ELECTRICAL IMPEDANCE MEASUREMENTS IN GASTRIC FUNCTION INVESTIGATIONS

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Abstract

Electrical bio-impedance can be considered as a physiological measurement of significant value in research and clinical applications. This is because certain structures of living organisms and the human body such as tissues and membranes are characterized by their specific impedance, which can be easily measured with existing technology. In addition, many body functions (physiological processes) involve changes in their ionic and electrolytic content and size, features that are followed by changes in their electrical characteristics indicated by their impedance values. The use of surface electrodes for measuring impedance in humans makes the method more attractive. Thus, during the 2nd half of the last century, impedance techniques have been developed to measure the blood flow in limbs, to form cross-sectional images of human body sections and to define body composition. The imaging techniques suffer from poor resolution due to the anisotropic electrical properties of the tissues.

Epigastrography, based on determination of electrical impedance changes, is a simple, inexpensive, radiation free technique, which can be repeated many times without any trauma to the patient and is a valuable research tool.

This work involves the generation, analysis and interpretation of epigastric electrical impedance signals. The signals recorded represent the epigastric impedance of a fasting volunteer before, during the oral intake of a liquid meal of typically 450 mL and for periods between 45 minutes to 2 hours post-prandially.

The half emptying times (T50s) were calculated for a variety of test meals, and the statistically significant differences between the T50s were found for meals which varied in calorific content in a total of 7 studies with at least 9 subjects per study. Significant differences between T50s were found also when testing the same meal but under different conditions, namely, intravenous infusion of peptides (GLP-1, loxiglumide) or placebo (physiological saline) and the release in the gastric cavity of amino acids, free or bound with an orally taken gelatine capsule.
However, the impedance T50s calculated were found to be considerably shorter than half emptying times presented in the literature using other techniques. Simultaneous application of scintigraphy confirmed that the T50s based on impedance were in comparison shorter. Similarly when experiments were carried out, simultaneously with the octanoic acid breath test and application of paracetamol absorption the same shorter, in comparison, values of T50s were obtained.

The comparison with scintigraphy strengthened the suspicion that impedance values were being strongly influenced by the presence of gastric acid. Gastric acid studies undertaken for a number of conditions supported the considerable influence that gastric acid played.

In vitro measurements of conductivity and pH of mixtures of test meals with gastric juice aspirates from subjects showed the acid influence.

Finally, the physiology of the gastric mucosa from the resting state to secretion leads to the hypothesis that epigastric impedance is in the main controlled by the conductivity of the gastric content rather than by the volume of the meal content in the stomach and is supported by the laws of physics underlying the electrical impedance of a bulk object.

In conclusion, both experimental and clinical results from the studies and investigations undertaken in this research convinced the author that the epigastric impedance reflects the conductivity and the acidity of the gastric content.

Further by carrying out signal processing of the epigastric impedance signals employing fast Fourier transformation information was extracted about the gastric contractions in the range of 2.5 to 4.5 cycles per minute, confirming the usefulness of the technique in the study of gastric motility.

In addition studies are necessary, to establish the validity of the results in the clinical environment and to answer conclusively the question "whether epigastric impedance can be developed as an external monitoring technique of gastric acidity and motility".
to my sons,

Nicholaos & Constantinos

for their understanding and support always.

Even in the most unpromising periods in life

something positive can be achieved.
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The stomach shows relative simplicity in its anatomical construction but its task is poly-synthetic.

The stomach is responsible of receiving and processing the raw material (food) and forwarding the chymified product to the intestine where nutrients and elements are absorbed necessary for the development and maintenance of the living body. The variety of the physico-chemical and biological properties of the substances the human stomach receives is vast, and its tasks undertaken are to kill most of the microorganisms ingested by the food, to eliminate the food particle size, to homogenize (chymification) it and propel it at a certain rate (emptying rates) to the duodenum. This poly-task becomes possible by the action of its secretions on the food and by its special mechanical activity.

Scientists tried to discover the secretes of the stomach's action since long ago by, for example, causing birds to vomit the food they had taken in at specific times and human beings to vomit at will.

First Stevens (Ste 1777), more than two centuries ago, studied the proteolytic activity of the gastric secretion in man. In 1824 Prout (Pro 1824) demonstrated and measured hydrochloric acid in the human gastric juice. About the same time (June 6 1822) "the man and the opportunity had met". "The man with a lid on his stomach" because of a gunshot injury created the circumstances and the army surgeon W. Beaumont grabbed the chance to study and describe the phenomena of the human stomach by observing and experimenting a living open human stomach for years.

Beaumont anticipated some of the most recent studies in the physiology of digestion. His written observations, experiments and conclusions were filtered into the text books of physiology. Until today not much has been added to the knowledge of the phenomena of the secretion of gastric juice and digestion in the stomach, and advanced technology only just verifies and quantifies Beaumont's observations in a solid way.
In 1871 the first clinical gastric secretion test by Leube was designed (Leu 1871). Professor Pawlow of St. Petersburg created ingeniously an isolated pouch at the fundus of the dog and it was possible to obtain gastric juice in a pure state. He confirmed Beaumont's words: "the gastric juice never appears to be accumulated in the cavity of the stomach while fasting".

Beaumont was the first to introduce and measure the "gastric emptying time" of various meals and relate the calorific content as regulator of the emptying rates of the gastric content and verify the hypothesis of Hippocrates that: "there are many kinds of aliments, but that there is at the same time but one aliment". Beaumont said instead: "the active solvent of the stomach should produce the same effect on all alimentary substances, is no more wonderful than that caloric should liquefy all kinds of matter".

Beaumont observed repeatedly and described in detail the mechanical activity of the stomach for trituration, mixing, homogenization and propulsion of its content in an amazing way. He first realized the quantity of the juice secreted depended on the emotions, exercise, and weather. He was the first who observed and described the "Gastric Function" in all its components and not partially.

But even these days according to the expert evaluation and after thorough reviews and discussions (Cam 98, Rea 89, Bar 78) clinical routine tests quantifying the parameters of gastric function have not been developed. Many tests based on different methods are conducted in various centres but from the researchers' interest and neither protocols have been established on the procedures nor values defining normality and abnormality. Additionally most of the methods are invasive, relatively expensive and risk inducing. Thus, the need of more work to be done for the improvement of the existing techniques and the development of new modalities that may give a better answer is evident.

During the last two decades scientists are trying to use the bioimpedance of the gastric area in order to study gastric function either forming images (electrical impedance tomography or applied potential tomography) or simply analysing the recorded bioimpedance signal over time. Bioimpedance is a meaningful magnitude of the human body because bioimpedance measurements depend on the electo-
physico-chemical processes and consequently can give information about size and physiological changes that occur at a particular site. Bioimpedance can distinguish living tissues from dead, large from small, moist from dry, normal from abnormal, resting from stimulated and so on. By applying different frequencies living tissues can be differentiated.

The stomach, being a body unit with a lot of physico-chemical changes due to its highly electrolytic secretions with a variable rate and to the changes of its volume because of the ingestion of food with a broad range of physico-chemical properties is offered to be studied with respect to its bioimpedance.

Indeed, since the beginning of the '80s clear epigastric biosignals are produced in a simple and convenient way and various parameters can be quantified. Relatively acceptable images of the stomach can be obtained, too. The main problem is what do these signals and images represent; are these signals reflecting a specific physiological process and which one; can they be quantified and in which way. The accelerated advanced technology detects and presents phenomena and their interpretation and evaluation must follow.

The present work is focused on the interpretation of the epigastric bioimpedance signals obtained using for the generation of these signals the so called Epigastrograph. The interpretation will be based on the functional gastric anatomy and physiology under various conditions, and comparison of the results obtained with the results of other methods. Specific knowledge on electrolytic substances, and electrochemistry was essential in addition to the physics of bioelectricity. The gastric functional anatomy and physiology needed for the interpretation of the epigastric biosignals are presented amongst other areas in this thesis. An effort of quantification of certain parameters has been made.

Finally, using Fourier transformation the periodicity in the generated signals was extracted. It was concluded that the mechanical gastric contractions were present in the signals and could be quantified; but detailed calculations of the power of the contractions was not made because it involves a tremendous load of work.
As a final comment, this work started mainly as a gastric emptying study, but it was finally a gastric function study. What is the difference between gastric emptying and gastric function, the author hopes, will be clarified at the end. But the author would like to point out that gastric function is related strongly to gastric acid secretion and the epigastric bioimpedance reflects the gastric phenomena controlled by the acid secretion.
2.1 Introduction

Life is dependent on energy. Energy consumption is necessary for the growth and maintenance of the human body. Muscle contraction, conduction of nerve impulses, secretion and absorption of various substances and nutrients by the cells involve consumption of energy. Energy, also, is needed for the chemical reactions in every cell to generate new tissue or to repair a damaged tissue. The Energy in the living organisms is provided by food. Food, as it is consumed, is not in a proper form to be used by the cells and it has to be processed through various stages, known as digestion. Digestion of the food is the breakdown of the food into smaller fragments by two ways, mechanical and chemical. The body organs that perform the digestion comprise the Digestive System, which includes the Gastrointestinal Tract (GI) or Alimentary Canal and the Accessory Digestive Structures (Fig. 2.1).

The gastrointestinal tract is a continuous tube approximately 4.5 m long, running through the ventral body cavity from mouth to anus, so that it is open to the outside body world (Fig. 2.1).

In particular, the GI tract includes the mouth, pharynx, oesophagus, stomach, small intestine and large intestine. The accessory structures or glandular organs namely are: salivary glands, liver, gallbladder and pancreas (Fig. 2.1).

