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STUDIES ON

# GASTRIC BICARBONATE SECRETION IN MAN

Henrik Forssell

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II Forssell H, Stenquist B, Olbe L. Vagal stimulation of human gastric bicarbonate secretion. Gastroenterology 1985; 89: 581-586

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Secretion of bicarbonate by surface mucus cells in the gastric epithelium is probably important in the mucosal defence against acid. The aim of this study was to develop a method for measurement of gastric bicarbonate secretion in man. The regulatory mechanisms in basal, vagally stimulated (sham feeding) and fundic distension (balloon) induced bicarbonate secretion have been explored.

The investigations were performed in healthy subjects and duodenal ulcer (DU) patients, the latter group before and/or after proximal gastric vagotomy (PGV). Gastric bicarbonate secretion was determined by use of a gastric perfusion system and computerized continuous pH and $PCO_2$ recordings.

Validation of the measuring system by small instilled amounts of bicarbonate gave a satisfactory correlation between added and recovered bicarbonate in the recorded range of bicarbonate determinations. Decreasing intragastric pH to between 3 and 4 did not affect the measured rate of bicarbonate secretion. Vagal stimulation increased gastric bicarbonate secretion in sixteen healthy subjects from $410 \pm 39$ μmol/h to $692 \pm 67$ μmol/h (mean $\pm$ SEM, $p < 0.001$). The muscarinic receptor antagonist, benzilonium bromide, almost abolished the sham feeding response while indomethacin left it nearly unchanged. Nine DU patients had identical basal and vagally stimulated bicarbonate output as healthy subjects. Basal bicarbonate secretion was significantly increased two months after PGV, whereas the bicarbonate output after sham feeding was unaltered. In the early postoperative period, anticholinergics reduced the enhanced basal bicarbonate secretion to a preoperative level. In six healthy subjects, graded fundic distension to volumes of 150 ml, 300 ml and 600 ml, each of 60 minutes, increased the bicarbonate secretion by 46 % ($p < 0.05$), 28 % (NS) and 84 % ($p < 0.05$), respectively. Continuous distension with 300 ml over 2.5 hours increased bicarbonate secretion, the peak response occurred at 45 minutes and gradually declined thereafter. Seven DU patients investigated after PGV had a response to graded fundic distension virtually identical to that in healthy subjects. Anticholinergics abolished the response to fundic distension, whereas indomethacin was without any significant effect. In healthy subjects, 16,16-dimethyl $PgE_2$ gave an about threefold greater response than vagal stimulation, fundic distension or carbachol.

It is concluded that gastric bicarbonate secretion in man is activated by cholinergic vagal nerves, the response being independent of intragastric pH above pH 2. There is probably an interplay of stimulatory and inhibitory mechanisms modulating basal and vagally stimulated gastric bicarbonate secretion. Fundic distension of the stomach stimulates bicarbonate secretion and the response is mediated by intramural neural cholinergic pathways. Vagal stimulation, fundic distension and carbachol appear to be submaximal stimuli of bicarbonate secretion or else may have both stimulatory and inhibitory actions on human gastric bicarbonate secretion.

*Key words:* gastric bicarbonate secretion; gastric secretion; gastric; bicarbonate; humans; duodenal ulcer; vagal stimulation; fundic distension; prostaglandin; carbachol

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# THESIS

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# GASTRIC MUCOSAL DEFENCE MECHANISMS

## INTRODUCTION

The stomach has a central role in the process of digestion. It is a reservoir and a mill and its glands secrete gastric juice containing hydrochloric acid and pepsin. Acidification of food destroys many ingested micro-organisms, begins the breakdown of food components and creates optimal conditions for protein digestion by pepsin. The stimulation of gastric secretion during digestion has been traditionally divided into cephalic, gastric and intestinal phases. During the cephalic phase, gastric secretion is evoked by stimulation of centres in the brain and impulses are mediated via vagus nerves. The gastric phase is initiated when food enters the stomach. The food volume stimulates stretch receptors in the gastric wall and food constituents interact with chemoreceptors resulting in further stimulation of acid secretion. The last stage, the intestinal phase, begins with the emptying of food and digestive products into the duodenum. This stage modulates both the secretion of acid and the emptying of food. The three phases overlap in time and are mutually interrelated in a complex nervous and humoral interplay.

Gastric juice is produced by billions of cells located in the glands of the mucosa. The parietal cells, being one predominant cell type, have the ability to secrete a hydrochloric acid solution of pH 0.8 *i.e.* the proton concentration of the primary gastric juice is about three million times greater than that of blood. The chief cells are another frequently occurring cell type in the glands and secrete pepsinogen, which is involved in the digestion of protein.

Both acid and pepsin have proteolytic actions on living tissue and these components of gastric juice are a potential threat to the mucosa in terms of autodigestion.

In the healthy stomach, there is a balance between aggressive factors and the protection provided by pre-epithelial, epithelial and subepithelial mechanisms of the mucosa. Secretion of mucus and bicarbonate by the surface epithelial cells constitute a mucus-bicarbonate barrier which is regarded to be a first line of defence. Other protective mechanisms comprise the inherent resistance of the epithelial cells to noxious stimuli as well as mechanisms for rapid tissue repair. The pathogenesis of peptic ulcer disease is multifactorial and far from established as yet. Apparently, the balance between aggressive factors and protective me-

chanisms in ulcer disease is upset in favour of ulcerogenesis. Possible mechanisms are excessive secretion of acid/pepsin, a reduced mucosal resistance or a combination of both.

Superficial mucosal damage induced by *e.g.* ethanol or acetylsalicylic acid is usually followed by increased passive diffusion of luminal hydrogen ions into the mucosa. This increased permeability induced by weak acids and some other substances was first recognized by Teorell (1939) and the sequence of changes in the mucosa was further described by Davenport (1964, 1966). In the experiments by Davenport on canine Heidenhain pouches, injury was induced by a variety of agents. Damage was estimated in terms of increased flux of sodium and potassium into the gastric lumen and a loss of luminal hydrogen ions. Within the mucosa, the acid released histamine, which increased the permeability of the capillaries and further stimulated acid secretion. This resulted in interstitial oedema with haemorrhage and an additional breakdown of the mucosal barrier (Davenport 1967, Davenport 1970).

Knowledge of gastric mucosal protection has increased rapidly during recent years. The following mechanisms have been suggested to be important and will be discussed in greater detail:

* pre-epithelial protection
  - mucus gel
  - bicarbonate secretion
* epithelial protection
  - hydrophobicity of luminal cell membranes
  - barrier to permeation
  - sulphydryl components
  - rapid cell turnover
  - restitution
* subepithelial protection
  - blood flow
  - tissue acid-base balance

## PRE-EPITHELIAL PROTECTION

### *Mucus gel*

The entire surface of the gastric mucosa is covered by a continuous layer of mucus gel, which has a variable thickness of less than 500 μm (Bickel and Kauffman 1981, Kerss *et al* 1982, Allen *et al* 1983). Both the surface mucus cells and the mucus neck cells in the upper part of the glands secrete mucus. Mucus is released predominantly by the process of exocytosis (Zalewsky and Moody 1979). The main components of gastric mucus are neutral glycoproteins, which contain sialic acid and mucopolysaccharides. The glycoproteins, constituting the greater part of mucus, are of particular importance for their specific properties such as gel formation and viscosity. Native glycoproteins have a high molecular weight of about two million daltons and are formed by polymerization of four glycoprotein subunits, which are joined by disulphide bridges (Allen 1981). The subunit consists of a protein core with carbohydrate side chains. Each molecule of the porcine gastric mucus glycoprotein contains over 600 of these carbohydrate side chains. The most important sialic acid is N-acetyl neuraminic acid. It is located in the terminal position of the carbohydrate side chain and has a strong negative charge. The rejection of neigh-

bouring negatively charged groups of the glycoproteins results in molecular expansion and an increase in viscosity. The most important feature of the glycoproteins is, however, the hydrophilic action of the carbohydrate side chains. Water molecules are strongly attracted to the matrix with most of the molecules trapped within the interstices of the gel. Accordingly, about 95 % of mucus consists of water.

Mucus has several functions. It lubricates the mucosa and constitutes a first line of defence against noxious gastric contents. Of importance in the latter respect is the active secretion of bicarbonate from surface mucus cells. In addition, mucus retards diffusion of hydrogen ions, the rate in mucus being about four times slower than that in unstirred water (Williams and Turnberg 1980). Thus the mucus gel accomplishes a zone of limited mixing between the gastric luminal contents and the relatively small amount of secreted bicarbonate. The existence of a pH gradient across the mucus layer has been shown *in vitro* in the stomach of rabbits (Williams and Turnberg 1981), frog (Takeuchi *et al* 1983) and *in vivo* in the rat (Ross *et al* 1981). A pH gradient has also been demonstrated in the human stomach, *in vitro* (Bahari *et al* 1982) and *in vivo* (Quigley and Turnberg 1985).

The mucus gel has a very low permeability to large molecules such as pepsin, which should also be inactivated by the alkaline environment. However, pepsin continuously hydrolyzes the lumen-facing part of the mucus gel layer with production of mucus glycoprotein subunits. These have lost much of their viscous and gel-forming properties. The thickness of the mucus gel is thus determined by the dynamic balance between mucus secretion and surface erosions by proteolysis and mechanical destruction. The gel is resistant to hypertonic NaCl, bile, ethanol and indomethacin (Bell *et al* 1985). Mucus secretion is stimulated, for example, by chemical irritants, carbachol, prostaglandins and the hormone secretin (Allen 1981).

