For centuries it was recognized that the stomach produces a juice, which has acidic properties, however, it was not until 1824 when Prout demonstrated the presence of hydrochloric acid in gastric juice. At the same time experiments on a patient with gastric fistula began by W. Beaumont showing alterations of acid secretion after meals and under various psychological conditions. After the discovery by L. Popielski in 1920 that histamine is a direct stimulant of oxyntic glands, histamine started to be used in the 1930s in gastric secretory tests. Then in 1949 the dose of histamine was established by K. Kowalewski to induce in humans maximal gastric secretion and in 1953 Kay from UK, using a similar dose of histamine (0.04 mg/kg), introduced augmented histamine test to determine maximal acid output. The digestive period of gastric secretion can be divided into 3 phases: cephalic phase, gastric phase, and intestinal phase. When an acidified meal reaches the antrum or proximal part of the small intestine, the inhibitory autoregulatory mechanisms are triggered. Using a peptone meal as a physiological stimulant of gastric secretion, Fordtran and Walsh designed in 1973 the intragastric titration method. Histamine stimulates H1 and H2 receptors, producing some side effects so Betazole (Histalog), an analogue of histamine was introduced, because of smaller side effects than with histamine. In 1967, pentagastrin, which contains a C-terminal amino-acid sequence of gastrin and does not exert serious side effects, was applied first in Poland as a stimulant of gastric acid secretion instead of histamine. At the present time, a 12 or 24 h pH-metry with a magnetic recording of gastric acidity using the Digitrapper was found to have a greater diagnostic value in assessment of gastric acid secretion under natural conditions including meal than classic gastric secretory tests. This technique has been widely used in detecting the duodeno-gastric or gastro-esophageal reflux (GERD) and testing various drugs affecting gastric acid secretion and healing acid-pepsin disorders.

Key words: gastric secretion, meal, histamine, pentagastrin, intragastric titration, pH-recording
INTRODUCTION

Archaic theories on gastric content

For centuries it was known that the stomach produces a juice, which has sour or acidic properties. However, it was not clear what kind of acid was produced in the stomach. The first hypotheses considered three possibilities: lactic acid, acetic acid, or butyric acid. Not until the XIX century, the detection of the presence of hydrochloric acid in gastric juice was made. The controversy concluded with the discovery of this acid in the stomach by Prout in 1824 (1).

Examination of gastric acid secretion in XIXth and XXth centuries

In 1822, Beaumont began his famous experiments on a wounded patient who developed a chronic gastric fistula (2). During the next eleven years, he collected gastric juice, obtained through a fistula from this patient and analyzed its content. He discovered the alterations in gastric secretion depending on the digestive state of the patient. These investigations initiated gastric secretory testing in man (1).

The first studies related to the stimulation of gastric acid secretion took place in 1876, when Leube applied a standard meal 7 h before gastric juice aspiration. The concept of a fractional test meal was introduced in physiology after the experiments of Ewald and Boas, Ehrenreich, as well as Rehfuss (2 - 4). Their investigations were carried out in the second part of XIXth century and the beginning of XXth century. They collected samples of gastric acid at frequent intervals after a standard meal.

Since 1930, the method of stimulated gastric secretion was developed. Polland and Bloomfield in 1931 (5), and then Ihre in 1938 (6) used histamine, which was known since L. Popielski's discovery in 1920 to be a potent and direct gastric secretagogue. Histamine was initially administered in a dose of 10 µg/kg to stimulate gastric acid secretion in humans. K. Kowalewski, working after WWII in the Free University of Brussels, attempted to establish the optimal dose of histamine to achieve the maximal stimulation of acid secretion, by using a dose of 0.03-0.04 mg/kg. He concluded that this dose fulfills the criteria for the maximal histamine test. Kay, using an identical dose to that of Kowalewski, that is 0.04 mg/kg, also reached maximal stimulation of acid secretion in humans in 1953 (7). Because of the increased dose of secretagogue was used and maximal acid output was reached, this test was called by Kay the "augmented histamine test". Kay attempted to explain the observed phenomenon of maximal histamine tests by correlating maximal acid output with parietal cell mass (number), which was subsequently confirmed in humans by Card and Marks in 1960 (8).

