

# Abolition of Pentagastrin-Stimulated Alkaline Tide Using the Carbonic Anhydrase Inhibitor Acetazolamide

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## Abstract

**Background:** Alkaline tide is the transient increase in blood and urine pH following stimulation of gastric acid secretion. It is attributed to  $\text{HCO}_3^-$  release from parietal cells in parallel with  $\text{H}^+$  secretion. The enzyme carbonic anhydrase is thought to be responsible for  $\text{HCO}_3^-$  production from  $\text{CO}_2$  and  $\text{OH}^-$  in the parietal cell.

**Objective:** To examine the effect of pretreatment with the carbonic anhydrase inhibitor, acetazolamide, on the alkaline tide phenomenon.

**Methods:** Ten patients with dyspepsia and demonstrable alkaline tide were tested on three separate days. The pH and base excess were determined in arterialized venous blood before and 45 minutes after an intramuscular injection of pentagastrin. The pH of the urine was measured before and 120 min after pentagastrin injection. Measurements were performed after pentagastrin alone on day 1, following pretreatment with acetazolamide 60 min before pentagastrin on day 2, and after the administration of acetazolamide alone on day 3.

**Results:** Following the administration of pentagastrin alone, the blood base excess increased by  $1.61 \pm 0.2$  mEq/L (mean  $\pm$  standard deviation) and the calculated alkaline tide at 45 min was  $33.99 \pm 4.49$  mEq. On day 2 with prior administration of acetazolamide, base excess decreased by  $0.21 \pm 0.39$  mEq/L, and the calculated alkaline tide was  $-3.28 \pm 7.57$  mEq, which was significantly lower than on day 1 ( $P=0.0001$ ). On day 3, following acetazolamide alone, the base excess values decreased by  $0.53 \pm 0.2$  mEq/L and the alkaline tide was  $-10.05 \pm 3.33$  mEq; there was no significant difference compared with day 2 ( $P=0.44$ ).

**Conclusion:** Pretreatment with acetazolamide abolished the alkaline tide induced by pentagastrin. This finding supports the view that carbonic anhydrase has a major role in the alkaline tide phenomenon.

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enzyme carbonic anhydrase, neutralizes intracellular hydroxyl ions produced as a result of luminal acid secretion. The bicarbonate generated is removed from the cell via the basolateral chloride bicarbonate exchanger [6]. The significance of the effect of bicarbonate produced during acid secretion on changes in plasma and urinary pH is still controversial [7]. There are conflicting data concerning the relationship between gastric acid secretion and the post-prandial decrease in urine acidity [3,8]. In addition, it has been argued that pancreatic bicarbonate secretion occurs in parallel to acid secretion and should neutralize the effects of gastric acid secretion [7]. However, we have shown in several studies that this phenomenon parallels acid secretion [4,9]. Thus, stimulation of acid secretion with pentagastrin increased base excess maximally after 45 minutes and these changes parallel peak acid output measured in gastric aspirate. Procedures that reduce acid output also reduce the alkaline tide. Examples include vagotomy [4,10],  $\text{H}_2$ -receptor antagonists [11], and calcium channel blockers [12].

The measurement of alkaline tide is a potentially convenient non-invasive and inexpensive test for gastric acid hypo- and hypersecretory states. Arterialized venous blood has been shown to accurately reflect pH in arterial blood, and serial samples are easily obtained with this technique [13]. Studies using this method have confirmed that the alkaline tide is measurable in both blood and urine [4]. Since the production of bicarbonate is dependent on the activity of carbonic anhydrase, inhibition of this enzyme should prevent the alkaline tide phenomenon. We used acetazolamide to investigate the role of this enzyme in producing the alkaline tide.

## Materials and Methods

### Subjects

The study group comprised 12 consecutive patients with negative serology for *Helicobacter pylori*, who were referred for investigation of dyspepsia. Pregnant and lactating women, patients less than 18 years of age and patients with renal or pulmonary disease were excluded. Informed consent was obtained before enrollment. The study was reviewed and approved by the Institutional Review Board of the Rabin Medical Center.

The transient increase in blood and urinary pH following gastric secretion [1–4] has been termed the alkaline tide phenomenon [5]. Carbonic acid, formed in the presence of the

## Design

All subjects were asked to stop medication that affects acid secretion or gastric acidity, for at least 30 days prior to the study. After a fast of at least 9 hours, blood and urinary pH were determined before and after an injection of 6  $\mu\text{g}/\text{kg}$  pentagastrin (Pepatavalon, Zeneca Limited, UK). Ten subjects who demonstrated an alkaline tide of  $>20$  mEq/L at 45 minutes continued to days 2 and 3 [Figure 1]. On day 2, the participants were tested following the administration of 375 mg acetazolamide (URAMOX<sup>®</sup>, Taro Pharmaceutical Industries, Israel) 60 min before the injection of pentagastrin. On day 3, blood and urine pH were measured following the administration of acetazolamide alone. On day 1, urine was collected before and 2 hours after pentagastrin injection. On days 2 and 3, urine was collected immediately before the administration of acetazolamide (60 min before the injection of pentagastrin) and 2 hours after the pentagastrin injection.