Another suggested property of mucus is facilitation of unidirectional flux of hydrogen ions from the gastric glands into the lumen. The transit of acid secreted by the parietal cells appears to occur through mucus which is more highly sulphated (Tasman-Jones 1985). Such mucus, with the highest negative charge contributed by sulphate and sialic acid radicals and produced especially by the mucus neck cells, should behave as a cation exchanger. A concentration gradient of sodium ions is proposed to be generated across the mucus layer by the continuous activity of a Na,K-ATPase at the basolateral membrane of the mucus cells. Sodium ions diffusing along this gradient should generate a diffusion potential positive at the cell-facing surface of the mucus gel. It is suggested that this potential moves hydrogen ions into the lumen and also prevents backwards diffusion (Smith *et al* 1985).

Gastric mucus is also important in the repair of superficial damage to the mucosa. After damage to the surface cells, there is a rapid release of copious amounts of mucus and plasma proteins which, together with cellular debris, form a continuous coat over the destroyed area (Wallace and Whittle 1986).

This mucoid cap provides a favourable micro-environment for repair by restitution.

## *Bicarbonate secretion*

Bicarbonate secretion in the stomac occurs by electroneutral $Cl^-/HCO_3^-$ exchange at the luminal cell membrane of the epithelial cells (Flemström and Garner 1982). The mucus cell contains most of mucosal cyclic GMP diesterase (Sung *et al* 1972) and carbonic anhydrase activity (Lönnerholm *et al* 1985). Carbonic anhydrase is localized to the apical cytoplasm and microvillar cores of the gastric mucus cells (Sugai and Ito 1980). The presence of these enzymes provides evidence that the secreted bicarbonate originates from the mucus cells. Acetazolamide, which inhibits carbonic anhydrase, has been shown to reduce active alkalinization by amphibian gastric fundic mucosa (Flemström 1977), decrease canine gastric bicarbonate secretion (Kauffman and Steinbach 1981) and to reduce the ability of the mucosa to resist acid (Werther *et al* 1965).

Cyclic GMP, but not cyclic AMP, stimulates the alkaline secretion by gastric mucosa *in vitro* (Flemström 1977) and cholinergic stimulation increases the bicarbonate secretion in the canine antrum and fundus with a concomitant elevation in the mucosal concentration of cyclic GMP (Cheung and Newton 1979). This suggests that cyclic GMP may serve as an intracellular secondary messenger. For each hydrogen ion secreted by the parietal cell, a molecule of $CO_2$ derived from arterial blood flow is converted to bicarbonate. The latter is released into gastric venous blood consequently provoking an alkaline tide after stimulation of acid secretion (Rune 1965). The vascular arrangement of the mucosa may facilitate the transport of the bicarbonate released by the parietal cells towards the bicarbonate secreting cells of the surface epithelium (Gannon *et al* 1984).

Gastric as well as duodenal bicarbonate secretion has been extensively studied during the last 10 years both in *in vitro* and *in vivo* preparations of various amphibia and common laboratory mammals, and especially by Flemström and his coworkers. It has been found that bicarbonate secretion from mucus cells is a metabolism dependent process rather than a passive diffusion and that it is stimulated and inhibited by a variety of physiological mechanisms and pharmacological agents (Flemström 1987). The rate of bicarbonate secretion from surface mucus cells is below 10 % of the maximum rate of acid secretion in several species (Garner *et al* 1983).

Measurements of human gastric bicarbonate secretion have been accomplished by titration of bicarbonate concentration, from recordings of pH and $PCO_2$ and from measurements of gastric juice osmolality. Gardham and Hobsley (1970) estimated basal bicarbonate secretion to be 236 ± 48 μmol/h (mean ± SEM) in patients with pernicious anaemia. The measurement was accomplished by means of back-titration of the alkaline gastric juice with hydrochloric acid. Andre and coworkers (1973) used a glycine buffer to trap hydrogen ions and bicarbonate secretion was subsequently determined with a titration technique in three steps, which included addition of HCl to the

gastric sample to neutralize bicarbonate, removal of $CO_2$ by boiling and finally back-titration to pH 7 with NaOH. They found the exceedingly high bicarbonate output of $7020 \pm 840$ μmol/h. It should be noted that the use of the glycine buffer caused an unphysiological pH of over 9 in the stomach. Furthermore, such a buffer probably traps tissue $CO_2$. In 1982, Rees and coworkers reported a method for the measurement of human gastric bicarbonate secretion in which gastric juice was collected in 10-min intervals and pH and $PCO_2$ were analyzed in the samples. This method was based on techniques developed for measurements of bicarbonate secretion in animals (Garner and Flemström 1978). The bicarbonate secretion was calculated using the Henderson - Hasselbalch's equation. Under basal conditions, it amounted to $347 \pm 100$ μmol/h. A similar method has been described by Johansson and collaborators (1983) who used a gastric instillation technique measuring pH and $PCO_2$ in the recovered instillate. Kleist *et al* (1985) measured gastric bicarbonate concentration by using the van Slyke technique and found a gastric bicarbonate secretion value of similar magnitude as with the pH - $PCO_2$ technique. Another approach for assessment of human gastric bicarbonate secretion has been described by Feldman (1983) using measurement of gastric juice osmolality. In healthy subjects, a basal gastric bicarbonate rate of $2600 \pm 600$ μmol/h was calculated. The method is based upon the fact that reaction between bicarbonate and hydrogen ions results in loss of osmoles and also on an assumed fixed relation between the osmolality of plasma and the osmolality of parietal and nonparietal secretions. Recently, both *in vitro* and human investigations have shown a several-fold overestimation by the osmolality method compared to both the pH - $PCO_2$ method and back-titration (Odes *et al* 1987).

Gastric bicarbonate secretion from amphibian mucosa and the mucosa of various mammals is stimulated by humoral substances such as dibuturyl cyclic GMP (Flemström 1977), calcium ions (Flemström and Garner 1980), cholecystokinin (Flemström *et al* 1982) and several other gut hormones, for example, pancreatic glucagon, pancreatic polypeptide and neurotensin (Konturek *et al* 1985). Gastrin and secretin have been found to be without any stimulatory effect (Flemström 1987). Gastrin (Feldman 1983) and secretin (Kleist *et al* 1985) were also unable to affect gastric bicarbonate output in man as was the case with histamine (Feldman and Schiller 1982). Cholinergic agents, on the other hand, stimulated bicarbonate secretion (Feldman 1983). Prostaglandins are potent stimulants of gastric bicarbonate secretion (Garner and Heylings 1979) and the synthetic analogue of prostaglandin $E_2$ has been shown to stimulate gastric bicarbonate secretion in man (Johansson *et al* 1983, Feldman 1983). Gastric bicarbonate secretion was increased by electrical vagal stimulation in the cat (Fändriks and Delbro 1983) and by physiological vagal stimuli such as sham feeding in man (Feldman 1985). Exposure to acid has been found to increase gastric bicarbonate output in experiments with bullfrog mucosa (Heylings *et al* 1984), in the canine Heidenhain pouch (Konturek *et al* 1984), in the rat by acid solutions of pH 1 but not pH 2 (Takeuchi *et al* 1986) and al-

so recently in man when intragastric pH was allowed to fall to 2 (Crampton *et al* 1986). A vagally induced rise in feline gastric bicarbonate secretion was enhanced by splanchnicotomy and/or ligation of the adrenal glands (Fändriks 1986a), indicating adrenergic inhibition of the cephalic stimulated gastric bicarbonate secretion.

## EPITHELIAL PROTECTION

### *Hydrophobicity of the epithelial lining*

Both the surface mucus cells and the neck mucus cells face outermost towards the lumen of the stomach and constitute an anatomical barrier restricting passive diffusion into the underlying tissue of hydrogen ions and of sodium and potassium ions along their concentration gradients into the lumen. This tissue barrier may also prevent autodigestion by pepsin. Surfactants such as amphoteric phospholipids have the ability to increase the hydrophobicity of biological membranes and have been identified in gastric juice and on the apical cell membrane of the surface epithelial cells (Hills *et al* 1983, Butler *et al* 1983). The acid secreting part of the stomach in particular has a highly hydrophobic surface and these phospholipids have been suggested to resist digestion (Slomiany and Slomiany 1980). The surfactant molecule is orientated in such a way that two hydrocarbon chains per molecule form a hydrophobic exterior surface. The opposite hydrophilic ends face the underlying cell, which is hydrophilic due to the preponderance of outward orientated hydroxyl and carboxyl groups in the membranes. The hydrophilic ends of the phospholipids are positively charged and form electrostatic bonds with the negatively charged cell membranes. Mucus makes a substantial contribution by stabilizing and replenishing the absorbed surfactant monolayer (Hills 1985).

Surfactants contribute to the surface epithelial protection by preventing the water-soluble agents in the gastric lumen from reaching and damaging the cells. Exposure of the luminal surface of the stomach to a high concentration of the damaging agents aspirin and sodium deoxycholate results in a rapid decrease in the hydrophobicity of the mucosa (Hills *et al* 1983). The reduction in surface hydrophobicity is effectively and dose-dependently reversed by synthetic analogues of prostaglandin $E_2$ (Lichtenberger *et al* 1985). Furthermore, a liposomal suspension of surface active phospholipids significantly protected the gastric mucosa of rats from experimental acid induced gastric damage (Lichtenberger *et al* 1983).