Pathological aspects of gastric acid secretion

In the 1920, when Pavlov's theories of nervism dominated in gastric physiology, L. Popielski, Polish professor of pharmacology at University in Lvov,
found for the first time that histamine is a direct and potent non-nervous stimulant of gastric acid secretion and may be useful in testing gastric secretory activity in humans. Basal acid secretion was thought at this time to be of neural origin (9). In 1932, Henning and Norjsoth (10) demonstrated for the first time that in patients with duodenal ulcers, an increase of gastric acid secretion occurred. The reasons of this enhancement of gastric secretion in ulcer patients remained unknown, until the discovery of *Helicobacter pylori* infection and its eradication in therapeutic regimen of ulcer disease starting in 1980s (11).

**Phases of gastric secretion**

Gastric secretion depends upon the intrinsic activity of gastric glands, autonomic innervation, gastrointestinal hormones and blood flow in the gastric mucosa. A meal appears to be the most potent, physiological stimulant of acid and pepsin secretion. The dual control of gastric secretion by vagus nerves and gastrin ensures optimal postprandial stimulation (12).

The digestive phase of gastric secretion can be classically divided into 3 phases: cephalic, gastric and intestinal phase.

**Cephalic phase of gastric secretion**

The cephalic phase of gastric acid secretion is caused by the action of stimuli on receptors in the head region, resulting in the activation of bulbar vagal centers. Then, vagus nerves convey impulses to gastric glands, stimulating secretion of hydrochloric acid and pepsin. A part of efferent vagal impulses passes to G cells through the postganglionic neurones releasing gastrin-releasing peptide (GRP) and increasing the release of gastrin and part of the impulses reach the enterochromaffin cells (ECL-cells) to release histamine. The oxyntic cells are equipped with gastrin (CCK₂), cholinergic (M₃) and H₂-receptors that respond to direct or indirect (histamine and gastrin) stimulation of vagal nerves, producing gastric acid secretion (*Fig. 1*). The evidences supporting the participation of vagal nerves in gastric acid secretion during the cephalic phase was first provided by Pavlov and his team at the famous Department of Physiology of Military Medico-Chirurgical Institute at St Petersburg (13).

Cephalic phase can be mimicked by pharmacological methods, for example by hypoglycemia evoked by insulin or by 2-deoxy-D-glucose (2DG) (14). These secretory tests have been widely used in testing the integrity of the vagal nerves and in the past to document the accuracy and completeness of a surgical vagotomy used for the treatment of peptic ulcer disease. As vagotomy is no longer performed for the surgical treatment of peptic ulcers, these tests have lost their significance in gastric secretory testing. Large doses of atropine or pirenzepine are capable of suppressing gastric acid secretion induced by cephalic-vagal stimulation and this has been called medical or pharmacological vagotomy.
Gastric phase of gastric secretion

The gastric phase occurs during the presence of a meal in the stomach. A major role in this phase has gastrin released from G cells in the antral part of the stomach; however, short- and long vago-vagal reflexes initiated by the distention of the gastric wall and products of protein digestion also contribute to gastric acid secretion. Gastrin was discovered by Edkins in 1905 (15). In the 1960's Gregory and his colleagues purified gastrin from antral mucosal extract and from a tumor of Zollinger-Ellison syndrome (gastrinoma) and revealed the chemical structure of gastrins (16, 17). The predominant form of gastrin in the antral mucosa and the circulating blood is 17-amino acid gastrin ("little gastrin") and 34-amino acid peptide ("big gastrin") with identical C-terminal sequence including Trp-Met-Asp-
Phe-NH₂, that by itself has similar acid stimulatory activity to the whole gastrin molecule. In the beginning of the 1970's, Yalow and Berson (18,19) detected other form of gastrins composed of larger molecule and naming it, "big big gastrin". In the case of gastrinoma, hypersecretion of gastrin results in a very potent and prolong stimulation of gastric acid secretion (Zollinger-Ellison syndrome). The role of gastric secretory tests in this syndrome will be described later.

**Intestinal phase of gastric secretion**

When a meal reaches the proximal part of the small intestine, the last phase of gastric secretion occurs - the intestinal phase. In the 1940's, Gregory and Ivy (20) proved the humoral nature of this phase. More recently, a hormone called 'enteroxyntin' has been held responsible for this intestinal phase but the role of gastrin released from duodenum cannot be excluded (21).