## Blood and urine samples

The determination of the alkaline tide was performed as previously described [9]. Briefly, a 21 gauge "butterfly" needle was introduced into a vein on the dorsum of the hand. Before each sample was taken the hand was immersed in a water bath at 43°C to arterialize the venous blood. At each sampling, 5 ml of blood were drawn and discarded. Two blood samples were then drawn anaerobically in heparinized syringes and analyzed for  $\text{pO}_2$  and base excess, within 5 min, in an automatic blood gas analyzer (AVL OMNI, Graz, Austria). Only samples with  $\text{pO}_2 > 70$  mm Hg (arterialized blood) were evaluated. Otherwise, the hand was re-immersed and the procedure repeated. Base excess recorded for each sample was the mean of two determinations. Urinary pH was measured using a pH meter (pH-Meter, 3310, Janeway, USA).

## Data analysis

AT (mEq) was calculated as  $\Delta \text{BE (mEq)} \times 0.3 \times \text{body weight (kg)}$ . Results are expressed as the mean  $\pm$  standard deviation. Observations were compared by paired Student's *t*-test and  $P < 0.05$  was considered significant.

## Results

Of the 12 patients who agreed to participate in the study, one withdrew after day 1 and another was excluded because his alkaline tide was  $<20$  mEq/L after pentagastrin injection. There were four women and eight men in the original group; the final group comprised three women and seven men aged 21–77 years.

Following pentagastrin injection alone on day 1, BE increased by  $1.61 \pm 0.2$  mEq/L at 45 min ( $P = 0.0001$ ). Calculated alkaline tide was  $33.99 \pm 4.49$  mEq (mean standard error) [Table 1]. There was no statistical difference between the alkaline tide

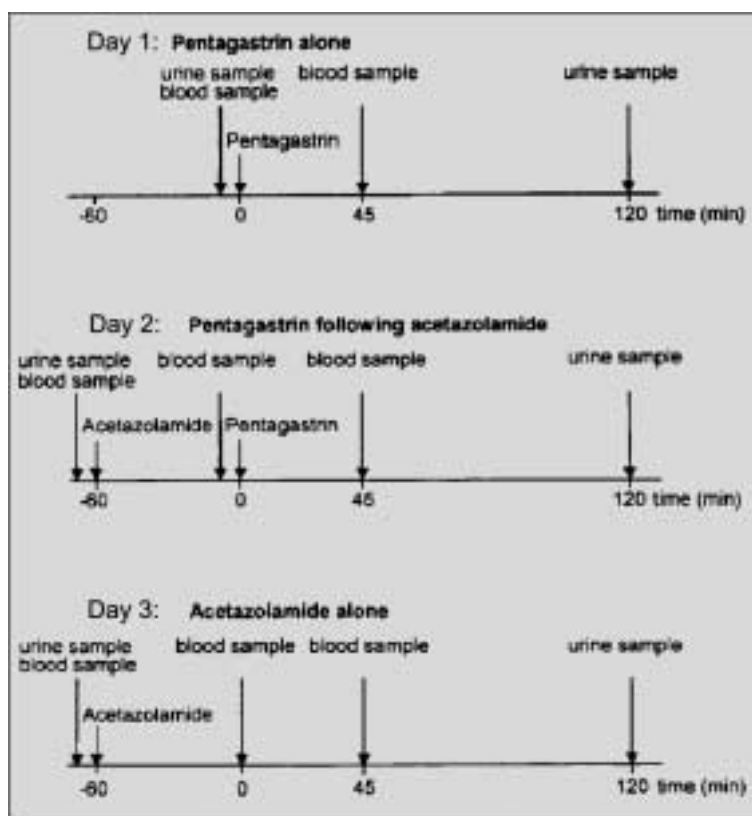


Figure 1. Graphical representation of experiments on days 1, 2, and 3.

response among men and women. Urinary pH increased significantly by  $0.88 \pm 0.26$  pH units ( $P = 0.002$ ) [Table 1]. On day 2 (acetazolamide + pentagastrin), BE decreased by  $0.21 \pm 0.39$  mEq/L, which was not a significant change ( $P = 0.7$ ). The calculated alkaline tide was  $-3.28 \pm 7.57$  mEq (mean  $\pm$  standard error) and the  $\Delta$  was significantly lower than on day 1 (pentagastrin alone) ( $P = 0.0001$ ). Urine pH increased by  $2.65 \pm 0.21$  pH units ( $P = 0.0001$ ) [Table 1], which is the expected independent effect on the kidney after acetazolamide. This was also reflected in the results on day 3 (acetazolamide alone) in which BE decreased by  $0.53 \pm 0.2$  mEq/L and alkaline tide was  $-10.05 \pm 3.33$  mEq, which was not significantly different from day 2 ( $P = 0.44$ ). Urinary pH increased by  $2.98 \pm 0.15$  pH units ( $P = 0.0001$ ).