### *Hydrogen ion permeation*

Hydrogen ion permeation across the epithelium may occur across the cell membranes or through tight junctions between the cells. The ion permeability of the tight junctions determines the electrical resistance of epithelia (Powel 1981). The resistance of the apical and basolateral cell membranes varies little between epithelia. The epithelium is moderately tight in the gastric fundus whereas it is more leaky in the antrum. Permeation of hydrogen ions across tight junctions may occur by proton diffu-

sion since protons can hop from one water molecule to another by rearrangement of hydrogen bonds. At neutral pH, the shunt paths are cation selective and relatively permeable to hydrogen ions. Protons and polyvalent cations such as $Ca^{2+}$ neutralize the negative charges that line the pathways thereby changing the shunt path instead being anion selective. Thus, the negatively charged groups govern the permselectivity of the aqueos tight junctions. Consequently, at low pH the tissue may exclude hydrogen ion permeation (Powel 1981). Hydrogen ions may also be transported across the apical cell membrane by $Na^+/H^+$ exchange controlled by intracellular calcium concentration (Benos 1982). When the mucosal surface epithelium is damaged, hydrogen ions diffuse from the lumen into the mucosa and sodium and potassium ions move from the tissue into the lumen. This increased permeability can also be observed as a marked decrease in the transmucosal electrical potential difference (PD). Acidification may damage the cells by interference with their ion transport mechanisms resulting in a loss of volume regulation and/or by denaturation of vital proteins.

## *Sulphydryl compounds and EGF in gastric mucosal protection*

Tissue ischaemia or injury produced by noxious agents such as ethanol may result in the accumulation of toxic free radicals. Ischaemia is followed by utilization of high energy compounds such as adenosine triphosphate and accumulation of adenosine monophosphate since oxidative phosphorylation is reduced. The adenosine monophosphate is catabolized further to hypoxanthine, which is accumulated (Younes *et al* 1984). Ischaemia also converts the enzyme xanthine dehydrogenase to the xanthine oxidase form. This enzyme requires the presence of oxygen for activity. For instance after rapid reperfusion, oxygen becomes available for the tissue and xanthine oxidase will act on hypoxanthine producing superoxide radicals ($O2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH\cdot$). These free radicals, each containing an unpaired electron in its outer shell, are highly reactive, potent oxidizing and/or reducing agents. Cell injury is presumably caused by the damage of various cell membranes with intracellular release of *e.g.* lysosomal enzymes (Itoh and Guth 1985). Nonprotein sulphydryl compounds have been found in high concentrations in the gastric epithelium. The major component is reduced glutathione, which is capable of binding reactive free radicals. Ethanol lowers the concentration of nonprotein sulphydryls in the gastric mucosa whereas reduced glutathione induces gastric cytoprotection while increasing sulphydryl levels (Szabo *et al* 1981). Thus glutathione peroxidase appears to be a natural scavenger. Sulphydryl blocking agents prevent the cytoprotective action of certain prostaglandins concomitant with a decrease in sulphydryl concentration (Szabo *et al* 1981).

Epidermal growth factor, EGF, which is a peptide of 53 amino acids, has been identified in salivary glands and in duodenal Brunner gland secretions. It is a powerful mitogen and stimulates DNA synthesis as well as ornithine decarboxylase activity. These observations suggest EGF to be important in

the process of tissue repair and growth and in the physiological maintenance of the gastroduodenal mucosa. In addition, EGF is an inhibitor of gastric acid secretion. It has been found that EGF reduces gastric induced lesions at doses not inhibiting acid secretion (Skov Olsen *et al* 1984). Approximately 10 % of the structure of EGF consists of SH-containing amino acids and it has been suggested that at least part of the protective effect of EGF in the gastroduodenal mucosa is due to these sulphydryl groups (Szabo 1984).

## *Gastric mucosal cell renewal*

The gastric mucosa has a very rapidly proliferating epithelium. The rate of production of new cells has obvious implications for mucosal protection both during steady state conditions and after mucosal damage. Cells migrate from the neck of the glands towards the lumen and differentiate into surface mucus cells. The origin most probably is undifferentiated neck cells. Other cells migrate down towards the depth of the gland and differentiate into parietal cells and chief cells. Human isthmus-pit cells divide about once every 36 hours and the time taken for these cells to reach the surface of the mucosa varies between 48 and 96 hours (Wright 1984). An increase in gastric cell turnover may contribute to mucosal defence, as damaged cells are more rapidly replenished. On the other hand, chronic administration of prostaglandins to the rat (Reinhart *et al* 1983) and humans (Tytgat *et al* 1986) induced trophic changes in the gastric mucosa, especially the gastric antrum. The mucosa was thickened due to an increase in cell size and number of all cells in the glands, but the trophic changes were not caused by an increase in cell turnover (Fich *et al* 1985). It is thus probable that prostaglandins of $E_2$ type retard senescence and exfoliation of mucosal cells, explaining the foveolar expansion in the presence of unaltered proliferation (Tytgat *et al* 1986).

## *Mucosal repair by restitution*

After extensive damage of the surface epithelial cells caused, for example, by hypertonic sodium chloride solution, repair occurs within a few hours (Svanes *et al* 1982, Rutten and Ito 1983). This process, called restitution, is unlikely to be due to a proliferation of cells in the isthmus of the glands which instead requires several days. The rapid re-epithelialization is characterized by migration of remaining viable surface mucus cells and mucus neck cells from the crypts to cover the damaged surface. These cells are squamous shaped and depleted of mucus granules. The first sign of restitution is the presence of extended thin cell processes that begin to cover the denuded basal lamina. With time, a continuous epithelial cell layer of flattened migrated epithelial cells is formed. Tight junctions are restored and, after a longer period of restitution, the cells become more and more cuboidal to form low columnar cells containing mucus granules. A return of electrical parameters such as PD accompanies the morphological stages of healing. In frog gastric mucosa, the restitution process is associated with a concomitant passive flux of bicarbonate across the epithelium. The rate is about half that of basal hydrogen ion secretion in the control

fundic mucosa. Luminal pH below 4 inhibits and high nutrient bicarbonate concentration enhances epithelial restitution (Svanes *et al* 1983). Pretreatment with indomethacin did not increase the mucosal damage caused by a hypertonic sodium chloride solution and pretreatment with exogenous 16,16-dimethyl prostaglandin $E_2$ did not affect the restitution process in any favourable way (Svanes *et al* 1984). In other studies, it was found that a largely restituted rat mucosa was not damaged by repeated insult of 70 % ethanol when the superficial mucosa was nearly completely re-epithelialized, unless the overlying necrotic gelatinous cap was removed (Lacy 1985).

## SUBEPITHELIAL PROTECTION

### *Mucosal blood flow*

The gastric mucosa, like all tissues, depends on blood flow for supply of nutrients and oxygen and for disposal of cellular waste products. Insufficient gastric blood flow is thus hazardous and a threat to tissue viability. When the gastric mucosa is exposed to an irritant that increases hydrogen ion permeation, tissue damage is greatly enhanced by a simultaneous decrease in mucosal blood flow. The exposure of canine gastric mucosa to sodium taurocholate and acid increased hydrogen ion diffusion and enhanced blood flow but occurred without gross damage to the mucosa (Ritchie 1975). However, simultaneous reduction in blood flow by vasopressin (Ritchie 1975) or by haemorrhagic shock (Ritchie and Shearburn 1977) caused marked damage to the mucosa. Mucosal blood flow is thus essential in the disposal of hydrogen ions and noxious agents which permeate the mucosa.The ratio of blood flow to hydrogen ion diffusion determines the degree of the ensuing mucosal injury (Cheung and Chang 1977). Measurement of intramural pH of canine gastric mucosa in the absence or presence of luminal acid during haemorrhagic shock further suggested that the protective function of mucosal blood flow is related to the disposal of hydrogen ions entering the mucosa (Kivilaakso *et al* 1978a). Intramural pH decreased only slightly during reduction of mucosal blood flow when luminal acid was absent. However, a much greater decrease in the intramural pH occurred in the presence of luminal acid and a particularly rapid and profound decrease in intramural pH was observed after the addition of sodium taurocholate.

### *Acid-base balance*

The intraglandular capillary supply of the stomach originates from submucosal arterioles which divide into capillaries at the base of the glands. These capillaries pass through the mucosa parallel to the glands, join to a capillary network just beneath the surface mucus cells and drain into mucosal venules (Gannon *et al* 1984). The mucosal venules do not receive further capillary tributaries deeper within the mucosa. This vascular arrangement ensures the maintenance of an unidirectional blood flow in the mucosal exchange vessels. Further, it may facilitate the protective role of alkaline tide by transporting bicarbonate from lower regions of the mucosal vasculature to the capillaries just below the surface mucus cells. In the oxyntic area of the stomach, the acid secretory pro-

cess utilizes carbon dioxide. For each hydrogen ion secreted, one ion of bicarbonate is released. These bicarbonate ions diffuse into capillaries surrounding the glands and are transported by blood flow to the surface of the mucosa. Here, bicarbonate is delivered to mucus cells, but is also available for neutralization of hydrogen ions which may have diffused into the mucosa. The buffering capacity of tissue and blood, especially as related to the availability of bicarbonate ions to surface epithelial cells, is thus important in the protection of the gastric mucosa against acid induced injury. Actively secreting gastric mucosa with its accompanying alkaline tide of bicarbonate tolerates luminal acid far better than does a resting or inhibited mucosa (Kivilaakso *et al* 1978b, Arvidsson and Haglund 1984).

In a rat model, parenteral administration of sodium bicarbonate effectively protected the gastric mucosa from luminal acid (Kivilaakso 1981). This protection depended on the presence of bicarbonate rather than on the alkalinity of the tissue induced by respiratory hyperventilation. Nor did other buffer species offer any protection. Ambient bicarbonate has also been shown to be important in the maintenance of gastric mucosal intracellular pH (Kivilaakso 1983), which was significantly higher when bicarbonate was present in the fluid surrounding isolated frog gastric mucosa than when it was absent. Furthermore, intracellular pH was higher than extracellular pH only when bicarbonate was present. Consequently, nutrient (blood) bicarbonate ions may enhance the ability of the mucosal cells to withstand acid.