Sokolov, in 1904 (22), showed that the presence of acid in duodenum has inhibitory influence on gastric secretion. This phenomenon was further investigated by Konturek and Grossman (23) in 1965. They found that the upper duodenum is involved in the inhibition of gastric secretion, and found that the excision of consecutive parts of duodenum leads to a gradual increase in acid production by the stomach. A total excision of the duodenum completely abolished the inhibitory effect of acid in the intestines on gastric secretion. Subsequently, in 1971, Konturek and Johnson (24) explained this mechanism by inhibitory reflexes between the duodenum and the stomach (intramural and vago-vagal duodenogastric reflexes).

*H. pylori* infection was reported to disturb the autoregulation of gastric acid secretion, and this has been explained by the decrease in the number and the secretory deficiency of the D-cells in the antral and duodenal mucosa which controls the release of gastrin from G-cells (11, 12). There is no particular test detecting the disturbance of gastric autoregulation due to *H. pylori* infection except the finding of the enhanced plasma level of gastrin in infected patients (11).

**Indications for gastric secretory testing**

In the 1970s the usefulness of various secretory tests in detecting gastric acid-pepsin disorders was widely discussed and their application in the diagnosis of secretory alterations, especially in hyperchlorhydria in peptic ulcerations and gastrinoma (Zollinger-Ellison syndrome) was considered (Fig. 2). With wider use of upper endoscopy and the gastric pH-metry, the usefulness of gastric secretory tests markedly declined.

**Diseases with gastric hypersecretion**

An increase of gastric hydrochloric acid secretion in patients with dyspepsia seems to be the most obvious indication to perform gastric secretory testing as a diagnostic procedure. The first ailment considered in this spectrum was duodenal ulcer disease. There was an observed increment of gastric acid production related
to duodenal ulceration, accompanied by hypergastrinemia, indicating that the normal control of gastrin release, by enhanced luminal acid concentration, is disordered. Elevation of gastric acid content in the stomach is, however, not pathognomonic, because in some cases of duodenal ulcer disease, acid concentration remained within normal limits (25, 26). The reason of hypergastrinemia, accompanying peptic ulcerations was revealed in the 1990s (27), when the role of \textit{H. pylori} in the antral regulatory processes was established.

The main area of usefulness for gastric secretory tests remains for detecting gastrinoma. The excessive gastric acid secretion in this syndrome was recognized before and it was found to be accompanied by very high levels of plasma gastrin. An elevated level of basal acid output, near the maximal acid output, appeared to be symptomatic for Zollinger-Ellison syndrome (Fig. 3). This property could be detected by gastric secretory tests.

\textit{Diseases with gastric hyposecretion}

A decrease of gastric acid secretion can also be easily revealed using secretory tests in certain diseases such as pernicious anemia, gastric atrophy or gastric cancer. In the 1970’s, gastric secretory tests were useful in the study of these cases. However, it is important to emphasize that these tests were performed for research purposes rather than for routine clinical practice.

\textit{Fractional test meals. The intragastric titration method}

A meal is commonly known as a potent, physiological stimulant of gastric secretion. In 1973, Fordtran and Walsh (28) designed the intragastric titration method to measure the postprandial (gastric phase) of gastric acid secretion.
Under normal conditions, the intragastric pH gradually decreases in response to a liquid, usually peptone meal. The first step of this test was ingestion of a meal. Then small samples of gastric content were aspirated at intervals every few minutes and the pH of the samples was determined. This procedure allowed measurement of intragastric pH and whenever this pH fell below pH 5.5, sodium bicarbonate (0.15 mmol/L) was added to the gastric liquid meal (usually 10% liver extract) in order to maintain a constant intragastrical pH at the original value of pH 5.5. The amounts of sodium bicarbonate solution instilled into the stomach, corresponded to the amount of hydrochloric acid secreted by the stomach after meal ingestion.

In 1974 Konturek et al. (29), at the Institute of Physiology Academy of Medicine in Cracow, modified this method, using a barostat to maintain a constant intragastric pressure and gastric emptying rate. The gastric acid secretion measured with this technique reached the value of augmented histamine or pentagastrin tests and was accompanied by a marked rise in plasma gastrin level. The peak acid secretory output obtained with liver extract meal was about twice as high as that induced by sham-feeding but comparable to that obtained with pentagastrin or histamine. This technique was very useful in detecting the alterations in gastric acid secretion induced by the addition of gastric inhibitors such as H₂-receptor antagonist or proton pump inhibitors (Fig. 4).