2.2 The stomach (role, location and anatomical features)

2.2.1 Role

The stomach is a temporary storage and process muscular sack with very sophisticated walls, so as to control and retain a specific lamina environment for the most effective digestion and absorption to occur. It exhibits special mechanical properties for the mixing, breaking and propulsion of the food, houses cells for the
production of the so called gastric juices for the chemical process of the food and responds to secretory stimuli or inhibition signals.

Fig. 2.1: The organs of the Digestive System; Gastrointestinal tract and accessory structures [reprod from Tor 96, p753].

The stomachs of vertebrates show similar structure but also remarkably morphological differences. The development of their stomach depends on the body size and shape, need for food storage, nature of diet and frequency of food intake. The function of the stomach is strongly related to its ability to secrete acid and pepsin.
2.2.2 Location

The stomach varies in shape and position in different persons and in the same individual, depending upon its content and whether the person is in the supine or erect position. In the supine posture, the stomach commonly lies in the left, upper body quadrant meaning that it lies in the upper left part of the abdominal cavity, nearly hidden by the liver and diaphragm. It tends to be higher up and run horizontally in short, stout people (a steer-horn stomach) and is often elongated vertically in tall, thin people (a J-shaped stomach).

2.2.3 Anatomical features

In particular, the stomach is an expansion of the GI tract just below the oesophagus and terminates at the duodenum, the beginning of the small intestine (Fig. 2.1). It is

![Diagram of the interior and exterior of the stomach showing the 4 regions cardia, fundus, body, and antrum](reprod from Tor 96, p767).
approximately 25 cm long in an adult and has a volume of 50 mL and a diameter of about 3.5 cm when it is empty which can expand even to 1500 mL and accommodate 4 L of food without any considerable change in the pressure. This is possible because when it is empty it collapses inwards throwing its mucosa and submucosa into large, longitudinal folds called rugae (Fig. 2.2).

The stomach usually is divided in three parts: fundus, body and antrum (Van 85) (Fig. 2.3). The body and fundic region are thinner walled than the antral. The superior wider part of the antrum, called pyloric antrum, narrows to form the pyloric canal, which terminates at the pylorus and pyloric sphincter. The proximal stomach has been shown secreting mainly acid and the distal mainly hormones.

![Diagram of the stomach showing the wall thickness in various regions.](image)

**Fig. 2.3:** Diagram of the stomach showing the wall thickness in various regions.

Histologically the stomach's wall consists of four tunics, the typical ones of the GI tract, namely as mucosa, submucosa, muscularis and serosa from the lumen and outwards (Fig. 2.2). Mucosa is tunic of interest in the present work.
2.3 Gastric Mucosa (Functional morphology)

2.3.1 General

The gastric mucosa because of its biological and clinical interest has been a subject of much investigation. The investigation studies concluded that stomach walls produce and secrete gastric juice consisting of hydrochloric acid for defending the ingested via food bacteria and for chemical break-up, pepsin for initial protein break-up as well as other electrolytes. Which cell type is responsible for HCl secretion or pepsin or for the rest of electrolytes? What forces determine the movement of the secretions from gland lumen to gastric lumen? The objective of this section is to give answers to these questions.

The specialised task of the stomach is due mainly to the most inner layer towards the lumen of its wall, mucosa. The rest of the layers, submucosa, muscularis and serosa play a supportive role in supply, maintenance generally and in differentiating the mechanical properties and strength of the gastric wall. The superficial layer of the gastric mucosa towards the lumen (surface epithelium) and the housed glands underneath are the most important parts of the gastric mucosa and a comprehensive description will follow.

2.3.2 Mucosal epithelium

The superficial lining of the mucosa towards the gastric lumen is made up of columnar epithelial cells appearing abruptly at the cardia of the stomach with the termination of the stratified squamous epithelium of the oesophagus. These cells have basally located ovoid nuclei and a large supranuclear portion filled with a varying amount of mucinogen-containing granules.

The epithelial layer invaginates below the surface to form pits and foveolae, which communicate with the gastric glands (Fig. 2.4). In this way the glands open into the gastric lumen via the gastric pits, which form shallow depressions occupying 25-50% of the mucosal surface. The gastric pits are partially lined by these cells (epithelial), which are then gradually replaced by a larger cell type, mucous neck cells considered
being the stem cells of the epithelium. More than one gastric glands are associated with a single pit; thus, when viewed from the lumen, the pit with its associated glands has the appearance of a tree, with three to five main branches. This infolding (invagination) effectively increases the surface area of the stomach about 20-fold.

Just below this layer is the lamina propria, a connective tissue housing small blood vessels, nerve fibres and lymphatic ducts. Another layer, the muscularis mucosa separates the lamina propria from the underlying tissues. The epithelial layer, lamina propria and muscularis mucosa consist the mucosa.

Fig. 2.4: Gastric mucosa from the body (A) and antral region (B); the relative absence of parietal is evident [reprod from Van 86, p484]
2.3.3 Glands of the gastric mucosa

The stomach is divided into two general functional portions, the proximal (fundus and upper body) and distal (lower body and antrum). The proximal stomach has been shown secreting mainly acid and the distal mainly hormones (Fig. 2.3). This due mainly to the type of glands each portion is lined with.

The mammalian gastric mucosa contains three distinct types of gastric glands, each lining a separate region of the gastric mucosa: the cardiac, oxyntic or gastric and pyloric glands (Fig. 2.4).

2.3.3.1

The cardiac glands occupy a small ring area of about 0.5-4.0 cm wide around the gastro-oesophageal junction and correspond with the gastric cardia. These glands are tubular, highly-branched and coiled in shape containing mucus cells and few or no peptic or parietal cells. Cardiac glands secrete mucosubstances and small amounts of electrolytes.

2.3.3.2

The oxyntic glands are the most distinctive glands of the stomach and they occupy mostly the fundus and the upper body of the stomach. Gastric glands are straight or slightly coiled tubules oriented perpendicularly to the gastric mucosa surface. From three to seven gastric glands empty through a slight constriction (the neck of the gland) into the deepest portion of each gastric pit, penetrating to about a quarter of the thickness of the mucosa.

It has been identified that at least five types of cells are housed in the gastric glands named as mucus neck cells, undifferentiated neck cells, parietal or oxyntic, peptic or chief and endocrine cells. A brief description of the oxyntic gland cells follows excluding the parietal cells that will be described later on because of their uniqueness, importance and role.
The mucous neck cells are relatively few in number and are located between the parietal cells in the neck and upper segment of each oxyntic gland. The apical cytoplasm of these cells is occupied in part by mucous granules, the stippling of which is less pronounced than in the surface epithelial cells and is progressively less in the cells further down the pits.

In the junctional area between the epithelial cells lining the pits and the neck region of the glands the undifferentiated cells are lying with numerous of mitoses. These cells appear to represent the “mother cells” of the surface epithelium and of the oxyntic glandular cells (Wil 73).

The peptic cells situated mostly in the lower half of the gland tubules are columnar or cuboidal with numerous of large granules, where the precursor of the enzyme pepsin, pepsinogen, is stored and released under appropriate stimulation.

A variety of endocrine cells are scattered between the basement membrane of the oxyntic gland and the peptic cells. Including small, dense and often basally situated cytoplasmic granules are related to the lamina propria and to the capillary bed. Four types of those have been identified, including G-cells for gastrin production, EC cells for substance P and motilin, D or A cells for somatostatin etc.

2.3.3.3

The pyloric glands are located throughout the pyloric area of the stomach and are extended into the body mostly along the lesser curvature for a variable distance. They are simply branched or extensively coiled tubules and composed of cells similar to mucous neck cells with few or no oxyntic cells. Their secretion is a mixture of alkaline mucous and some electrolytes such as calcium phosphate, sodium and potassium bicarbonates and chlorides.

Between the oxyntic and pyloric glands area there is a transitional zone of mixed glands (pyloric and others) which may be the site of gastric ulceration. The mucosa of the pyloric gland area develops much deeper gastric pits than the other gastric regions (Fig. 2.4).
In the middle third of the glands and to some extend in the neck region are scattered G-cells as it has been shown by immuno-fluorescence which secrete gastrin. The G-cells are in pyramidal shape with a narrow apical pole covered by microvilli packed in a bouquet and projecting into the glandular lumen (Fig 2.5). The role of microvilli may be the transmission of stimulatory or inhibitory signals from the lumen to the cell. At the base of G-cells adjacent usually to a capillary, there are numerous dense granules under resting conditions and empty granules after stimulants of gastrin release such as food. It has been suggested that gastrin is stored in the secretory granules and discharged into the cytoplasm upon stimulation to be released at the cell base into the capillary bed (For 69).

**Fig. 2.5: Gastrin cell. The apical microvilli open to the lumen [reprod from Joh 87, p844].**

The number of G-cells may increase in pathological conditions with gastric acid secretion as in achlorhydria. In duodenal ulcers (DU) the number of G-cells appears to be normal but there are a higher number of empty granules suggesting greater than usual functional activity.
2.3.4 Parietal or oxyntic cells.

Parietal or oxyntic cells are the most distinctive and characteristic gastric cells and one name (parietal) comes from their appearance in their location site and the other (oxyntic) form their secretion.

2.3.4.1 Location & morphology

There are numerous at the gland neck just below the pits and the upper half of the gland segment. They are moderately large, oval to pyramidal in shape measuring up to 25 μm in diameter. They are often bulging outwards from the walls of gastric glands. They are placed peripherally and their conical shape (Fig. 2.6) allows the cells to be inserted into the core of the gland and to communicate with the lumen of the gland. Their nucleus is centrally located and a large number of mitochondria consist a sign of high-energy demand.