# AIMS OF THE PRESENT INVESTIGATION

The ability of the gastric mucosa to resist autodigestion is of multifactorial nature. Both mucus and bicarbonate are actively secreted by the mucus cells of the gastric mucosa and constitute a mucus-bicarbonate barrier, which is regarded as a first line of defence. While gastric bicarbonate secretion has been extensively characterized *in vitro* and *in vivo* in several mammals, the nature of this bicarbonate secretion has been very little explored in humans. The aims of the thesis were

1. to develop and validate a method for measurement of gastric bicarbonate secretion in man
2. to study the influence of vagal nerves on gastric bicarbonate secretion
3. to study the effects of fundic distension on gastric bicarbonate secretion
4. to study the mechanisms involved in regulation of gastric bicarbonate secretion by use of anticholinergics and cyclooxygenase inhibitors

# MEASUREMENT OF BICARBONATE SECRETION

Measurement of gastric bicarbonate secretion in the present study was based on the assumption that bicarbonate appearing in the gastric perfusate reflected the secretion of bicarbonate into the mucus gel layer. Acid inhibition by histamine $H_2$-receptor antagonists increased intragastric pH to a level above pH 6. At pH 6.10, *i.e.* at the pKa of carbonic acid, half of intragastric bicarbonate exists in the form of free bicarbonate ions, the rest being converted to carbon dioxide by the reaction of bicarbonate with acid. Consequently, use of a higher intragastric pH of around 6.5 further decreased the formation of carbon dioxide and thus minimized the possible loss of $CO_2$ by eructation, passage through the pylorus or diffusion into the gastric wall. The measuring system contained electrodes which simultaneously measured pH and $PCO_2$. The concentration of free bicarbonate was calculated according to the formula of Henderson (1908) and Hasselbalch (1916). A proportion of free bicarbonate was always neutralized by hydrochloric acid with formation of $CO_2$. The $t_{1/2}$ for this reaction is about 14 seconds (Flemström and Garner 1987). The concentration of $CO_2$ was added to obtain total bicarbonate concentration according to the formulae:

TOTAL BICARBONATE CONCENTRATION

$$= [HCO_3^-] + [CO_2]$$

where

$$[HCO_3^-] = S \times PCO_2 \times 10^{pH - pKa}$$ and

$$[CO_2] = S \times PCO_2$$

The solubility coefficient, S, for $CO_2$ and the dissociation constant, pKa, for carbonic acid, both depend on the solution and its chemical and physical properties. For example, the solubility coefficient increases with decreasing temperature. At 37 °C, 0.033 mmol per mm Hg of $CO_2$ is dissolved in 1 litre of water. The corresponding values at 30 °C and 20 °C are 0.039 and 0.052 mmol per mm Hg, respectively. Presence of ions in the solution depresses the S value (van Slyke *et al* 1928), especially at ion concentrations above 0.3 M. The dissociation constant, pKa, will increase slightly with decreasing temperature but is affected more by the ionic strength of the actual solution. Hence, the pKa of carbonic acid is reduced by 0.5 x the square root of the ionic strength of the solution (Hastings *et al* 1925). The pKa in water solutions at 37 °C is 6.31 and the corresponding value in plasma is 6.10.

## The measuring system

A Salem sump tube no. 12 was positioned with its tip in the upper part of the gastric antrum and was coupled to a measuring system. Its outlet was connected to an Egnell suction pump, which produced an intermittent negative pressure of 15 - 20 kPa once a second (Fig. 1, I). Physiological sodium chloride solution at room temperature was continuously infused into the stomach through an additional thin tube, parallel to the main tube. The perfusion port was located just below the cardia of the stomach, *i.e.* 12 cm proximal to the tip of the Salem sump tube. The perfusion solution contained phenol red as a marker. This enabled calculation of the volume of gastric contents using the formula:

$$\text{VOLUME OF GASTRIC CONTENTS} = \frac{\text{perfusion volume x phenol red conc. of the perfusate}}{\text{phenol red conc. of the gastric aspirate}}$$

The concentration of phenol red in the perfusate was 8 mg/l. Concentration of phenol red in the aspirate was measured spectrophotometrically at a wavelength of 560 nm. A high perfusion rate of 440 ml/15 min facilitated mixing of secreted gastric juice with the perfusate. In addition, the high rate diluted secreted acid and the rapid washing-out shortened and allowed easy detection of episodes of duodenogastric reflux of bicarbonate.

## The measuring chamber

The measuring chamber was made from translucent plastic material and occupied a volume of only 9 ml (I). The pH and $PCO_2$ electrodes were placed in such a manner that air bubbles did not interfere with the recordings. The outlet of the measuring chamber was at the top allowing air bubbles which were intermingled with the gastric juice to pass along the side wall to the top of

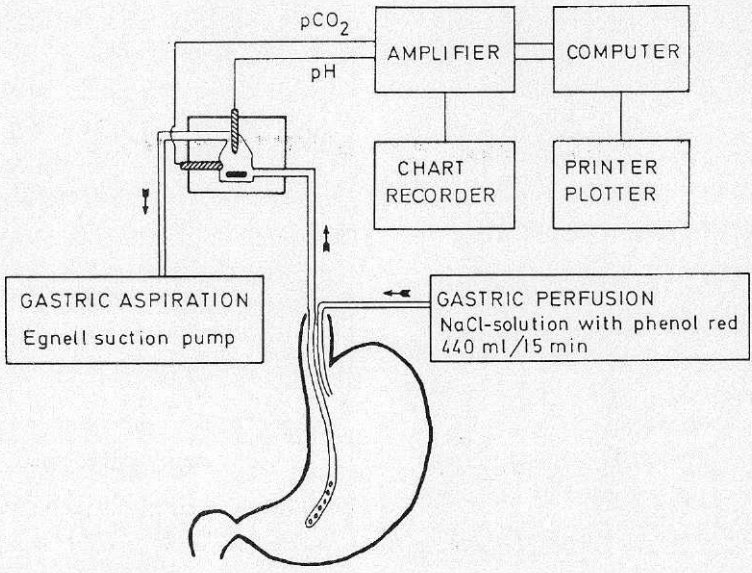


Fig. 1 Schematic diagram of the measuring system. From I.

the chamber without disturbing measurements of pH and $PCO_2$. The pH electrode and its reference (Radiometer, Denmark: G2040C and K8040 respectively) both had screw-caps allowing gas-tight insertions into the measuring chamber. A magnetic stirrer inside the measuring device ensured thorough mixing of the passing gastric aspirate.

## The $PCO_2$ electrode

The $PCO_2$ electrode (Radiometer, Denmark: E8001) is basically a pH electrode placed in bicarbonate electrolyte behind a teflon membrane of 12 μm thickness. This membrane is impermeable to ions such as $HCO_3^-$ and $H^+$ but permeable to gas molecules. The $CO_2$ dissolved in the gastric perfusate diffuses into the bicarbonate electrolyte until an equilibrium is reached, changing its pH. This pH change is converted into a $PCO_2$ reading in the amplifier (Radiometer, Denmark: PHM 73) by using a linear calibration curve which relates log $PCO_2$ to pH. The $PCO_2$ electrode was always calibrated against two carefully analyzed gas mixtures which contained $CO_2$ in the range of 1 to 3 % in $N_2$ for alternate 15-min periods in the few hours before the experiment.

## The computer recording

The pH and $PCO_2$ signals from the amplifier were stored digitally in a computer (Commodore, USA). The amplifier was provided with an analog-digital converter which was interfaced between the output of the amplifier and the computer. The binary pH and $PCO_2$ signals were preceded by identification signals to enable the computer programme to differentiate between pH and $PCO_2$ signals. The computer calculated total bicarbonate concentration every 30 seconds. Since the $PCO_2$ electrode is rather slow-reacting compared with the pH electrode, the computer programme compensated in the calculations by using the pH value recorded 90 seconds before the $PCO_2$ value. The computer was programmed to use a pKa = 6.10 and a S value = 0.039 in the calculation of total bicarbonate concentration since the aspirate had a temperature of about 30 °C. During measurement of gastric bicarbonate secretion the computer stored 252 recordings and performed 256 calculations per hour.

## Salivary bicarbonate

The salivary concentration of bicarbonate varies from a few mmoles/litre to about 25 mmoles/litre. The subjects were instructed not to swallow their saliva and it was collected with the aid of a dental suction set. The volume of saliva collected during unstimulated conditions was up to 20 ml per 15 minutes. Even when saliva was expectorated, the involuntarily swallowed salivary bicarbonate amounted maximally to 13 % of the measured bicarbonate in gastric contents (Forssell *et al* 1984). The amount of involuntarily swallowed saliva was estimated in the present investigation by measuring the concentrations of amylase in the saliva and the 15-min gastric aspirates:

$$\text{SWALLOWED SALIVARY VOLUME} = \frac{\text{volume of gastric contents x gastric amylase conc.}}{\text{salivary amylase conc.}}$$

Any contribution to amylase by duodenogastric reflux was assumed to be negligible

as long as bilirubin was absent from the recovered gastric perfusate and/or any rapid transient increases in bicarbonate concentration from duodenogastric reflux episodes (see below) did not occur. The total salivary bicarbonate concentration was determined from measurement of salivary pH and $PCO_2$ as described for gastric samples. The amount of swallowed salivary bicarbonate was calculated from the volume of swallowed saliva and its bicarbonate concentration:

SWALLOWED SALIVARY BICARBONATE

= salivary bicarbonate conc. x swallowed salivary volume

Finally, the amount of bicarbonate in swallowed saliva was subtracted from the calculated total gastric bicarbonate output to obtain the amount of bicarbonate secreted by the stomach:

NET GASTRIC BICARBONATE OUTPUT

= (total gastric bicarbonate conc.
x volume of gastric contents)
- swallowed salivary bicarbonate

## Duodenogastric reflux of bicarbonate

The occurrence of alkaline duodenogastric reflux was detected from bile staining of the gastric perfusate passing through the transparent measuring device and tubes. Coincident with the visual observation of bile reflux was a short rise in recorded pH and $PCO_2$ values (I). Such spikes were superimposed on the basal level of bicarbonate secretion and were usually of only a few minutes duration. These spikes were excluded in the calculations of gastric bicarbonate output by a computer-editing programme. Moreover, measurement of bilirubin concentration at alkaline pH in the collected 15-min gastric aspirates excluded continuous modest duodenogastric reflux. Periods with a bilirubin concentration › 1 μmol/l were rejected.

