3
32	688	770	114	10	2	3

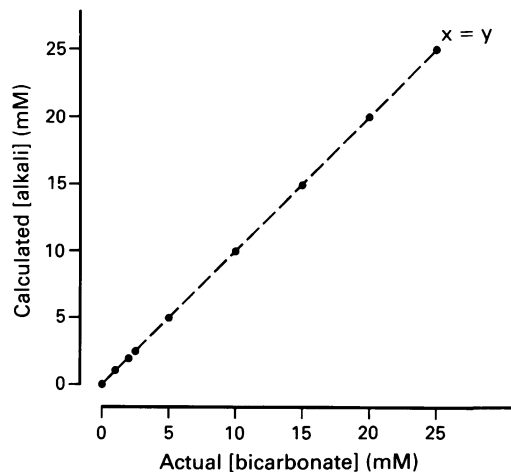


Figure 6: Accuracy of the sensitive back titration technique in determining the alkali content of standard bicarbonate solutions ($n=27$, $r=1.0$, $p=0.0001$, average % difference = 0.2%).

minutes in eight subjects, no symptoms were reported and mean salivary bicarbonate secretion was unaltered (basal 5665 $\mu\text{mol}/\text{hour}$, after acid bolus 5337 $\mu\text{mol}/\text{hour}$, $p=0.94$).

In 12 subjects the oesophagus was perfused continuously with 0.1 M HCl for 30 minutes. Two subjects did not experience symptoms due to acid perfusion and no increase in salivary bicarbonate output occurred. Ten other subjects experienced nausea and heartburn in association with acid perfusion of the oesophagus and median salivary bicarbonate secretion increased from a basal 401 $\mu\text{mol}/15$ minutes to 497 μmol ($p=0.02$) and 1100 μmol during the two 15 minute periods of acid perfusion (Fig 7). Salivary bicarbonate concentrations did not increase significantly and the increased bicarbonate secretion was largely due to an increase in salivary volume from median 34 ml/15 minutes basal to 46.5 ml/15 minutes ($p=0.006$) and 55.0 ml/15 minutes during the two periods of acid perfusion. The difference in baselines between these two groups results from the large degree of individual variation in salivary secretion.

Discussion

In the oesophagus, mucosal integrity depends on a balance between aggressive factors such as

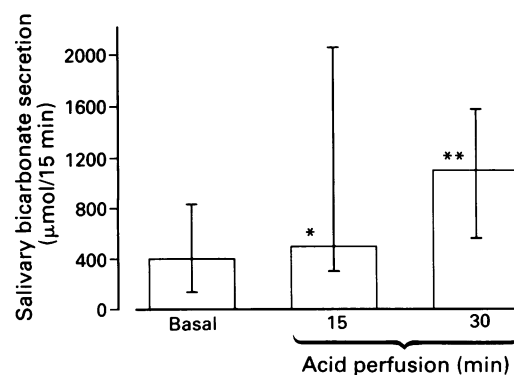


Figure 7: Oesophageal perfusion with 0.1 N HCl (5 ml/minute over 30 minutes) is associated with symptoms in 10 subjects (nausea and heartburn), and enhancement of salivary bicarbonate secretion ($*p=0.019$; $**p=0.009$). Median values and 95% confidence intervals are given.

refluxed acid and pepsin,¹² and protective mechanisms that include salivary and oesophageal bicarbonate secretion,^{4,5} salivary and oesophageal mucus,¹³ mucosal resistance and permselectivity,^{14,15} proliferative activity of the epithelium,¹⁶ mucosal blood flow,¹⁷ and motor activity that enhances clearance and maintains competence of the gastro-oesophageal junction.¹⁸ After an episode of acid reflux, secondary oesophageal peristalsis can reduce the remaining bolus to less than 1 ml. Studies have shown that exogenous radiolabelled acid instilled in the oesophagus can be cleared after two swallows.¹⁹ Despite these clearance mechanisms, a small volume of acid remains, reducing oesophageal intraluminal pH and creating an environment for mucosal injury.

Swallowed saliva is now regarded as an important mechanism for neutralising acid within the oesophageal lumen and oesophageal pH exhibits an incremental increase with repeated swallows.^{20,21} The major source of the neutralising capacity of saliva is its bicarbonate content,²² but there is now evidence that gland bearing oesophageal mucosa can also secrete alkali, a phenomenon first documented in the opossum oesophagus in vivo.⁵ This alkali can contribute to acid neutralisation and the intra-oesophageal pH increases with time in response to acid even in the absence of salivary secretion.⁵ Similar experiments on rabbit oesophagus, which is devoid of submucosal glands, failed to show alkali secretion suggesting that these glands are the source of oesophageal alkalisation. These experimental findings may be relevant to humans, as similar submucosal glands have been described in the oesophagus for more than 80 years. Several lobules of small tubuloacinar glands drain into a common duct that leads to the surface of the squamous epithelium, and with histological techniques, use of acid toluidine blue has identified irregular longitudinal rows of ductal ostia in the human oesophagus.²³