**Other methods**

In the 1970's, Go et al. (30) and Malagelada et al. (31) described other method for measuring of gastric acid secretion. This method required placement of 2 tubes: one in the stomach and another in the duodenum. The basis of this technique differed from intragastric titration in that the pH of the liquid meal in
the stomach was not maintained at its constant and original value. Instead, the intragastric pH was allowed to reach its natural level in response to gastric acid secretion. A series of equations were necessary to calculate gastric acid secretion. It should be mentioned that in 1976 MacGregor et al. (32) independently described a comparable method.

Rune (33) had a different approach for measuring of meal-stimulated acid secretion. He determined arterial pH and pCO₂, at frequent intervals after a meal to calculate base excess resulting from the acid secretion into the lumen of the stomach.

**Ethanol and caffeine tests**

Ethanol and caffeine were previously employed as stimulators for gastric acid secretion. However, the response of a stomach to ethanol or caffeine was much smaller, as compared to pentagastrin, histamine or a meal. Therefore, ethanol and caffeine are no longer used for routine gastric secretory testing in humans (34).

---

**Fig. 4.** Effects of therapeutic dose of cimetidine, ranitidine or omeprazole used in lower (20 mg) or higher dose (40 mg) on gastric acid secretion in duodenal ulcer patients following sham-feeding, liver extract meal, pentagastrin or histamine. (Konturek S.J. et al., 1976)
AUGMENTED (MAXIMAL) GASTRIC SECRETORY TESTS

Histamine or Histalog secretory tests

Since the discovery of histamine as gastric secretagogue by L. Popielski, it is well known as potent stimulant of parietal cells to produce hydrochloric acid. It acts entirely through the activation of H₂ receptors on these cells. However, histamine also stimulates H₁ receptors, producing severe side effects, such as hypotension, dizziness, nausea, palpitations, and headache. To avoid the above mentioned side-effects, antihistamine drugs (e.g. promethazine), which block H₁ receptors, should be administered prior to examination. A patient's position plays an important role during testing, because of the risk of hypotension. The most appropriate position is a recumbent one or, at least, a sitting position during the histamine secretory test (35).

Following its description by Kowalewski in 1949 and then by Kay in 1953, the augmented histamine test was introduced into routine clinical practice. Histamine was administered in a dose of 40 µg/kg in this test. Previously histamine was applied in lower doses but this did not produce maximal acid output. After intravenous or subcutaneous administration of dose 40 µg/kg/h, histamine can

---

**Fig. 1. Dose-response curves to histamine and pentagastrin in DU patients**

*Fig. 5. Effects of graded doses of histamine (10-40 µg/kg) or pentagastrin (0.24 - 6 µg/kg) on gastric acid secretion in patients with duodenal ulcers. (Konturek S.J. et. al., 1976)*
induce acid secretion reaching maximal acid output. This dose was established as a standard in further investigations. After histamine administration the peak secretory effect usually occurred during the first postinjection hour. Nevertheless, because of danger for patients, caused by side effects, a derivate of histamine, betazole, replaced native histamine in secretory stimulation.

Betazole, an analogue of histamine, is better known as Histalog. It was administered in higher doses than histamine, i.e. 2 mg/kg (36). It produced the peak effect later than histamine, i.e. during 2nd hour. It was reasonable to replace histamine by betazole in gastric secretory testing due to lower rate of side effects of betazole (37). Therefore, in this case, administration of the antihistaminic drugs is not necessary. However, when at the end of the 1960's, pentagastrin began to be used in gastric secretory tests, the histamine and its analogue were quickly replaced by pentagastrin for gastric secretory testing (38). The reason for this situation was a markedly lower intensity of side effects, a faster production of peak secretory response, and a lower dose required to produce a maximal secretory response.

**Pentagastrin secretory test**

As mentioned above, in 1967, pentagastrin was approved as a stimulant of gastric acid secretion instead of histamine. Pentagastrin appeared to be safer for patients and the secretory response to pentagastrin was found to be identical to that of gastrin and histamine.