## Experimental procedure and protocol

Bicarbonate secretion was measured after an overnight fast. Before the experiment, a histamine $H_2$-receptor antagonist was given orally to the subjects. Two alternative premedication regimens were used in the experiments, either cimetidine, 400 mg, one and three hours before the investigation or ranitidine, 150 mg, two and fourteen hours before start of the experiment. Both regimens gave an increase in intragastric pH to around 6, which usually lasted for about four hours.

The pH electrode was calibrated at pH 6.00 and 7.00 with two buffer solutions. Thereafter, the $PCO_2$ electrode was calibrated against two different concentrations of $CO_2$ for alternate 15-min periods. The subject was seated in a semirecumbent position and the Salem sump tube with the additional small tube for perfusion was introduced into the distal part of the stomach. Residual gastric juice was aspirated and discarded. The perfusion system and the computer based system for measurement of gastric bicarbonate concentration were subsequently commenced. Aliquots of fifteen minutes aspirate were used for marker determina-

tion and for measurement of amylase and bilirubin concentrations. The gastric bicarbonate output values and the amount of swallowed saliva were stored in a computer database (Digital Equipment Corp., USA).

Statistical analyses were performed with the aid of an RS/1 computer programme (Bolt Beranek and Newman Inc., USA). Results are expressed as mean ± SEM. Statistical significance was evaluated using Wilcoxon's matched-pairs signed rank test.

# RESULTS AND DISCUSSION

## The measuring system

In *ex vivo* control experiments, acid was added to bicarbonate solutions in the chamber of the measuring device. The time required to receive stable recordings of $PCO_2$ varied from 0.5 to 3 minutes, depending on the amount of $CO_2$ formed during the process of neutralization. In contrast, stable recordings of pH were obtained almost immediately. Thus, the computer had to compensate for the slower reaction of the $PCO_2$ electrode by using the pH value recorded 90 seconds before that of $PCO_2$.

In other *ex vivo* experiments, the interference of air bubbles in the perfusate was tested. The recordings of pH and $PCO_2$ were disturbed only when large amounts of air bubbles occurred in the effluent. A high rate of gastric perfusion with few air bubbles from the air inlet of the Salem sump tube would thus provide more reliable measurements in human experiments. Two perfusion rates were therefore tested in seven healthy subjects. Rates of 220 ml/15 min, which is the rate routinely used in our acid secretory studies, and 440 ml/15 min were evaluated. Less variation in the recorded basal secretion of bicarbonate was observed with the higher perfusion rate (I). This may reflect the reduced number of air bubbles contained in the effluent. The higher perfusion rate of 440 ml/15 min was thus used in the subsequent studies of gastric bicarbonate secretion.

To further validate the measuring system, exogenous bicarbonate in amounts varying from 50 to 400 μmoles was instilled into the stomach of healthy subjects. The correlation coefficient, r, between added and recovered amounts of bicarbonate was 0.91 ($p < 0.001$, I). This indicates that the bicarbonate output was satisfactorily measured over a wide range of secretory rates.

With a luminal pH at the pKa of carbonic acid, 50 % of bicarbonate occurs as free ions and the rest reacts with acid to form $CO_2$. At values of intragastric pH below 4, virtually all bicarbonate is in the form of $CO_2$. The effect of decreasing intragastric pH on recorded values of bicarbonate output was studied in healthy subjects by adding 250 μmol HCl to the perfusate during one 15-min period. The intragastric pH fell to between 3 and 4 but no change in bicarbonate output was observed during the 15-min period of acid load or in subsequent 15-min periods (I). It could thus be concluded that the system adequately measured bicarbonate even if the intragastric pH fell to between 3 and 4 and that a moderately low in-

tragastric pH did not stimulate bicarbonate secretion. Similar validation experiments were performed by Johansson and coworkers (1983) when testing their technique using gastric instillations. These investigators were unable to demonstrate a stimulation of bicarbonate secretion by a pH of about 3. Recently, Crampton *et al* (1986) reported the stimulatory effect of an intragastric pH of about 2 on gastric bicarbonate output in man. In animal experiments, stimulation of bicarbonate secretion by luminal acid occurs both in the stomach and the duodenum, but the required acidity of the gastric solution needs to be about pH 2 (Heylings *et al* 1984) or even lower (Takeuchi *et al* 1986).

The present results also indicate that loss of $CO_2$ by eructation, passage through the pylorus or diffusion into the gastric tissue is small and presumably negligible. Similar findings have been made by Fändriks in cats (1986b). He reported a modest underestimation of bicarbonate secretion during active acid secretion. Moreover, animal experiments have shown that diffusion of $CO_2$ out of the stomach as well as diffusion of $CO_2$ into the stomach from tissue and blood is small (Garner and Flemström 1978, Kauffman and Steinbach 1981). Hence, the findings of a high intragastric $PCO_2$ value, in the present investigations sometimes above 40 mm Hg and thus exceeding that in blood, and even higher values reported by others (Schierbeck 1892, Rune and Henriksen 1969, Garner and Flemström 1978) indicate that $CO_2$ is produced in the gastric lumen by neutralization of bicarbonate with acid. To minimize the risk for loss of $CO_2$ in the present experiments, inhibition of gastric acid secretion by histamine $H_2$-receptor antagonists was used, particularly since cimetidine has been found to exert no direct effect on bicarbonate secretion in animals (Garner and Flemström 1978, Flemström and Turnberg 1984). It should be mentioned, however, that a high intragastric pH may to some extent decrease gastric bicarbonate secretion by removing the stimulatory effects of luminal hydrogen ions, as discussed above.

The measuring system enables detection of alkaline duodenogastric reflux episodes as indicated by a short rise in both pH and $PCO_2$. These spikes frequently coincided with the visual observation of bile reflux and were superimposed on the bicarbonate secretion level. The short duration of these reflux-generated bicarbonate spikes of only a few minutes very probably reflects the use of a high rate of gastric perfusion to washout alkaline reflux. Moreover, measurement of bilirubin concentration in the collected gastric samples excluded every continuous modest duodenogastric reflux. In a series of experiments in sixteen healthy subjects (mean age 30 years, range 20-46 years, II), no duodenogastric reflux was observed at all in 5 subjects. Eleven subjects had a mean of 4.6 episodes (range 1 - 12) over 105 min of measurement of basal bicarbonate secretion. Less than half of these episodes occurred without simultaneous bile staining of the aspirate. These results indicate that the present method distinctly demonstrates duodenogastric reflux, which thus can be eliminated enabling correct calculation of gastric bicarbonate secretion.

In summary, the various validation tests

have shown that the computerized measurement of human gastric bicarbonate secretion satisfactorily determines bicarbonate output. Continuous measurement of pH and $PCO_2$ seems preferable to discontinuous recording of these parameters. Moreover, the method detects duodenogastric reflux without intubation of the duodenum. A high gastric perfusion rate facilitates the identification of duodenogastric reflux episodes and shortens their duration by rapid washing-out.

## Basal bicarbonate secretion

The rate of basal bicarbonate secretion in 34 healthy subjects (10 women and 24 men) with a mean age of 32 years (range 20 - 46 years) amounted to 366 ± 23μmol/h (mean ± SEM). These subjects have been investigated on a total of 107 different occasions and the intra-individual coefficient of variation was 30 %, a value much less than the 80 % (Feldman and Richardson 1981) and 110 % (Lind *et al* 1986) reported from studies on human basal acid secretion. The larger variation observed in basal acid secretion may be attributed to the influence of a varying vagal tone affecting the parietal cell mass. Basal acid secretion can be inhibited by anticholinergics or vagotomy (Gillespie *et al* 1960, Stenquist *et al* 1979). In contrast, a rather high dose of anticholinergics (benzilonium bromide) was unable to change basal gastric bicarbonate secretion in healthy subjects (II). This may suggest that any cholinergic drive at the mucus cell level does not modulate basal gastric bicarbonate secretion in the intact human stomach. The cyclooxygenase inhibitor, indomethacin, did not significantly affect basal gastric bicarbonate secretion (II), indicating that basal gastric bicarbonate secretion does not depend on the release of endogenous prostaglandins. This is in contrast to the findings in the human duodenum (Selling *et al* 1987) where indomethacin reduced both basal and acid stimulated bicarbonate secretion.