The gland is inserted into a network of capillaries, thus assuring the blood supply to this area of the tissue. There is also complex innervation of the gastric glands where nerve fibers arising from the intramural plexus contact both the parietal and peptic cells. There is evidence that several neurotransmitters, such as acetylcholine (Ach) and epinephrine that modulate the function of the cells are also present.

At the light microscope level the presence of an infolding of the apical plasma membrane is evident and it is called intracellular or secretory canaliculus. The special nature of this structure and the large H⁺ gradient generated by the stomach clearly suggest an association between the canaliculus and H⁺ secretion and transport.

Nowadays, it is well known that these cells secrete the gastric acid and remarkable changes occur during acid secretion in the parietal cell: changes in the cell morphology, changes in the metabolism, and activation of the proton pump.

Evidently, the other name, oxyntic, refers to their ability to secrete acid.
Fig. 2.6: The parietal cell in non-secreting (A) and in secreting (B) state. The long microvilli of the canaliculi on stimulation is striking [reprod from Joh 81, p531]
Studies showing that HCl acid was found only in stomachs containing parietal cells, and that a larger number of oxyntic cells resulted in higher acid production supported the parietal cell as source of the gastric HCl, before it was well postulated. Also, the presence of acid in the fetal stomach coincides with the differentiation of parietal cells (Sal 62). The cytoplasm and cytoplasmic membrane of the parietal cells exhibit specific properties and certain aspects in detail are presented below.

2.3.4.2 Cytoplasm of parietal cells:

An abundance of large mitochondria consist the major component of the cytoplasm of parietal cells occupying 30%-40% of the cytoplasmic volume (Hel 72, Ito 74). The mitochondrial cristae are transversely oriented and may have distinct angular configurations and small, dense intramitochondrial granules are common in the matrix. These are associated with high-energy consumption during the production of the HCl acid as well as the exceptionally high oxidative activity of the gastric mucosa (Fig. 2.6). Isolated parietal cells showed five times higher oxygen consumption than the mucous cells (Sol 78).

2.3.4.3 Cytoplasmic membrane of parietal cells:

Electron microscopic examination of the parietal cell does reveal unique structures, namely the tubulovesicular membranes and the intracellular secretory canaliculi.

In particular, the abundant and conspicuous cytoplasmic membrane component of parietal cells in the non secreting stomach consist the tubulovesicular membranes. These membranes have been characterised as agranular endoplasmic reticulum, vacuoles, vesicles, vesicotubules or bulbotubules and during the early period of electron microscopy there was an ambiguity in the true morphology of the system. This question still remains. Certain methods of preparation (fixation and embedding) favor the vesicle nature, others as the rapid freezing and fixation by freeze substitution, the tubular profiles. Ito et al. (Ito 77) prefer to use as terms, the tubulovesicular system or the tubulovesicular membranes, since the tubular system may be more representative of the living state, and because there always are some vesicles and focal regions of some tubules that are enlarged.
In most gastric mucosae, either in fasting or feeding status of the animal, a variation in the abundance of the tubulovesicular system in different parietal cells has been noted. But under the maximum gastric secretion, there is a rapid depletion of these membranes followed by an increase in the number and size of microvilli of the secretory canaliculi (Fig. 2.6) as both ways, simple observation or stereo-measurements show (For 75, Hel 72-71, Ito 74, Sch 79, Zal 77). The membrane of the microvilli lacks the prominent morphological coating or glycocalyx present on the other cells in gastric glands.

The morphological transformation from rest to secretion in parietal cells in isolated gastric glands has been observed (Ber 80, Ito 77, Sch 79). In particular, Ito and Sedar and their associates separately (Ito 77, Sch 79), in their morphological studies on the parietal cell, observed that the intracellular canaliculus may become completely internalised and close off its opening to the lumen of the gastric gland (Fig. 2.6(A)). Although this was not a constant feature of all parietal cells in the non-secreting configuration, some of these cells with distended canaliculi lined with a few stubby microvilli and numerous tubulovesicular membranes exhibited clear evidence of this feature. In some glands, the lumen was filled with a dense product, which appeared to be secreted pepsinogen from chief cells. In nearby parietal cells, the intracellular canaliculi were clear and void of the dense lamina material. This indicates that these parietal cell canaliculi must be truly internalised and intracellular in these inactive cells.

The process or the mechanism by which the parietal reverts from one morphological state to the other (Fig. 2.6) when they pass from the inactive status to fully secreting, is not exactly established. It can be explained by "direct continuity", so that the membrane is exteriorised by membrane flow or exchange (Ito 60, Lee 71-73, Sed 62). This has not been demonstrated by tracers and there is no evidence of this transformation in mammalian parietal cells (Ito 74) except if the process is so fast that the existing at that time technology was inadequate. Another way may be "direct transfer" of membranes (Ito 74, Sch 79). This mechanism is supported by the freeze-fracture replicas of microvillar and tubulovesicular membranes (For 71-72, Ito 74, Lee 71).
Finally, it can be considered that the intracellular canaliculus may become completely internalised and close off its opening to the lumen of the gastric gland.

Summarising, the parietal cell secretion passes into the canaliculi, which start at the base of the cells and after a tortuous course open into the apical cell surface. These intracellular canaliculi are lined with microvilli and surrounded by the cytoplasm with numerous tubovesicular structures. The microvillus membrane and the tubovesicular apparatus undergo markedly changes in the transition from the resting to active secretory. These changes include marked increase in the number, size and complexity of the microvilli combined with a marked decrease in a number of tubovesicles (Fig. 2.6). Thus, with the onset of gastric secretion, the tubovesicles are transformed into the microvillous membrane resulting in a marked increase in the area of the secretory membrane (Hel 74).

Thus, the parietal cell is the most logical candidate for secretion of HCl acid. The secretion must occur across the parietal cell microvillar membrane. The acid then flows out from the intracellular canaliculus into the gastric gland lumen and into the stomach lumen. But still the accepted role of parietal cells as the source of the gastric acid is based on indirect evidence (1981). Under a variety of conditions, the HCl acid at maximum secretion from the parietal cells approximates 160mmol and it is almost isotonic to plasma.

Which cite of the parietal cell is the most probable for the production of gastric acid? The apical cell membranes, the cytoplasmic vesicles or the mitochondria. Only the apical cell membranes are in contact with luminal contents, so that the site of osmotic flow of water is also accessible to lamina solutions (Dut 79).

2.4 Electrical properties of the gastric mucosa:

The gastric mucosa, if immersed into Cl⁻ solutions, shows a potential difference (PD) with the negative in the lumen side under acid secretion condition or not. Measurements of cell membrane potentials of the surface cells, show a negative well with the apical membrane less polarised than the basal (Spe 75,74a-b). Changes of K⁺ or Cl⁻ concentration on the basal surface show that this membrane is selectively
permeable to these two ions (Sac 71). Also, the change of the potential across the baso-lateral surface is almost exactly reflected by a change across the apical membrane (Spe 74b), and this means that the paracellular pathway contributes little to the tissue conductance. The same is observed with \( \text{Na}^+ \) and \( \text{HCO}_3^- \) ions (Reh 72).

When \( \text{Cl}^- \) is removed (in the frog mucosa) and replaced by sulphate in resting tissue, the PD drops to low values and the tissue resistance rises (Hei 75); but in the presence of acid secretion, the PD actually reverts and is then linearly related to the rate of acid secretion (Hei 75).

### 2.5 Blood supply of the gastric mucosa:

There is a rich blood supply to the stomach and during the last decades there were developed techniques to measure gastric mucosal blood flow and total gastric blood flow (Fig. 2.7).

![Fig. 2.7: Diagrammatic representation of the arterial supply of the stomach](reprod from Joh 81, p711).

The supply is derived primarily from the coeliac artery playing the role of axis and arising from the front of the aorta. Tree branches namely as left gastric, hepatic and splenic arise from the coeliac artery. As Fig. 2.7 shows, two main arterial branches
are formed by the right and left gastric arteries along the lesser curvature and by the right and left gastroepiploic arteries along the greater curvature. Most of the arteries supplying the anterior and posterior walls of the stomach arise at right angles from these branches and pass through the muscular coat to form an extensive plexus of vessels in the submucosa. The arteries, arising as branches of the submucosal plexus, penetrate to the mucosa, divide where the gastric pits join the gastric glands and form a network of tortuous capillaries which continue into the subepithelial capillary plexus.

Studies revealed (Jac 66) that, when the stomach is stimulated to secrete, mucosal blood flow increases. If blood flow to the stomach is reduced sufficiently, secretion decreases; but increasing blood flow (low dose of isoproterenol) does not increase secretion if not any other stimulant is applied. Thus mucosal blood flow plays a supportive and permissive role in gastric secretion (Gut 78, Kni 77, Har 68).

There is also evidence that mucosal blood flow plays an important role in the prevention of injury of the gastric mucosa. Increased blood flow protects against injury and vice versa under the same conditions (Rit 75, Whi 77, Mer 73, Moo 77).

Blood flow measurements after the ingestion of a high protein meal (45 minutes) showed increased blood flow of the whole stomach which was due to increased mucosal blood flow (Bon 79). Food in stomach increased also antral and corpus blood flow and with progressive starvation the antral to corpus flow ratio decreased (Tay 76).

Studies with rats showed a decline in blood flow by age after a peak was reached. Also the weight of the alimentary canal relatively to the whole body weight decreased by age (Var 76).