It is commonly known, that gastrin stimulates specific receptors on ECL cells to release histamine, which then acts on H₂-receptors of parietal cells to stimulate acid secretion. There may be some gastrin receptors on the parietal cells but the major pathway of the stimulation of gastric acid is through the release of histamine from ECL cells and activation of H₂-receptors of parietal cells (12). With isolation and purification of gastrin by Gregory, it was conceivable to use native gastrin in order to stimulate gastric acid secretion. However, native gastrin is a 17 or 34 amino acid containing peptide, which is rather expensive. In 1976, when gastrin was already synthesized, Walsh et al. (39) used it as a secretagogue in gastric secretory testing in humans, however, its production was too expensive to be employed in routine clinical investigations.

Because of these problems, it was necessary to find an analogue of gastrin which would possess the same biological activity as native gastrin. The most effective was found to be a 5-amino acid peptide containing the C-terminal portion of the gastrin molecule which produced a maximal response of gastric acid secretion. Therefore, it was enough to synthesize a peptide composed of these 5 C-terminal amino acid sequence of natural gastrin in order to obtain a potent stimulation of parietal cells. Due to the structure of this substance, it was called pentagastrin (38, 40). In 1966, the usefulness of pentagastrin and tetragastrin was thoroughly examined as gastric secretagogues in our institute in
Cracow (41). It was established that tetragastrin had the same physiological properties as pentagastrin and it also produced minimal side effects (42).

Pentagastrin has a property of inducing of maximal gastric secretory response similar to that evoked by histamine, betazole, or native gastrin. Therefore, it can be used in testing maximal gastric acid secretion. Pentagastrin was administered either subcutaneously or intravenously as a single bolus or as continuous infusion of graded doses. A range of effective doses was from 0.24 to 6 µg/kg (38, 40). It was established that the standard dose in intravenous infusion of pentagastrin producing maximal gastric acid secretion was about 1.2 µg/kg/h (Fig. 5) (12, 41). After pentagastrin application, the peak secretory effect took place within 45 to 60 minutes (43). To summarize we can conclude, that pentagastrin has advantages, as compared to histamine and its analogue, i.e. smaller side effects and an earlier stimulatory peak response. However, after pentagastrin administration rarely side effects can also be observed, such as nausea, diaphoresis, hypotension, even syncope. It is important to emphasize that hypotension after pentagastrin application rarely occurs as compared after a histamine injection (44). Pentagastrin-dependent side effects increase with an elevated dose of this peptide and they often take place after rapid intravenous injection of pentagastrin.

![24 h record of intragastric pH before and after administration of omeprazole](image-url)

*Fig. 6. 24 h record of intragastric pH before and after administration of omeprazole. The time periods of lunch, dinner and breakfast as indicated by arrows.*
The pentagastrin-stimulated gastric secretory test maintains its clinical value until present time. It is useful, as an additional examination, in diagnostic procedures, in the case of diseases with hypochlorhydria or hyperchlorhydria.

At present time

Gastric analysis with fractional test meals, histamine or pentagastrin administration were performed predominantly from 1960s to 1980s. They were, at that time, experimental methods, which were tested in order to assess their usefulness in the diagnostic regimen related to gastric diseases. However, improvement in gastroscopy, led to a decrease in the practical value of gastric secretory tests in clinical circumstances. Introduction of novel techniques, such as 12 or 24 h intragastric pH-metry, gave the possibility of accurately measuring gastric circadian pH changes (Fig. 6). It appears to be undoubtedly better to measure acidity in the stomach under natural conditions than by the classic gastric secretory tests. The pH-metry allows the determination of intragastric acidity under normal daily and nocturnal conditions that is in the interdigestive periods, such as, after food intake, and at night when in some patients, despite gastric inhibition, periods of acid production occurs, resulting in nocturnal pain and gastroesophageal reflux.

CONCLUDING REMARKS

1. Gastric acid secretory testing serves to determine the secretory capacity of the gastric glands to produce acid that depends upon the number of active parietal cells;
2. Most popular secretory tests are induced by histamine and pentagastrin, which are used to determine the gastric secretory activity in various gastric diseases, especially in peptic ulcer and gastrinoma;
3. At present these secretory tests are replaced by 12 or 24 h gastric pH-metry which allows for the examination of the gastric secretion under natural conditions without any risk of side effects which have been described both in tests with histamine or pentagastrin.

REFERENCES


Received: November 15, 2003
Accepted: December 15, 2003

Author's address: S. Kwiecien, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka St., 31-531 Cracow, Poland. Phone: (12) 421 1006; fax: (12) 4211578