The rate of basal gastric bicarbonate secretion in healthy subjects as determined in the present investigations agrees with the findings by other laboratories using recordings of pH and $PCO_2$ to calculate the bicarbonate secretion (Rees *et al* 1982, Johansson *et al* 1983). Much higher rates of basal bicarbonate secretion in humans, about 2500 μmol/h, however, have been reported by Feldman (1983). Measurements of gastric volume, hydrogen ion concentration and osmolality of both gastric juice and plasma were used to calculate secretion by the method of Feldman. The calculation is based on a two component model of gastric secretion and the assumption of a fixed relation between the osmolalities of plasma and parietal and nonparietal secretions. However, this relation may not be constant and, moreover, has been examined only in the stimulated canine stomach. Another explanation for the quantitative discrepancy in rates of basal gastric bicarbonate output is that hypotonicity of gastric juice could arise from both the neutralization of secreted bicarbonate by acid and the processes for secretion of acid and water within the gastric glands (Flemström 1985). Although it seems that the osmolality method overestimates secretion of human gastric bicarbonate (Odes *et al* 1987), results based on osmolality and pH - $PCO_2$ measurements, respectively, seem to be qualitatively similar.

The basal bicarbonate secretion rate in nine patients with duodenal ulcer disease (mean age 52 years, range 33 - 69 years) was 414 ± 57 μmol/h (III) and did not differ significantly from that in healthy subjects. Basal gastric bicarbonate secretion was increased by 30 % to 539 ± 74 μmol/h ($N = 9$, $p < 0.01$) about 2 months after proximal gastric vagotomy (III). Hypothetically, the effect of partial denervation of the stomach by proximal gastric vagotomy may be consistent with removal of an inhibitory mechanism modulating basal gastric bicarbonate secretion. In support of this hypothesis, benzilonium bromide restored the enhanced basal bicarbonate secretion after vagotomy to a preoperative level (III). Benzilonium bromide had no effect on basal gastric bicarbonate secretion in the intact stomach (II), possibly due to an active inhibitory mechanism.

The enhanced basal gastric bicarbonate secretion must originate either from the innervated and/or denervated region of the stomach. However, the present investigation was unable to further clarify which area of the stomach that had increased its bicarbonate secretion. The increase may originate from the antrum in analogy with the regulation of canine antral secretion of gastrin (Debas *et al* 1975) where a vagally dependent oxyntopyloric reflex inhibition exists. The increased gastric bicarbonate secretion may thus be explained by an enhanced secretion from the antrum caused by removal of such a hypothetical inhibitory mechanism following proximal gastric vagotomy. The antrum accounts for about 25 % of the total surface area of the stomach (Ito 1981) and evidence has been presented (Flemström 1977, Konturek *et al* 1985) that the bicarbonate secretion in the antrum and the fundus-corpus region of the intact stomach is similar. Assuming that the basal gastric bicarbonate secretion from the vagally denervated fundus and corpus area is unchanged after proximal gastric vagotomy, the increase in basal bicarbonate rate from the antrum would be about 120 %. This figure would correspond well with the increase in gastric bicarbonate secretion, about 125 %, two months after vagotomy in response to sham feeding (III). It is reasonable to presume that this response originates only from the antrum.

In the conceptual model of gastric bicarbonate secretion (Fig. 2), one of the final transmittors close to the mucus cell level most probably is acetylcholine. The nature of the inhibitory mechanism likely to affect the intramural ganglia is not clear. It may be mediated via noncholinergic transmission since a rather high dose of benzilonium bromide did not affect the basal bicarbonate secretion of healthy subjects with intact vagal nerves. The influence on gastric bicarbonate secretion of noncholinergic vagal fibres would accord with a previous demonstration in the cat, in which the transmitter was suggested to be substance P (Fändriks and Delbro 1983). Moreover, a noncholinergic vagal release of gastrin and a cholinergic inhibitory mechanism controlling gastrin release have been shown in man (Feldman *et al* 1979, Stenquist *et al* 1979). A vagal cholinergic stimulatory effect on the gastric motor activity and a vagal noncholinergic, probably VIP-ergic, relaxatory effect on the stomach have also been demonstrated (Martinson and Muren 1963, Fahrenkrug *et*

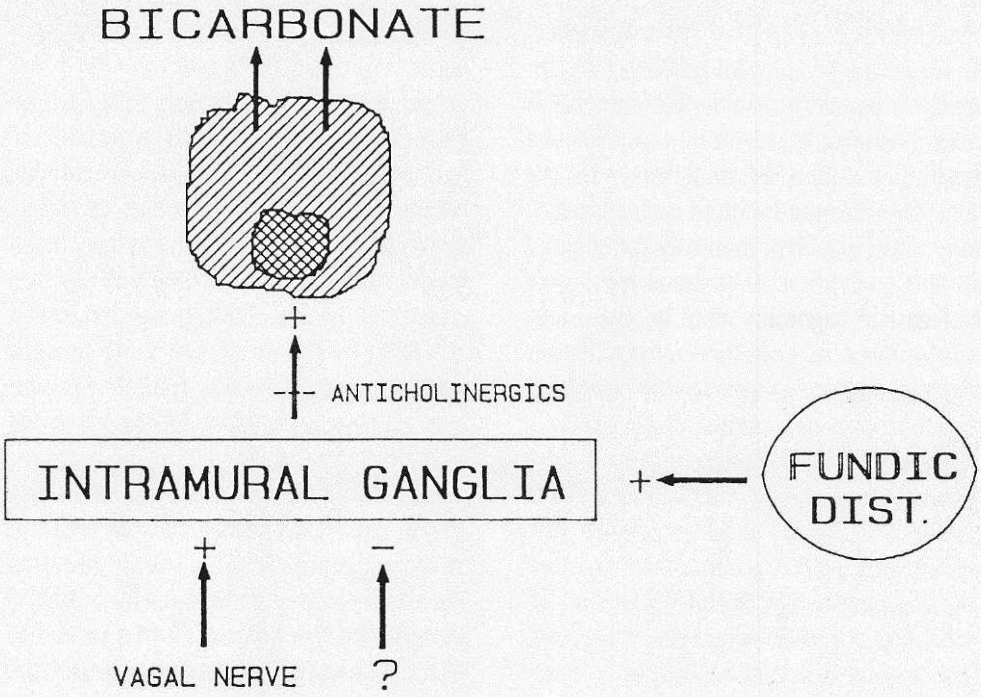


Fig. 2 Conceptual model of the regulation of human gastric bicarbonate secretion.

*al* 1978). The inhibitory mechanism may consist of vagally dependent oxyntopyloric reflexes, as discussed above, (Debas *et al* 1975) which are eliminated by proximal gastric vagotomy.

Alternatively, a sympathetic inhibitory mechanism may influence the gastric bicarbonate secretion as demonstrated in the cat (Fändriks 1986b). Some of the postganglionic sympathetic neurons that accompany the blood vessels to the stomach and also innervate the mucosal glands (Furness and Costa 1974) are cut by the proximal gastric vagotomy. In the cat, the vagally stimulated increase in gastric and duodenal bicarbonate secretion was inhibited by an intact splanchnic nervous supply or by administration of an alpha$_2$-adrenoceptor agonist, clonidine. Furthermore, an enhanced bicarbonate output was observed after administration of guanethidine or yohimbine (Fändriks 1986b).

Nevertheless and irrespective of what the nature of this inhibitory mechanism may be, the basal bicarbonate secretion had declined and was at the preoperative level about one year after the proximal gastric vagotomy, suggesting a gradual regress of the non-inhibited basal gastric bicarbonate secretion.

## Vagal stimulation of gastric bicarbonate secretion

Sham feeding is a physiological, vagally mediated stimulus of gastric acid secretion in man (Stenquist *et al* 1978). Modified sham feeding is accomplished by allowing the subject to chew and spit out a meal (Noring 1951). The acid response to modified sham feeding is about 50 % of the maximal acid response to pentagastrin. It is of a similar magnitude to that produced after adequate sham feeding, determined in the postoperative period in patients in whom food was swallowed and subsequently drained via a gastrostomy avoiding chemical stimulation of the stomach by food (Stenquist *et al* 1978). The acid response to sham feeding can be inhibited by about 65 % but not abolished by anticholinergics, suggesting that the neurotransmission at acid-secreting cells is only in part cholinergic and that peptidergic transmission or amines may also be involved (Stenquist *et al* 1979, Stenquist *et al* 1987).

Vagal stimulation by sham feeding, performed identically as in acid secretory studies, was used in the present study to examine whether stimulation of the surface mucus cells increased bicarbonate secretion. Vagal stimulation in sixteen healthy subjects increased gastric bicarbonate output by 69 %, *i.e.* from 410 ± 39 to 692 ± 67 μmol/h (mean ± SEM, $p < 0.001$, II). This increase was mainly caused by an increase in bicarbonate concentration, and to a lesser extent, by an increase in volume. In most experiments, the gastric bicarbonate response to sham feeding began only a few minutes after start of the vagal stimulation with a peak bicarbonate output in the 15-min period of sham feeding (Fig. 3). In cats, electrical stimulation of vagal trunks induced a rapid in-

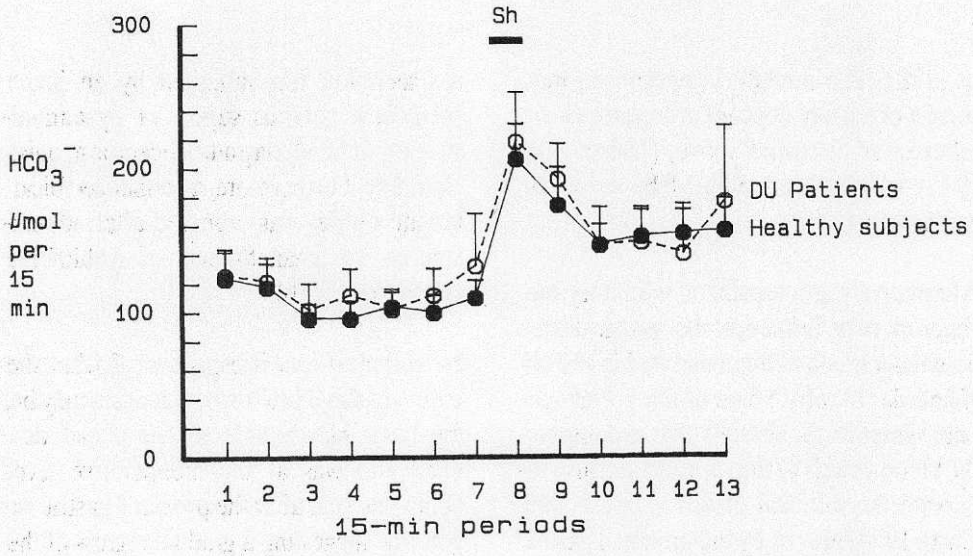


Fig. 3 Basal and sham feeding stimulated gastric bicarbonate secretion in sixteen healthy subjects (closed circles) and nine duodenal ulcer patients (open circles). Mean + SEM. From II and III.