It has been postulated that the increase in mucosal blood flow may be a secondary defensive response to mucosal damage (Bru 79, Aug 70, Che 75).
2.6 Mucosal secretions

2.6.1 Introduction

The stomach besides serving as a "temporary storage area" for the ingested food, continues the demolition job begun in the oral cavity by farther degrading the food, by both ways, mechanical (physical) and chemical and then delivers the chyme, the product of its activity, into the small intestine at an appropriate rate for absorption, assimilation and disposal. The chemical process into the stomach is due mainly to gastric secretions known as "gastric juice" and lipase in the infants.

Under normal conditions the gastric mucosa pours into the stomach 2 to 3 L of gastric juice every day, containing a variety of substances, such as mucus, lipase, pepsins, intrinsic factor and a large number of ions mainly H⁺, K⁺, Na⁺, Cl⁻, HCO₃⁻ moving into water. The gastric lumen of an empty stomach is acidic (pH~2) (Gan 97-page 459). Under normal conditions gastric juice may contain swallowed saliva and refluxed duodenal secretions. The mechanisms of production and flow across the gastric mucosa of the most important constituents in the gastric juice follow.

This section is dealing with the mechanisms of production and flow of most important constituents of the gastric juice (secretions).

2.6.2 Production and transport of hydrogen ions (H⁺).

In 1925 Beaumont realised the acidic environment of the gastric lumen and Prout in 1924 found that HCl acid was the dominant substance in the human stomach investigations started to define the mechanism and location of the production and the transport of gastric acid. The most satisfactory theory started being developed by in '70s in the previous century. Measurement of gastric acidity had started before the source of it was known (Bar 78). Observations that the acidity was correlated with the number or the mass of the parietal cells gave the hint that probably those cells were responsible of the gastric acid production.
In 1970 the histochemical localisation of ATPase activity on mammalian parietal cell microvillous membrane was observed by Rubin (Rub 70). Saccomani et al. later observed that the antibody to the major membrane fractions of hog stomachs with the K⁺ATPase containing H⁺ transport function was localised on the apical and canalicular microvilli of parietal cells. Only weak reactions were evident on the zymogenic cells or on the basal and lateral cell surfaces (Sac 79,77). The conclusion from this study was that the proton translocation ATPase is in the regions of parietal cells, which were the presumed sites of acid secretion. The tubulovesicular system did not react to this enzyme.

In the past, two primary energy sources have been considered for ion transport in mammalian cells, namely, ATP and oriented redox systems (Her 74). On this basis the energy source for H⁺ secretion is either an ATPase or a redox pump. According to an earlier observation that acid secretion was absolutely dependent on O₂ consumption, a reluctant attitude was created in accepting a completely ATP-dependent H⁺ pump.

The development of isolated cell models, such as the rabbit gastric glands and the use of probes of acid accumulation within the secretory canaliculus (either aminopyrine or acrydine orange) have provided a direct test of the ATP dependence of acid secretion. Studies by Lee et al. (Lee 74) demonstrated that the addition of ATP resulted in H⁺ transport into these gastric vesicles confirming the role of this enzyme in acid secretion.

In a more descriptive way, the vesicle membrane defines an intravesicular space. The addition of ATP to gastric vesicles results in up-take of H⁺ into the vesicle space only if there is intravesicular K⁺. The loss of H⁺ from the medium is monitored by a pH electrode (Lee 74). There is a considerable discrepancy in the loss of H⁺ from the medium and the appearance of free protons in the vesicle interior. This shows the presence of buffering in the vesicle space (Rab 78, Sac 76). The effect of K⁺ on the exterior of the vesicle as opposed to the interior can be determined by fixing internal K⁺ and varying external K⁺ by dilution into a choline medium or Na⁺ medium with the Cl⁻ held constant. It has been shown that both external K⁺ and external Na⁺ are inhibitory to the transport reaction and the effect is on the rate of transport but not on
the maximal gradient (Wal 80). The H\(^+\) transport data are consistent with the ATPase kinetics that have been described and with the presence of a K\(^+\):H\(^+\) exchange mechanism.

With the model that has been developed for the gastric vesicles [a KCl entry step and then an active K\(^+\):H\(^+\) exchange] the K\(^+\) is cycled across the secretory membrane. The earlier model for the basolateral membranes, where both K\(^+\) and Na\(^+\) cycles across this membrane were postulated, can readily be combined with the model developed for H\(^+\) transport by adding the K\(^+\) cycle on the secretory canalicular surface. The modification that occurs with secretion is the export of Cl\(^-\) across the secretory surface and the transport of HCO\(_3^-\) across the basal surface.

It also was observed that in the frog gastric mucosa, even at resting conditions, there is considerable reduction of the cytochromes, including cytochrome oxidase (Her 71). This shows that there is some feature of redox chain in the gastric mucosa. With the stimulation of secretion the redox components undergo reduction but this can be expected.

Summarising, the net result of all metabolic studies performed on the gastric mucosa, lead to the conclusion that ATP in the form of H\(^+\)/K\(^+\) ATPase, the enzyme involved at the final step of H\(^+\) secretion, is located within the cell membrane of the intracellular canaliculi and it is required for acid secretion and that the source of energy is probably mitochondrial metabolism (Joh 81)

2.6.3 Bicarbonate secretion

The existence of non-acidic HCO\(_3^-\) secreted content in the gastric lumen was proposed by Danish physiologist Schierbeck in 1892, when he measured the gastric pCO\(_2\) in dogs (Joh 81, pp603).

Bicarbonate enters the gastric lumen by active secretion, by diffusion and probably occasionally by bulk flow of interstitial fluid. It also may enter in the gastric lumen with the saliva and duodenal content. Bicarbonate production in humans was measured to be 0.4 \(\mu\)Eq/cm\(^2\)-hr or the human gastric secretion measurements showed
8 to 20 mMol of HCO$_3^-$ under basal conditions (Kri 75). The electrolyte composition of fundic alkaline secretion is similar to that of the antrum.

It was proposed that active HCO$_3^-$ transport protects the gastric epithelium from damage by alkalization of the surface boundary of the mucosal membrane (Fie 80,78,77). This concept has gained support from findings that several potential ulcerogens inhibit gastric HCO$_3^-$ secretion (Fle 78) and antulcer agents increase the HCO$_3^-$ (Gar 79).

If the difference in magnitude between the normally large H$^+$ secretion and the HCO$_3^-$ smaller transport (5-10% HCO$_3^-$ /H$^+_{max}$) is taken into consideration, the alkalinity of HCO$_3^-$ would be ineffective if secreted directly into the intragastric bulk solution; the rise in pH would be very small. Thus, an active transport of HCO$_3^-$ into a surface boundary zone with low turbulence would be more effective (Fle 78, Teo 36-47). A counterdiffusion of hydrogen and bicarbonate ions in a membrane model was considered by Teorell (Teo 47). The viscoelastic mucous gel at the mucosal surface also has physical properties to support HCO$_3^-$ and H$^+$ gradients. The concentration profiles within the surface boundary would depend on the amounts of H$^+$ and HCO$_3^-$ available for diffusion, the presence of other ions and the structure and electrical charge of the mucus. Measurements with microelectrodes across gastric mucous layer, show a mean pH of 2.36 at the lumen facing side and 7.59 at the cell facing side support the boundary zone model (Wil 80).

An output of HCO$_3^-$ of 1 to 2% of maximal H$^+$ output would be sufficient to account for acidity regulation in humans. This ratio was calculated from the experimental data and mathematical analysis of acidity regulation (Mak 66, Obr 48) based on “volume of alkalinity secretion and HCO$_3^-$ concentration” and of “primary acidity and permeability coefficient for H$^+$ ions”. This means that the amount of HCO$_3^-$ transported by the mucosa is probably quantitatively sufficient to account for the well known continuous loss of H$^+$ ions from acid gastric contents.

Because of the higher mobility and usual excess of H$^+$ ions, the process of neutralisation for the mucosal protection should occur in the immediate vicinity of the lamina cell membranes.
Sodium ions may be transported together with HCO$_3^-$ or diffuse into the lumen when H$^+$ is neutralised.

2.6.4 Water (in)flow

2.6.4.1 Secretion & Transport.

It may be stated that the stomach is designed to secrete. As it is expected, by any measure, water is the most abundant component of gastric secretion. The mechanism of water flow is profoundly an essential part of the gastric secretory process. Usually it is assumed that water is driven osmotically across epithelia in sufficient quantity by gradients formed when a solute is secreted or absorbed. The achievement of steady state gradients has resulted in the evolution of a variety of morphological arrangements in the epithelia. It seems that the intercellular channels of the gastric mucosa exhibit a dynamic character in their permeability, which can be altered even reversibly with various kinds of treatment. A small change in interstitial pressure can modify these channels directly, as in the opening and closing of a valve.

More specifically, water flows across the gastric mucosa in response to osmotic gradients created within the tissue by the active transport of solutes and mainly HCl (Moo 72). Water flow can be maintained against hydrostatic as well as external osmotic gradients of 100 mOsmol/L as it was shown by in vitro studies with gastric mucosa of amphibians, i.e. frog (Dav 49, Dur 56).

The osmotic transport of water was studied also, by modifying the endogenous osmotic gradient from the luminal side.

After instillation of isotonic solutions of the buffer glycine, impermeable electrolytes or non-electrolytes on the luminal surface of a histamine stimulated canine mucosa the concentration of the secreted acid was calculated. The concentration of the secreted acid increased beyond its limiting value in normal secretion. The increase was due primarily to a reduction in the rate of net water flow towards the lumen because of the isotonicity (Moo 65, Hei 54, Lin 47).
Durbin working with unilateral amphibian mucosa using hypotonic instillates (10% of normal) showed abolishment of the net water flow (Dur 79).