Perfusion techniques have been used to investigate secretion and absorption in the human gut in vivo. The ideal system involves isolating a segment of gut, thus preventing contamination from above or below and allowing 100% recovery of perfused solution. The technique that most closely approaches these ideals, is that used by Isenberg's group to investigate duodenal bicarbonate secretion.⁸ The oesophagus is a muscular segment, the main function of which is the passage of ingested food and fluid from the pharynx into the stomach. The response to bolus distension within the oesophagus is proximal contraction and distal relaxation and the generation of an aboral contraction wave. Inflation of an intraoesophageal balloon to between 2 and 2.5 cm diameter (8 to 9 ml), causes pain in normal subjects and is associated with peristaltic activity.²⁴ These findings are consistent with our early experience, when attempts were made to isolate a 10 cm segment of oesophagus between two occluding balloons in six subjects. This system was not tolerated in pilot experiments, and we decided to perfuse an open segment of oesophagus and make appropriate corrections for contaminating

saliva and refluxed gastric fluid.²⁵ Another group has published preliminary findings on alkaline secretion in a balloon occluded segment of human oesophagus.²⁶ The potential advantages of balloon occlusion were not realised, however, with appreciable contamination by saliva (20%) and incomplete oesophageal fluid recovery.

In our study an open segment of oesophagus was perfused and recovered fluid analysed for bicarbonate contents, with deductions made for salivary contamination and refluxed gastric fluid. In 32 healthy subjects without symptoms median net oesophageal bicarbonate output was 416 $\mu\text{mol}/\text{hour}/10\text{ cm}$ with contaminating saliva and refluxed gastric fluid contributing 25% and 2.5% of total bicarbonate measured in the oesophageal segment by the pH/pCO₂ method. About 50% of the infused solution was recovered. There was also an excellent correlation between the quantity of exogenous bicarbonate infused into the oesophagus and that measured and calculated from oesophageal aspirates. Evidence is also presented which suggests that amylase determination in oesophageal fluid is an accurate reflection of salivary contamination. In vitro studies show only a 2% reduction in amylase activity when salivary pH is reduced from 8.2 to 7.2.

One potential criticism of the pH/pCO₂ method is that investigators may be measuring dissolved CO₂ in perfusing solution that has diffused as CO₂ gas down a mucosa to lumen concentration gradient, rather than occurring due to secreted bicarbonate ions. A sensitive back titration technique was therefore used in 15 experiments to determine salivary and oesophageal alkali outputs, and to compare these with bicarbonate outputs as determined by the pH/pCO₂ method. In vitro experiments show back titration to be very accurate and precise in determining the alkali content of standard bicarbonate solutions. Median oesophageal alkali output was 563 $\mu\text{mol}/\text{hour}/10\text{ cm}$ (back titration) compared with a median bicarbonate output of 489 $\mu\text{mol}/\text{hour}/10\text{ cm}$ (pH/pCO₂ method). The two methods correlated ($r=0.69$) and did not give significantly different overall outputs ($p=0.38$). With back titration, swallowed saliva accounted for a median 17% of alkali within the oesophagus whereas refluxed gastric alkali contributed 1.0%. The pH/pCO₂ method slightly overestimated oesophageal fluid alkali concentration but the error was much greater for salivary analysis due to its higher pH. Less variable results were obtained with back titration and for these reasons, we suggest that back titration is the analytical technique of choice for determining alkali concentrations in saliva and oesophageal fluid.

Our findings have important implications for oesophageal defence against acid and pepsin injury. The human oesophagus secretes bicarbonate at about 400 $\mu\text{mol}/\text{hour}/10\text{ cm}$, which is a similar order of magnitude to gastric bicarbonate secretion. The finding that oesophageal acidification increases salivary bicarbonate is of particular interest and is in agreement with previously published results by Helm and coworkers.⁵ These studies imply the existence of an oesophagosally reflex that may be responsible for

the symptom of 'waterbrash'. Whether this response occurs only in symptomatic subjects remains controversial as one group of investigators have reported acid stimulated salivary bicarbonate secretion in the absence of symptoms.²⁷

The data from these studies do not allow us to comment on the mechanism of human oesophageal secretion, but evidence in mammals suggests the importance of submucosal glands. Finally, the physiological relevance of oesophageal bicarbonate secretion remains to be investigated as a mucus-bicarbonate barrier may not exist in the oesophagus, by contrast with the stomach and duodenum.²⁸

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