crease in bicarbonate output. This rise in bicarbonate secretion occurred somewhat earlier than that of hydrogen ion secretion, suggesting anticipatory protection by bicarbonate against acid (Nylander *et al* 1987). In the present investigation, vagal stimulation evoked a similar increase in gastric bicarbonate output irrespective of intragastric pH, range above 2 to about 7 (II). This indicates that the bicarbonate response during sham feeding is not secondary to a concomitant increase in luminal hydrogen ion concentration. The anticholinergic drug, benzilonium bromide, which has a minimal ability to cross the blood-brain barrier, inhibited the rise in gastric bicarbonate secretion in response to sham feeding by 88 % (II). The bicarbonate response to vagal stimulation was thus almost abolished by benzilonium bromide, suggesting a mainly cholinergic excitatory effect by sham feeding on human gastric bicarbonate secretion.

The vagal stimulation of gastric bicarbonate output in nine duodenal ulcer patients did not differ significantly to that of healthy subjects (Fig. 3), the increase being 67 % above basal bicarbonate secretion *i.e.* from $414 \pm 57$ to $691 \pm 83$ μmol/h ($p < 0.01$, III). After proximal gastric vagotomy, which was complete in all patients since no increase in acid output was observed after a separate sham feeding test, there was still a significant increase ($p < 0.01$) in gastric bicarbonate secretion, which amounted to 29 %, in response to vagal stimulation. Presumably, this response originated from the still vagally innervated antral part of the stomach. The bicarbonate output, however, is about twice that expected from the antrum. This seems consistent with the observation of an increased secretion also during basal conditions and may be due to the loss of an antral inhibitory mechanism. A third sham feeding test was performed about one year after proximal gastric vagotomy. The response to vagal stimulation was then abolished by anticholinergics which implies a remaining susceptibility to anticholinergic blockade (III).

Quantitatively, the human gastric bicarbonate secretory response to vagal stimulation is much smaller than the acid response to sham feeding. This indicates, on allowing for the neutralizing effect of the secreted bicarbonate, that the amount of acid produced in conjunction with sham feeding clearly is still below the maximal acid secretory capacity determined by a pentagastrin test. Consequently, this implies that sham feeding in man is either a submaximal stimulus of acid secretion or affects both stimulatory and inhibitory acid secretory mechanisms. The gastric bicarbonate response to sham feeding may also be submaximal or sham feeding may affect both stimulatory and inhibitory mechanisms. A much greater response, about a 200 % increase, was thus observed after instillation of 16,16-dimethyl prostaglandin $E_2$ (I, Johansson *et al* 1983). The occurrence of inhibitory actions would be consistent with findings in the cat and rat of a sympathetic reflex inhibition of vagally mediated gastric and duodenal bicarbonate secretion (Fändriks 1986b, Jönson and Fändriks 1986).

## Stimulation of gastric bicarbonate secretion by fundic distension

The second phase of gastric acid secretion is the gastric phase, initiated by food reaching the stomach. Both chemical and mechanical stimuli are involved in this process. Graded distension by stepwise inflation of a balloon located in the fundic area of the stomach elicits a volume-related acid secretory response (Grötzinger *et al* 1977a). This acid response to fundic distension was similar both in healthy subjects and duodenal ulcer patients and the observed peak acid response averaged about 50 % of the peak in response to pentagastrin stimulation. Graded fundic distension to volumes of 150 ml, 300 ml and 600 ml increased the acid output in six healthy subjects by 94 %, 106 % and 194 %, respectively (Grötzinger *et al* 1977a).

Experiments with graded fundic distension of the stomach in six healthy subjects evoked an increase in gastric bicarbonate output amounting to 46 % ($p < 0.05$), 28 % (NS) and 84 % ($p < 0.05$) over 60 minutes of distension to volumes of 150 ml, 300 ml and 600 ml, respectively (Fig. 4). Obviously, no dose-dependent bicarbonate secretion was observed with this experimental design, which was identical with that used in the acid secretory experiments by Grötzinger and coworkers (1977a). Instead, a peak in bicarbonate secretion occurred after 45 minutes of distension with 150 ml, followed by a decline. During distension to a volume of 300 ml, bicarbonate secretion was lower than in the preceding period with 150 ml

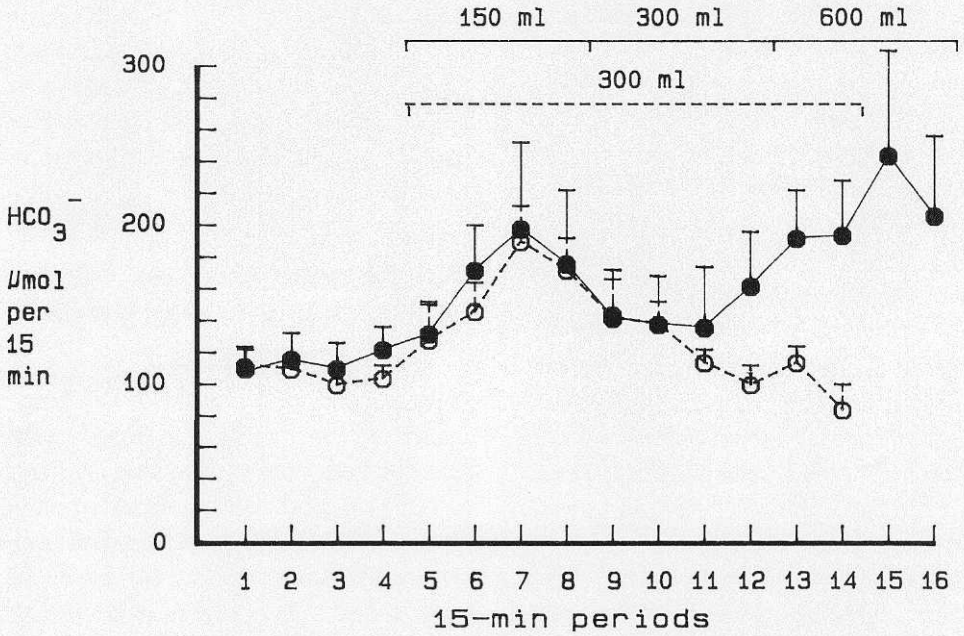


Fig. 4 Basal and fundic distension stimulated gastric bicarbonate secretion. Graded fundic distension in six healthy subjects (closed circles) and continuous fundic distension with a volume of 300 ml during periods 5 to 14 in four healthy subjects (open circles). Mean + SEM. From IV.

and not significantly different from basal secretion. A second peak occurred 45 minutes after start of distension with 600 ml. A series of experiments were performed in four of the six healthy subjects to further clarify the nature of this biphasic response to graded fundic distension. Distension with a volume of 300 ml was maintained for 150 minutes. Again, there was a peak after 45 minutes. Bicarbonate secretion then gradually declined and reached basal bicarbonate levels 60 minutes later despite the continued distension (Fig. 4). These results indicate that fundic distension elicits a fading bicarbonate response, possibly reflecting volume adaptation of the gastric wall. Another possibility to explain the decrease in bicarbonate output during sustained fundic distension would be a somewhat slower activation of inhibitory mechanisms. In fact, Schöön *et al* (1978) have shown that distension-induced inhibitory mechanisms operate in the regulation of gastric acid secretion. Moreover, experiments in healthy subjects as well as in vagotomized duodenal ulcer patients with fundic distension failed to enhance pentagastrin-induced acid secretion. Instead the distension inhibited acid output in some subjects (Grötzinger *et al* 1977b).

In seven duodenal ulcer patients, who previously had undergone a proximal gastric vagotomy, graded fundic distension resulted in virtually an identical biphasic response as in healthy subjects (IV). All patients were considered to be completely vagotomized, since postoperative tests showed no acid increase in response to sham feeding. On the reasonable assumption that the two groups are comparable, it seems that the distension-induced bicarbonate response is mediated mainly by short enteric intramural neural pathways and not by long vagovagal reflexes (Fig. 2). It was also observed that the fading response to graded distension remained after proximal gastric vagotomy.

The response to fundic distension with 150 ml for 30 minutes was abolished in healthy subjects by pretreatment with the muscarinic antagonist, benzilonium bromide, while pretreatment with the cyclooxygenase inhibitor, indomethacin, was without any effect (IV). These results would suggest a purely cholinergic reflex mechanism which is not mediated by endogenous prostaglandin liberation. The latter accords with findings in earlier studies of gastric bicarbonate secretion in man (II, Feldman and Colturi 1984) but contrasts results from studies of human duodenal mucosal bicarbonate secretion (Selling *et al* 1987). In the latter study, indomethacin was given orally in the same dose as in the present investigation, but was shown to reduce both basal and acid-stimulated duodenal bicarbonate secretion.