Durbin, using the results of the mentioned studies with isotonic and hypotonic instillates, calculated the osmotic permeability to be about 45 nL/cm²/hr/mOsm. This value is similar to that extracted using exogenous osmotic gradients. These values probably underestimate the osmotic permeability, because the infolding of the mucosa into tubules and the parietal cells into canaliculi increases the velocity of the secretory flow and prevents the access of instilled solutes to the sites of osmotic equilibration. In this way only a fraction of the applied osmotic gradient reaches the lamina surface of the parietal cells.

In the dog, 88% of the effective pore area of the gastric mucosa is occupied by pores with a radius of 2.5 Å and 12% with a radius of 90 Å and the same is observed in the frog's mucosa (Vil 63). Thus the distribution is skewed to the favour of smaller pores but it is probably continuous between the two extremes. The larger pores may represent transient defects resulting from cell turnover or from experimental damage.

The flow of water in response to hydrostatic gradients is due mainly to the small set of large pores. According to Poisseuille's law, the flow is proportional to radius. Flow in response to osmotic gradients is mainly via the large set of the small pores. This explains partly why the hydrostatic permeability is 100 times greater than osmotic permeability (Dur 78, Moo 69).

2.6.4.2 Models of water transport

Models of water flow in epithelia usually consider an intermediate compartment into which solute is actively transported. The intermediate compartment is retained hyper-osmotic and as a result draws in water. The description of two such models follows:

-The double membrane model proposed by Curran and Durbin in 1960 (Cur 60, Dur 60) shown in Fig. 2.8A, accepts that membrane (1) is responsible for active transport into the space between the two membranes; this space and the membrane (2) are undefined to a certain extent and could represent the unstirred layer bordering membrane (1). A macroscopic version of the double membrane model has been
constructed of cellulose and sintered glass membranes and confirmed the theory of osmosis in leaky membranes (Dut 79, pp128).

The parameter $L_p$ had been introduced in these membrane transport models to describe the net flow of solution per unit area of membrane caused by unit pressure difference across the membrane. The driving pressure gradient may be osmotic ($L_{op}$) or hydrostatic ($L_{hp}$). It has been found that $L_{hp}$ values were considerably higher than $L_{op}$.

$L_{op}$ in gastric mucosa was measured by instilling hyperosmotic solution into the lumen. Osmotic flow of water dilutes the solute concentration on the side with the higher concentration and on the same time osmotic removal of water increases the concentration on the opposite side. In fact, the bulk flow of secretion (water) sweeps external solute away from the site of osmosis. This “sweeping” causes difficulties in measuring the $L_{op}$. Moody and Durbin (1969) measured the effect of hydrostatic pressure applied to the laminal surface of dog gastric mucosa between chambers. They found that hydrostatic pressure was about one hundred-fold more effective than osmotic pressure in producing net volume flow (Moo 69).

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**Fig. 2.8:** Double membrane model (A) for osmotic flow of water; the arrows show active solute transfer at membrane (2). Rehm's model (B) representing the pits (black area) and tubules; the pits assumed impermeable and the tubules permeable to $H^+$ and water [reprod from (A): Dut 79, p 127; (B): Joh 81, p559]
Instillation of isosmotic sucrose, mannitol or NaCl solutions in the chambered dog stomach resulted in hyper-osmotic acidity (Moo 65), which was attributed to a deficit of water flow rather than to an excess of HCl production. Instillation of isosmotic HCl on the other hand gave a distinctly lower secreted acidity in conditions similar to those under which gastric juice is normally collected.

In conclusion, accepting the double membrane model the "instillate" effect depends on the greater or not restriction offered by membrane (2) (Fig. 2.8) to the substituted solute. The "instillate" also effect indicates that the primary site of water flow is accessible to external bathing solutions, which strongly suggests that the site of acid production in gastric mucosa is the apical cell membrane.

-Rehm et al. first considered the tubules as the main sites of H⁺ diffusion and osmotic water transport (Bor 59, Reh 70, Reh 53, Thu 56). Rehm considered the surface epithelial and mucous cells which line the pits (average depth 100m) to be impermeable to H⁺ and water, in contrast to the parietal cells which line the tubules (Fig. 2.8B). Because tubular cells are separated from the main lumen by a long, narrow, unstirred pathway, they are exposed to only a fraction of H⁺ and osmotic gradients present in the main lumen. If acid solutions are instilled in the stomach, H⁺ diffuse toward the tubules and from there cross the mucosal cell layer; Na⁺ enter the tubular lumen at the same site. Because the mobilities of H⁺ and Na⁺ are different a Na/H profile is created in the pits and tubules, which retains the blind tubular end transiently hypertonic and obligates the entry of the water. The entry of the water in the main lumen in turn results to a transient hypotonicity.

Rehm's model is expressed by the following equation (2.1)

\[ C = C_L e^{-vd/D} \quad (2.1) \]

where \( C_L \) is the initial concentration of solute in the main lumen; \( C \), the concentration of the solute at a distance \( d \) in the pits and tubules; \( D \), the diffusion coefficient of the solute and \( v \), the velocity of flow in the tubules calculated from the secretory flow rate and mean cross-sectional area of the tubules.
When the rate of secretion is high, the concentration profile within the tubules and pits is rapidly dissipated and the solute (or $H^+$, if the instillate is acid) is swept back to the main lumen. ($H^+$ derived from secretion occupy the tubules).

The principles of both models are similar and during active secretion the solutes from the lumen in diffusing process towards the mucosa are getting swept back to the gastric lumen because of the net secretion flow. The knowledge of the parameter $L_p$ is incomplete for the gastric mucosa. The calculated values using the osmotic pressure give a lower limit and those using hydrostatic pressure several different upper limits, possibly reflecting different states of the intercellular channels.

In conclusion, the instillate effect indicates that the primary site of water flow is accessible to external bathing solutions which, in turn, strongly suggests that the site of acid production in gastric mucosa is the apical cell membrane (Dut 79).

### 2.7 Mucosal protection from secretions

#### 2.7.1 Introduction

How do the parietal cells and the other epithelial cells protect themselves from secreted 0.1 N HCl? The mucus secreted by the mucous neck and surface mucous cells do not form an impermeable shield over the epithelium. Resistance to damage is complicated further by the outpouring of pepsinogen from the chief cells and the resulting potent proteolytic effect of pepsin (Ito 81).

Teorell first in 1933 reported that the gastric mucosa behaves as a semipermeable membrane permitting acid secreted into the lumen to diffuse back into the mucosa (Teo 33) and in 1947 he expressed the concept of the "gastric mucosal barrier" (Teo 47). Several investigators followed him emphasising the existence of an ion diffusion barrier but it was not till the publications of Davenport's experiments in 1964 (Dav 64a,b), when a much higher interest between the investigators aroused about the concept of the mucosal barrier and its clinical significance. Until 1980s the gastric mucosal barrier was thought in terms of passive permeability of ions but research since then started to uncover the complexity of the mechanisms with which gastric
mucosa protects itself against its own secreted acid and other substances (Joh 81, Fromm, p733).

In 1970 Davenport showed the significance of the mucosal barrier in the development of gastric diseases. Davenport using dogs with fundic pouches investigated the barrier properties of the mucosal barrier using various substances in contact with the oxyntic gland mucosa (instillation). The damage of the barrier was manifested by a decrease in the electrical potential difference, increased loss from the gastric lumen of hydrogen ions, increased permeability of the mucosa to sodium ions with accumulation of these ions in the gastric lumen, and leakage of interstitial fluid and plasma into the gastric lumen. Between the substances damaging the mucosal barrier are hydrochloric acid in high concentration, aliphatic acids (acetic and propionic), detergents (bile salts and lysolecithin), ethanol in high concentrations, salicylates in acid but not in neutral form (aspirin, salicylic acid), local anaesthetics, restrain or surgery, etc.

2.7.2 The structure and mechanisms of the mucosal barrier

The "mucosal barrier phenomenon" can be explained if two separate entities will be considered: the mucosal (locus) barrier and the mucous barrier which should not be confused. Hydrochloric acid diffuses freely through the mucous layer and the mucus is not a barrier to the diffusion of the hydrogen ions (H⁺).

The locus of the mucosal barrier it is assumed that it is formed by the apical, lipoprotein membrane of the surface epithelial cells and the close junctions between adjacent cells. The harmful action of the barrier-breaking substances is probably attributable to their ability to attack the lipoprotein membrane on the surface of the gastric epithelial cells. This disruption of the barrier is associated with serious pathophysiological consequences.

The locus barrier houses the mucous barrier. Mucus is a viscoelastic gel supported by the locus barrier and provides a flexible protective adherent to the mucosal surface. The bulk of mucus consists of water, up to 95% by weight, with about 1% by weight of dialyzable salts, with an electrolyte composition close to plasma (Hol 63). The
remainder 5% consists of non-dialyzable glycoproteins, proteins and nucleic acids (Cre 78, Hol 63, Hor 77). It is not water-soluble. The gelatinous nature and the structure of complex glycoprotein molecules make difficult the study of the gastric mucus and consequently the understanding of its biology, physiology and pathophysiology. Figure 2.9 represents schematically the mucosal barrier.

Mucus can be obtained by the collection of gastrointestinal juice from fistulas and pouches, by intubation and aspiration of the secretions or by scraping the mucus gel from the surface of isolated mucosa or in small amounts by biopsy. Mucus in gastrointestinal juice is partially degraded (All 78) and it is contaminated with non-mucous secretions (Lam 73-71). When mucous gel is obtained by scraping the mucosal surface, heavy contamination often results from simultaneous removal of the mucosal cells.