## Discussion

Pentagastrin induced a measurable alkaline tide ( $>20$  mEq) in 10 of the 12 patients. The fact that not all patients responded to pentagastrin is as yet unexplained. Acetazolamide, which inhibits carbonic anhydrase, abolished this effect and caused a metabolic acidosis. This was more marked when acetazolamide was given alone than when given together with pentagastrin, although the difference was not significant, probably due to the small number of patients studied. Although it is difficult to

BE = base excess

**Table 1.** Alkaline tide and urine pH changes after pentagastrin injection (day 1), pentagastrin following pretreatment with acetazolamide (day 2), and after acetazolamide alone (day 3)

Patient no.	Alkaline tide (mEq)			Urine pH change (pH units)		
	After pentagastrin injection	Pentagastrin following acetazolamide	Acetazolamide alone	After pentagastrin injection	Pentagastrin following acetazolamide	Acetazolamide alone
1	23.46	-36.72	0	0.51	2.39	2.67
2	21.84	-17.74	-17.74	0.78	1.49	2.78
3	24.42	5.94	-25.74	-0.02	2.72	2.64
4	27.54	-39.01	-15.3	0.78	2.79	3.37
5	60.42	10.26	-3.42	1.03	2.79	2.73
6	51.6	42	-18.0	-0.01	2.22	2.89
7	39.37	0	0	0.89	2.96	3.18
8	23.94	-10.08	-21.42	2.11	2.93	2.38
9	45.54	5.94	1.98	1.21	2.18	3.04
10	21.73	6.61	-0.95	1.51	4.05	4.11
Mean	33.99	-3.28	-10.05	0.88	2.65	2.98
±SE	±4.49	±7.57	±3.33	±0.26	±0.21	±0.15

SE = standard error

dissect the renal from the gastric effects of acetazolamide, the data suggest an interaction between them. Thus the metabolic acidosis produced from decreased bicarbonate absorption in the proximal tubule is partly neutralized by the alkaline tide.

Acetazolamide affects bicarbonate production in many tissues due to the widespread distribution of carbonic anhydrase [14]. As a result, there are effects on the acid/base balance within the cell and within different organs. In the gastric mucosa these interactions are complex since bicarbonate is secreted into the lumen by gastric mucosal cells and into the bloodstream by the parietal cell during acid secretion [15]. While luminal bicarbonate secretion may represent only 5–10% of acid secretion, it may be of importance in mucosal protection against acid [16]. Interestingly, bicarbonate secretion in the stomach is not affected by acetazolamide whereas acid secretion is diminished following sham feeding [15]. However, different studies have shown that bicarbonate secretion induced by prostaglandin E<sub>2</sub> is reduced [17] and not affected by acetazolamide [18].

The basolateral Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger is important for intracellular homeostasis in the parietal cell during acid secretion [6]. Inhibition of the exchanger by stilbene derivatives reduces acid secretion [19]. The inhibition of acid secretion by acetazolamide may be twofold. Firstly, reduced bicarbonate production reduces the availability for exchange with extracellular chloride and thus the parietal cell becomes depleted of this anion. Secondly, acetazolamide may directly inhibit the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger, as has been shown in the kidney [20].

Although the cellular effects of acetazolamide suggest that it may be a useful drug for reducing acid secretion, the pathophysiological effects are difficult to predict [21]. In high doses it has been shown to cause gastric ulceration [22]. There are conflicting data as to whether the ulceration is dependent, at least in part, on the metabolic acidosis produced by the renal effects of the drug [23,24]. Acetazolamide has also been shown to aggravate indomethacin-induced gastric ulceration [17] but to

be protective against ethanol-induced gastric mucosal damage [25]. There appears to be a cytoprotective effect that may be mediated by an increase in mucosal prostaglandin synthesis [25].

We have shown that measurement of alkaline tide is useful in the determination of acid secretion and follows predictable response patterns. Inhibitors of gastric acid secretion, such as H<sub>2</sub>-receptor antagonists, vagotomy and calcium channel blockers, reduce the alkaline tide. The effect of acetazolamide on alkaline tide is in keeping with these earlier data and confirms that carbonic anhydrase plays an important role in acid secretion.

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