## Comparison between stimulants of gastric bicarbonate secretion

In the experiments reported (I,II,IV), bicarbonate secretion in four of the healthy subjects had been stimulated by sham feeding, as well as by fundic distension and by prostaglandin administration. An additional series of experiments were performed in these subjects. The effects of increasing doses of carbachol in the range of 100 to 200 µg subcutaneously were tested. The purpose was to compare their maximal re-

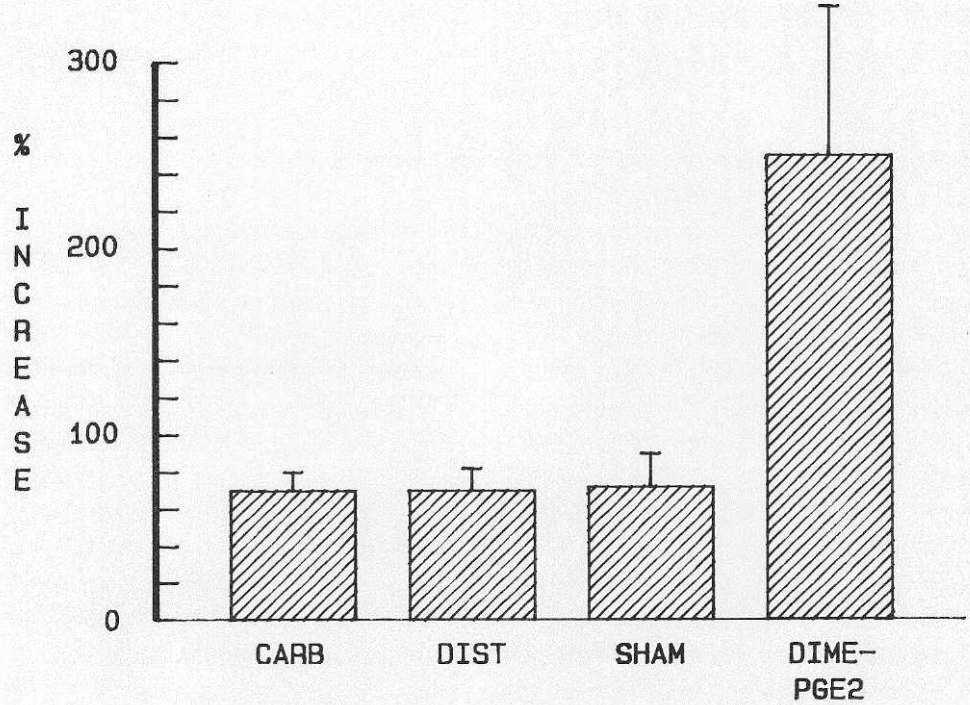


Fig. 5 Increase in gastric bicarbonate secretion in four healthy subjects in response to carbachol 150-200 μg subcutaneously (CARB), fundic distension to a volume of 600 ml (DIST), 15 minutes of sham feeding (SHAM) and gastric instillation of 80 μg 16,16-dimethyl prostaglandin $E_2$ for 5 minutes (DIME-PGE2). Per cent increase above basal level. Mean + SEM.

sponses to the different stimuli. The maximum dose of carbachol tolerated was 200 μg, which caused a slight bradycardia, an increase in salivary concentration of amylase and some abdominal motor discomfort. Sham feeding for 15 minutes, fundic distension to a volume of 600 ml and 150 - 200 μg of carbachol all increased bicarbonate output by about 70 % above basal level. Instillation of 16,16-dimethyl prostaglandin $E_2$, 80 μg for 5 minutes, gave an about threefold greater response (Fig. 5). These findings suggest that vagal stimulation, fundic distension and carbachol are submaximal stimuli of human gastric bicarbonate secretion or that they have both stimulatory and inhibitory actions on bicarbonate secretion.

# FUTURE PERSPECTIVES

The findings in the present investigation suggest a complex interplay between stimulatory and inhibitory mechanisms influenzing basal as well as stimulated human gastric bicarbonate secretion. Further measurements of human gastric bicarbonate secretion in more defined subgroups of subjects or patients may improve the possibility of clarifying these mechanisms. The complexity of the regulation of bicarbonate secretion is highlighted by the vagally dependent stimulatory effect of the muscarinic $M_1$-antagonist, pirenzepine, on mucosal bicarbonate secretion in the rat duodenum (Säfsten and Flemström 1986). The role of a sympathetic nervous influence on gastric bicarbonate secretion is of considerable interest and calls for further investigation. Such a sympathetic nervous control of vagally stimulated gastroduodenal bicarbonate secretion has been shown to exist in cats (Fändriks 1986b). Current experiments in healthy subjects in our laboratory also suggest a sympathetic tone to regulate basal gastric bicarbonate secretion. Since the pathogenesis of peptic ulcer disease is multifactorial and the disease, in many respects, is of psychosomatic nature, an enhanced sympathetic drive associated with mental and physical stress may contribute to a decreased epithelial bicarbonate secretion and result in a defective mucus-bicarbonate barrier. Recently, it has been shown that duodenal ulcer patients may have an impaired proximal duodenal mucosal bicarbonate secretion (Isenberg *et al* 1987). Since duodenal ulcer patients in the present investigation had a normal gastric bicarbonate secretion during basal or vagally stimulated conditions, the findings may indicate that ulcerogenesis is caused by local pathological factors in the duodenum and be due to an increase in aggressive factors combined with a decrease in local mucosal resistance. Patients with prepyloric gastric ulcers have acid secretory values similar or slightly lower than those of duodenal ulcer patients but heal their ulcers very slowly and their treatment is often a considerable challenge to both the physician and surgeon. On the other hand, in the pathogenesis of gastric ulcer disease, acid and pepsin secretion are usually within normal limits or reduced. Hence, a defect in the local gastric mucosal defence mechanism is suggested to operate in the development of both prepyloric and gastric ulcers, prompting the need for further investigations of gastric bicarbonate secretion in these patients. If such a defect in gastric bicarbonate secretion can be revealed, therapy may also be directed to the development of agents that enhance gastric bicarbonate secretion and thus strengthen the protective mucus-bicarbonate barrier.

# SUMMARY

A method for measurement of human basal and stimulated gastric bicarbonate secretion was developed in the present investigation. The mechanisms involved in the regulation of basal, vagus nerve stimulated as well as fundic distension induced bicarbonate secretion were studied.

The investigations were performed in healthy subjects and duodenal ulcer patients, the latter group before and/or after a proximal gastric vagotomy operation. Healthy subjects as well as ulcer patients were premedicated with a histamine $H_2$-receptor antagonist and gastric bicarbonate secretion was determined by use of a gastric perfusion system in combination with computerized continuous recordings of pH and $PCO_2$. The contribution of alkaline saliva to the measured gastric bicarbonate secretion was minimized by continuous salivary suction and correction was made for swallowed saliva by measurement of amylase in the gastric aspirate. A high rate of gastric perfusion facilitated the identification of alkaline duodenogastric reflux and also eliminated its influence on the measurement of gastric bicarbonate secretion.

Validation of the measuring system by instillation of small amounts of bicarbonate showed a satisfactory correlation between added and recovered bicarbonate in the range of bicarbonate determinations usually recorded. Decreasing intragastric pH to between 3 and 4 converted all secreted bicarbonate into $CO_2$, but did not affect the measured value of bicarbonate secretion. Vagal stimulation accomplished by sham feeding increased gastric bicarbonate secretion in sixteen healthy subjects from 410 ± 39 μmol/h to 692 ± 67 μmol/h (mean ± SEM, $p < 0.001$). This response was independent of intragastric pH in the range of 2 to 7. The muscarinic receptor antagonist, benzilonium bromide, almost abolished the sham feeding response while indomethacin left it nearly unchanged. Nine duodenal ulcer patients had identical basal and vagally stimulated bicarbonate output as healthy subjects. Two months after proximal gastric vagotomy, the basal bicarbonate secretion was significantly increased, whereas the output in response to sham feeding was unaltered. In the early postoperative period, anticholinergics reduced the enhanced basal bicarbonate secretion to a preoperative level. In six healthy subjects, graded fundic distension with a balloon to volumes of 150 ml, 300 ml and 600 ml, each distension period lasting 60 minutes, increased the bicarbonate secretion by 46 % ($p < 0.05$), 28 %

(NS) and 84 % ($p < 0.05$), respectively. Continuous distension with 300 ml over 2.5 hours increased the bicarbonate secretion with a peak at 45 minutes, whereafter the response gradually declined. Seven duodenal ulcer patients investigated after proximal gastric vagotomy had a response to graded fundic distension virtually identical to that of healthy subjects. Anticholinergics abolished the response to fundic distension, whereas indomethacin was without any significant effect. In healthy subjects, 16,16-dimethyl $PgE_2$ gave an about threefold greater response than vagal stimulation, fundic distension or carbachol.

It is concluded that human gastric bicarbonate secretion is activated by cholinergic vagal nerves, the response being independent of intragastric pH at levels above pH 2. There seems to be an interplay of stimulatory and inhibitory mechanisms modulating basal as well as vagally stimulated gastric bicarbonate secretion. Fundic distension of the stomach stimulates bicarbonate secretion and the response is mediated by intramural neural cholinergic pathways. Neither vagal stimulation nor fundic distension apparently involves endogenous prostaglandin production. Vagal stimulation, fundic distension and carbachol appear to be submaximal stimuli of bicarbonate secretion or else may have both stimulatory and inhibitory actions on human gastric bicarbonate secretion. The increase in bicarbonate secretion during vagal stimulation and fundic distension parallels that of acid secretion and may be regarded as a physiological response to reinforce the protective mucus-bicarbonate barrier.

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