A clear function of gastric mucus is to protect the delicate mucosal epithelium from mechanical damage by the passage of the food and vigorous forces that developed during the digestion. It also provides a slimy lubricant for the transport of solid material and yet with its strongly adhesive properties ensures that much of the gel remains firmly stuck to the mucosa in order to provide protection from the next round of mechanical abuse. It can retain water and in this way provides constantly the required aqueous environment to the mucosal surface. The mucus resists excessive swelling and solubilization by the luminal solutions.

Isolated gastric mucus secreting cells are sensitive to low pH and cease to synthesise mucus or even to respire in an environment with pH lower than 5 (Sna 72). In vivo, only the mucous gel separates these cells from the luminal side.

The gastric mucous gel does not provide an impermeable barrier to acid, since it is permeable to $H^+$. Heatly proposed that mucus protects the mucosa by acting as a barrier to gross mixing and by ensuring that a mucosal alkaline secretion within the gel was restricted to the luminal surface and neutralized the acid diffusing in from the lumen (Hea 59). Later, the demonstration of $HCO_3^-$ secretion by the gastric mucosa did support this hypothesis.
More specifically, if mucus and its contained HCO$_3^-$ consist an effective defense against acid, the mucous gel should satisfy two conditions:

a) it should provide an unstirred layer and in this way to maintain the secreted HCO$_3^-$ on the mucosal surface at a high concentration and prevent mixing with the bulk of the lamina HCl acid. The relatively dense molecular structure of the mucous gel makes it well suited to provide such an unstirred layer (All 76).

b) The flux of H$^+$ from the lumen through the unstirred layer of the mucous gel must not exceed the flux of HCO$_3^-$ from the mucosal surface. Calculations suggest that free diffusion of H$^+$ through an unstirred layer is a rapid process and under conditions of maximal stimulation, gastric HCO$_3^-$ secretion is unlikely to be more than 10% of the HCl acid output (Dav 67). Of course much of the secreted acid will be buffered by food and lost from the stomach by gastric emptying.

Both conditions are fulfilled as measurements with micro-electrodes showed a gradient of pH from 2.36 to 7.59 across the mucus on isolated rabbit gastric mucosa (Wil 79). The buffering capacity of the mucus in the absence of HCO$_3^-$ is minimal and not a major factor in protection of the mucosa from acid.
2.7.3 Injury of the gastric mucosa

A number of drugs and bile salts not only increase the permeability of the gastric mucosa but also decrease the production, secretion and quality of gastric mucus (Men 69, Rai 78, Wal 78). Alteration of gastric mucus may lead to ulceration but the protective capacity of mucus against lamina H⁺ is believed to be minimal (Men 69). Mucus has a limited buffering capacity and concentrated films of mucus contribute to a limited extent to the back diffusion of HCl acid (Hea 59, Wil 78). On the other hand solubilization of mucus using n-acetylcysteine is not altering the mucosal permeability in vivo (Dav 71) and drugs decreasing mucus secretion do not increase mucosal permeability (Men 69, Chv 72). Thus, it is concluded that mucus may add an unstirred layer containing a steep pH gradient but it is generally believed that affords little protection (Hea 59, Wil 78).

Davenport, also, proposed that acid diffusing back stimulates the gastric motility by activation of intrinsic plexuses and also causes the secretion of pepsinogen from the peptic cells and the damage and degranulation of mast cells resulting in histamine release. Histamine and probably other substances liberated during acid back-diffusion cause increased capillary permeability, vasodilation, edema, leakage of submucosal capillaries and obviously gastric bleeding. Such acute damage is easily reversible, but repeated exposure ceases the normal mucosal differentiation and chronic gastritis starts being developed.

Generally, agents that affect fundic mucosal permeability and active transport processes also affect antral mucosal in a similar way; subtle differences occur as well as differences of the degree of the change. As an example, mucosal damage because of shock usually is more intense in the proximal stomach than in the antral area. The oxygen demand in the upper stomach is higher than in the lower (Sat 78). The parietal cell bearing mucosa lacks glycogen and has a relative inability to utilise anaerobic glycolysis as an alternate source of energy (Men 74). In complete ischemic conditions, dephosphorylation of the adenylate pool is less (marked) in the antrum than in the upper stomach (Men 74).
There also are spontaneous permeability differences between fundic and antral mucosa as well as between species. In dogs the antral mucosa shows a higher permeability than the fundic (Dyc 69, Wer 70), but in rabbits the opposite happens (Fro 75).

Concluding, it is no longer proper to refer to the barrier as a simple entity, since there are at least two component-barrier; one is related to permeability itself and the other to the buffering ability of the mucosa. Simple reference to permeability or buffering would avoid confusion and allow stricter definition as well as the possibility for description of other mechanisms by which the mucosa protects itself against its own secreted acid.

It is worth at this point to mention the intramural pH, which is in the neutral zone and is a crucial factor in the ability of the mucosa to neutralise diffusing $H^+$.
3 GASTRIC DIGESTION

3.1 Digestion

The stomach besides serving as a "temporary storage area" for the ingested food, continues the demolition job begun in the oral cavity by farther degrading the food, by both ways, mechanical (physical) and chemical and then delivers the chyme, the product of its activity, into the small intestine at an appropriate rate for absorption, assimilation and disposal.

The chemical process into the stomach is due mainly to gastric secretions known as "gastric juice" and lipase in the infants.

The mechanical to the special structure of the wall muscles and pacemaker located in the greater curvature.

This preparation of the food inside the stomach is called Digestion. The digestion due to gastric juice is characterised as chemical digestion. The mechanical action results to gastric emptying.

The objective of this chapter is to present the main principles of the gastric secretions and gastric emptying in humans related with the epigastric electrical impedance measurements.

3.2 Gastric juice

3.2.1 Composition

Under normal conditions the gastric mucosa secretes into the stomach 2 to 3 L of gastric juice every day, containing a variety of substances, such as mucus, lipase, pepsins, intrinsic factor and a large number of ions mainly $H^+$, $K^+$, $Na^+$, $Cl^-$, $HCO_3^-$. The gastric lumen of an empty stomach is acidic with pH about 2) (Gan 97, Wes 98,
Under normal conditions gastric juice may contain swallowed saliva and refluxed duodenal secretions.

It is difficult to obtain a pure sample of the product excreted by the parietal cells, without any contamination of other gastric secretions and the purest possible specimens that have been analyzed, were almost isotonic with $H^+$ concentration equivalent to 0.17 N HCl and pH as low as 0.87. Thus, parietal cell secretion may well be an isotonic solution of essentially pure HCl acid containing 150 meq of Cl$^-$ and 150 meq of $H^+$ per litre.

The hydrochloric acid kills many ingested bacteria, initiates the protein digestion providing the necessary acidic environment for the precursor pepsinogen to enzyme pepsin as well as stimulates the flow of the bile and pancreatic juice.

The lumen of the stomach is electronegative by 30 to 80 mV relative to the serosa and $H^+$ ions are pumped against a concentration gradient about 1 million fold ($7>pH>1$); consequently, a lot of energy is required for the transport of both $H^+$, Cl$^-$ and other secretions into the gastric lumen (Fig. 2.9).

It was described in the functional morphology section that $H^+$ ions are secreted into the gastric lumen from the parietal cells with the electrogenic $H^+:K^+$ ATPase pump and Cl$^-$ transport is also implicated to form HCl acid with a pH even of 0.87. This production involves also the transport towards the gastric lumen of $K^+$, $Na^+$ and $HCO_3^-$. The ionic concentration of the gastric juice in respect to flow rate of the gastric secretion is given in Fig. 3.1 (Joh 81) It is apparent that the $H^+$ and Cl$^-$ ions dominate the gastric juice in higher flow rates.

Pepsinogen is a precursor of pepsin. It is converted to pepsin at pH lower than 5 and begins the digestion of protein by splitting interior peptide linkages. Pepsinogen in two forms (I and II) is secreted by peptic and mucous cells. The strongest stimulant of pepsinogen secretion is Ach.

Intrinsic factor is another constituent of the gastric juice, secreted by the parietal cells. It combines with vitamin $B_{12}$ in the stomach, forming a complex that is necessary for the absorption of this vitamin by the ileal mucosa.
3.2.2 Osmolarity of the gastric juice:

Osmolarity of the gastric juice is an important property as regulating the inflow forces (water flow section). In vitro experiments concluded that gastric secretion is isoosmotic with the solution directly bathing the mucosa (Dur 79-56, Mak 73). When the flow rate is low and acid concentration declines, secretion becomes distinctly hypotonic. A minimum is reached, about 20% lower than the osmolarity on high secretion rates (Lif 43).

The osmolarity of acid secretion can be calculated based on the Cl concentration according to expression: \( \text{Osmol} = 2 \times [\text{Cl}] \times \text{cof} \) where \( \text{cof} = 0.944 \) is the osmotic coefficient.

The osmolarity of histamine stimulated acid secretion is about 6% higher than that of the arterial plasma and can be made to increase or decrease in parallel with it by oral or intravenous administration of saline or water (Thu 56). The osmolarity of gastric venous plasma is higher than that of arterial plasma because of the clearance of water with H⁺ formed de novo in the mucosa (Alt 69). Gastric secretion appears to be isoosmotic with the gastric venous plasma.
The factors that determine the development of hypotonicity are the proportion of water to HCl, neutralisation by bicarbonate, which dissipates osmotically active H⁺, and the lower mobility of NaCl diffusing into the lumen relative to the mobility of HCl diffusing out of the lumen.

Higher osmolalities occur if specific solutions are instilled in the gastric lumen (Teo 40). Instillation of 0.2 M glycine caused an average acidity of 208 mM in a feline stomach. Instillation of isoosmotic sucrose, mannitol or NaCl solutions in the chambered dog stomach gave also hyperosmotic acidities (Moo 65) and this was attributed to a deficit of secreted water. Instillation of isosmotic HCl acid resulted in distinctly lower secreted acidity under similar rest conditions, causing normal gastric secretion.

3.3 Gastric acid secretion in humans

3.3.1 Historical aspects

Beaumont and Prout are the pioneers in the discovery of the acidity of the gastric secretions.

Beaumont for three years from April 1824 was lucky by the circumstances and was able to perform hundreds of experiments on the same person; the young man after an injury with a bullet injured in his chest found himself having a stomach with an opening to the outside world. Beaumont, his doctor, grabbed the chance parallel in keeping him alive to explore the human stomach. He was in the fascinating position (what an irony) even to visualize the interior of the stomach during a variety of conditions. His conclusions were drawn by observing, tasting and smelling the gastric contents, and were dependent completely on his senses. He used to insert the food via a string into the stomach through the opening in the chest (closed with a valve in the rest of the time) and take it out with the food altered (or otherwise) in its physico-chemical properties, due to the digestion process. This would happen every so often until the food disappeared, concluding that the stomach had passed the food into the duodenum. But even to this day nothing really more has been added except that the contents can be identified scientifically and instead of using strings sterilized
sophisticated tubes are inserted in the gastric lumen quite often controlled by radiological screening.

In 1824 Prout (Pro 1824) demonstrated the presence of hydrochloric acid in human gastric juice, measured the concentration of acid by "exact neutralisation" with strong alkali and expressed his concentrations as "grains in one pint". He also established the equation expressing the following status:

\[\text{[Total chloride]} = \text{[Neutral chloride]} + \text{[Titratable acid]}\]

In 1886 Jaworski and Gluzinski used 0.1 M NaOH and litmus paper and expressed concentrations as cc N/10 NaOH per 100 cc gastric juice or degrees of acidity. Later this unit was named the "clinical unit" and numerically was identical with mEq/L, mN and mmol/L (SI).

Ewald, in 1892, measured gastric acidity with titration using litmus paper or phenolphthalein and Congo red paper for the free acid. In 1912 Christiansen used Sorensen's pH scale and it was possible to titrate to a specific end point. Later, in 1917, Michaelis realised the difficulty between the relationship of pH and titratable acidity because of the presence of buffers and especially meals in the gastric juice. In 1920 Shohl and King set as end points of free acidity pH values from 1.2 to 3.0 and for free alkalinity from 8.0 to 9.6.

The most comprehensive study of the methods for measuring the acidity of gastric juice was made by Hollander in 1931. He based his method on the theory of titration of buffer-containing solutions and also discussed electrometric titration as the "existing simple and cheap set-ups" of nowadays were not available to Hollander.

Today with pH meters most of researchers measure the hydrogen ion activity either in vitro or in vivo, and define the titratable acidity using a base e.g. NaOH of 0.1 M to pH 7.0 to 7.4 again either in vitro or in vivo.

But even today, whichever test is in use, the questions of "what the end point of the titration should be" and "what the concentration of the titrating agent should be" as well as, "is the free hydrogen ion concentration or the total acid concentration more meaningful or important from the physiological point of view" still remain.
3.3.2 Terminology in human gastric secretions

The rate of the gastric acid secretion varies considerably among individuals probably because the total number of parietal cells in the stomach of normal individuals varies significantly according to their body size and weight and the content of the stomach. The terms “basal acid output" (BAO), “peak acid output" (PAO) and “maximum acid output" (MAO) are often used.

The term BAO is referred to the amount of gastric acid in mmol/L of an empty (fasting) stomach without any stimulating condition.

The term MAO gives the acid output in mmol during an hour under stimulation conditions, and the term PAO shows the maximal acid output under stimulating conditions for a shorter time, usually 10 minutes (mmol/L/10 min).

The unstimulated human stomach secretes acid at a rate of 10% to 15% of that at a maximal stimulation. The empty stomach contains a relatively small volume of gastric juice (60 to 100 mL) with a pH usually less than 2. Thus, the gastric mucosa is acidified.

The ion concentration of the gastric juice relatively to the rate of secretion is given in Fig. 6 (West). It is clear that at high secretion rates the H⁺ and Cl⁻ ions dominate and the gastric juice it is almost isotonic, but at low rates it is hypotonic.

It is evident the major role of gastric acid on gastric digestion. The factors controlling the gastric acid secretion in response to ingested food are following:

3.3.3 Phases of gastric secretion and controlling factors:

The human stomach is nearly always secreting acid at rates from nearly zero to 30% of the maximal secretory capacity. During the course of a meal increasing gastric secretory activity is the rule (stimulation) and three well distinguished but probably partially overlapping phases have been defined: cephalic-vagal, gastric and intestinal phase.
The secretion of gastric juice is regulated by both nervous and hormonal mechanisms that occur in three possibly overlapping phases: cephalic, gastric and intestinal (Fig. 3.2).

Fig. 3.2: Graphic representation of the 3 partially overlapping phases of gastric acid secretion along the course of a meal [reprod from Che 98, p30].

-Cephalic phase: even before the food enters the stomach the sight, smell, taste or thought of food cause increase of the secretions from the secretory cells and glands housed in the stomach's mucosa. Emotions such as anger, fear, anxiety may slow down the digestion, because through the sympathetic nervous system they inhibit the gastric activity (secretions).

-Gastric (mechanical, chemical) phase: sensory receptors in the stomach get activated when food reaches the stomach and initiate nervous and hormonal mechanisms to ensure that gastric secretion continues. Food, any kind, reaching the stomach may cause distention of it and/or an increase in the pH since food, may buffer some of the stomach acid; as a consequence, the stretch receptors and chemoreceptors are activated and stimulate the secretions of the parietal, chief and mucus cells. As the pH of the stomach chyme returns to a low-level pH and as the stomach walls are getting less distended, because chyme is pushed to the duodenum, the secretion slows down. Gastrin, also, acts as hormonal regulator. It's secretion is inhibited at a gastric pH less than 1.5, followed by inhibition of the oxynthetic cells.
function. The opposite happens for pH>2. It is evident that the acidity of the gastric contents regulates itself.

**Intestinal phase (mechanical, hormonal):** the acidity of the chime in the duodenum acts reversibly on the gastric acid secretion, meaning low acidity (pH>3) stimulates gastric secretions and vice versa. Distension, also, causes stimulation of HCl acid secretion, but presence of fat digestion products and hypertonicity inhibit the gastric secretions.

A more detailed consideration on the factors regulating the gastric acid secretion in response to ingested food follows:

a) **Cephalic-vagal phase:** Stimulation of acid secretion is activated by the thought, smell, sight, and taste of appetizing food. The message goes through the vagal nerves that innervate parietal cells in the fundus and gastrin cells in the antrum. Since vagotomy is interrupting this reflex it can be concluded that cephalic phase is mediated via the vagi (Far 28, Fel 79b, Knu 73, Pav 10).

![Figure 3.3: Acid output before and after modified sham feeding in 22 healthy subjects (mean ± SE) [reprod from Joh 81, p698]](image)

Fig. 3.3: Acid output before and after modified sham feeding in 22 healthy subjects (mean ± SE) [reprod from Joh 81, p698]
Conventional "sham feeding" (i.e., sham feeding that includes swallowing) and "modified sham feeding" (i.e., chewing and expectorating the food) offer an easy and successful way to evaluate the cephalic phase of gastric acid secretion in humans (Knu 74, May 74, Mig 74, Moo 79, Ric 77, Ste 78). The results of a trial of modified sham feeding are shown in Figure 3.3.

b) Gastric phase: This phase starts with the ingestion of the food and it involves mechanical and chemical changes that stimulate gastric acid secretion.

- Mechanical stimulation: As the food enters into the stomach distension of the stomach walls commences via the so called receptive relaxation. Distension is followed by stimulation of gastric acid secretion with activation of the long vagovagal reflexes and short intramural reflexes. Two methods have been used to measure the dependency of acid secretion by distension: gastric distension: using a balloon or a liquid meal.

Fig. 3.4: Effect of gastric distention with a balloon (A) or with saline (B) on gastric acid secretion (mmol/hr, mean±SE). It is clear an upper limit of volume distention on acid output. The Y axis in both graphs corresponds in acid output in mmol/hr [reprod from Joh 81, p700].

The fundal distension by a balloon evokes acid secretion (Fig. 3.4A) without increasing serum gastrin concentration suggesting that there is a local oxynto-oxyntic
reflex (Gro 77b). The acid secretion is markedly reduced with atropine or by proximal gastric vagotomy (Gro 77a) and this shows a simultaneous vagal reflex.

Antral distension by balloon does not increase acid nor gastrin secretion (Sch 78).

Distension studies by liquid meals (Fig 3.4B.) resulted in results comparable with those by balloons (Coo 70, Hun 52). Both methods showed that there is a limit at the rate of acid secretion versus volume with the maximum value at about 600 mL distension (Gro 77-a, Fel 79b).

![Graph](image)

**Fig. 3.5:** Acid secretion in response to 600 mL intragastric saline or to 600 mL intragastric food in 12 healthy subjects. The chemical stimulation by food causes a higher secretion [reprod from Joh 81, p701].

- Chemical stimulation: Studies lead to the conclusion that there is a chemical catalytic reaction between the food constituents and the gastric mucosa which either promotes or inhibits the gastric acid secretion (Fig. 3.5).

Carbohydrates and fat inhibit gastric acid secretion and the secreted acid during the digestion is due to distension (Ric 77, Mac 76, Moo 80, Gro 78, Chr 79, Ric 76).

Proteins, as food constituents, act as stimulants of gastric acid secretion. Undigested proteins, as albumin, are week stimulants but peptic digests of protein, such as polypeptides, peptones and amino acids are potent stimulants of acid secretion. All
amino acids are not equally effective in stimulating acid secretion. Phenylalanine is a potent stimulant, glycine not so (Ric 76, Byr 77, Ipp 76).

Amino acids and peptides stimulate gastric acid secretion mainly by releasing gastrin (G-17). Amino acids within the gastric lumen might stimulate parietal cells directly (not yet demonstrated in humans). The rising in plasma G-17 concentrations in response to a mixed amino acid meal is sufficient to account for the entire acid secretory response to a meal (Fel 78, Lam 80).

e) Intestinal phase: Presence of fat in the duodenum inhibits gastric acid secretion.

There are two ways by which meals containing amino acids and peptides could stimulate gastric acid secretion in the intestinal phase. Once amino acids are absorbed from the intestine into the circulation, they are capable of stimulating acid secretion (Ise 78). Intravenously infusion of amino acids also results to a secretion rate that is 30% to 35% of peak acid output. This suggests that the “intestinal phase” of acid secretion may be due largely to absorbed nutrients.

Amino acid stimulated acid secretion is also possible via release of a secretogogue from the small intestine, the entero-oxyntin.

Studies also showed that intravenous fat can inhibit acid secretion stimulated by intravenous amino acids as well as equal amounts of intraduodenal fat (Var 79).

The results of a study (Ric 77) on the contribution of cephalic and gastric phases separately on gastric acid secretion as well as all the stimuli simultaneously are presented in Table 3.1.

During the first hour the vagal stimulation is higher (almost 50%) but during the second hour the chemical dominates. The fact that the response to all stimuli acting simultaneously is higher than the sum of the separate stimuli suggests potentiation between stimuli (Ric 77).
Table 3.1: Gastric acid secretion in mmol (%), due to various mechanisms of stimulation separately and simultaneously after normal feeding in nine healthy subjects [reprod from Ric 77].

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>First hour</th>
<th>Second hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalic-vagal</td>
<td>12.0 (47%)</td>
<td>3.3 (11%)</td>
</tr>
<tr>
<td>Distension</td>
<td>6.3 (25%)</td>
<td>5.6 (19%)</td>
</tr>
<tr>
<td>Chemical stimulation</td>
<td>5.8 (23%)</td>
<td>12.7 (43%)</td>
</tr>
<tr>
<td>Stimuli acting simultaneously</td>
<td>25.6</td>
<td>30.0</td>
</tr>
</tbody>
</table>

3.4 Characteristics of meals that influence the gastric secretory response.

It appears that many physical and chemical properties of meals are likely to determine the magnitude and duration of the gastric secretory response. The following have been observed:

a) The external characteristics of a meal namely, smell, colour, taste and the individual preference matching to food play important role. These features determine the magnitude of the cephalic phase. There is evidence that unpleasant food produces virtually no cephalic stimulation (Jan 50).

b) Route of ingestion is another factor. Chewing stimulates (promotes) the cephalic phase and enhances salivation. Saliva increases the intragastric volume (distension is stimulus) when swallowed and being alkaline neutralises some of the secreted acid.

c) The nutrient-chemical composition of the meal. Carbohydrates and fat are not stimulants of acid secretion; these are considered more as exerting inhibitory actions in secretion and emptying. Also, delaying the gastric emptying may prolong the secretory response and offset any direct inhibitory effect. In contrast, protein is a stimulatory nutrient of food for acid secretion. The buffering capacity of a meal influences the inhibitory effect on acid secretion (Wil 68).
d) Specific dietary components such as coffee (Deb 71, Coh 71), tea and calcium-rich foods are stimulants but alcohol in moderate quantities has no any effect.

e) Physical state of the meal. Liquidification seems that accelerates the gastric acid secretion.

f) The total volume of the meal. Larger volume meals exert higher and longer secretory response.

In conclusion, it is likely that solid-liquid meals, which tend to be more appetising, require chewing and stay longer in the stomach would result in a greater net secretory response than nutrients ingested in soluble or suspended form.

3.5 Methods of measuring the acidity of human gastric juice:

The tests can be characterised as “by intubation” and as “tubeless”. Of course intubation of the stomach causes discomfort but gives more reliable results. Under this schemes the tests can be in vitro or in vivo with the best reliability with the in vivo ones. A brief description of the tests in use is following:

3.5.1 Tests by intubation:

3.5.1.1 In vitro titration of gastric aspirations.

Aspiration of gastric contents was the adopted way since the interest started. In the earlier studies that was possible in subjects with gastric fistulas but since that was happening rarely introduced the insertion of a tube into the stomach through the nose or the mouth. The ideal condition would be if the aspiration procedure withdrew all $H^+$ would be secreted. Thus fluoroscopy usually is used to control the positioning of the port of the tube; but even with the optimum placement some acid from 10% to 20% escapes aspiration due to emptying, neutralisation and $H^+$ back diffusion (Fel 79a, Hob 69). Then, either a base is used for the titratable acidity of the aspirations with the controversy as to which pH to assign the titration or to use a glass electrode to measure the $H^+$ activity ($\alpha_{H^+}$), which can be converted to $H^+$ concentration ($c_{H^+}$) by the following Equation 3.1:
\[ c_{H^+} = \frac{\alpha_{H^+}}{\gamma_{H^+}} \quad (3.1) \]

where \( \gamma_{H^+} \) is the activity coefficient for \( H^+ \) in gastric juice.

Hydrogen ions present a lower activity in different concentrations as well as in polyionic solutions than in pure HCl solutions.

3.5.1.2 In vivo intragastric titration

In 1973, Fordtran and Walsh introduced in vivo intragastric titration as a new technique (For 73) and it was possible, stimulated by food, for gastric acid secretion to be measured. A double-lumen nasogastric tube is inserted into the stomach and either a meal is eaten normally or is ingested in the stomach after being homogenised. Every 2 to 3 minutes, a sample of the gastric content (meal plus secreted fluid) is obtained, its pH is measured by a glass electrode and depending on the pH a base (e.g. sodium bicarbonate) is infused into the stomach in a quantity to achieve the initial pH of the food. The quantity of the titrating substance and the time course can give the secreted acid. The main disadvantage is that the gastric content is kept technically at the initial pH of the food and it is assumed that a proper mixing of the gastric content with gastric secretion takes place.

A study of basal acid secretion using both methods resulted in two fold secretion rates with in vivo titration compared to aspiration titration (Fel 79a). This can be attributed to the added amounts of the titrating agent (NaCl) which even in small quantities may cause stimulation of the secretions by distension.

3.5.1.3 In vivo intragastric pH measurements

With the development of miniature pH electrodes continuous intragastric pH measurements started since mid 80s (Eti 85, Fim 85, Sav 87, Kap 87, Emd 86, Fuc 88, Mer 88). The advantage of indwelling pH electrodes over nasogastric aspiration is the possibility to make and record continuous physiological measurements without causing stimulated gastric acid secretion or inducing duodenogastric reflex by repetitive aspiration. Glass electrodes are mostly in use and contact with the mucosa does not influence the pH; but if there is an impact of the electrode into the mucosa
then more than a 2 units rise on pH is measured. For a steady position into the stomach tethered electrodes are used and fluoroscopy can be used for optimum placement. More than one pH electrode can be inserted in the tube and the pH at pre-selected locations can be monitored simultaneously (Flexiflo, Ross Laboratories, Columbus, Ohio).

3.5.2 Tubeless tests:

Because of the discomfort intubation usually causes, several attempts have been made by researchers to develop clinical tests without intubation. Thus, there were developed:

i) Dye tests using Congo Red Thread (swallowed and retrieved), or azopyridine (gastro-ortho test) or azuresin, which dissociates in the presence of H\(^+\) and colors the urine blue after absorption (Bea 68, Kna 64, Seg 50).

ii) Radiotelemetry tests: Radio-capsules have been developed containing electrodes, pH-sensitive, which can broadcast the pH to a receiver outside the subject. This kind of telemetry may suggest normal, hypo- or hypersecretion and lacks the precision for reliable diagnostic purposes (Con 64, Ewe 68, Noi 59, Sta 69, Wil 69).

iii) Radionuclide tests: These tests are based on \(^{99m}\)Tc up-take, which is correlated with maximal acid output but cannot detect anacidity (Bar 67, Ber 73, Irv 67).

In conclusion, tubeless tests give a gross indication of the intragastric acidity and they are less reliable than the tests where intubation is involved (Bar 78).

3.6 Morphological factors in human gastric acid secretion

3.6.1 Introduction

Scientists since early in the past century tried to relate the secreted amount of acid in the stomach with the dimensions of the body, sex, age and ethnic origin. This is logical, given that all these parameters are related with the function of the stomach.
Usually the quantity of the intake food, we believe, depends on our appetite and this is related to our taste and the emptiness or not of the stomach and contributes significantly to body size or perhaps better to the body surface. The impression and the tendency for women is to eat less than men because women care more about an elegant appearance but also because they generally are smaller in size. The diet differs considerably between the nations and not only on the quantity, quality and nutritional value of the food. As soon it was realised that gastric acid is one of the most vital physiological entities in the digestion process and consequently in human body growth and appearance, researchers started to relate the human’s body external characteristics with the amount of the secreted acid.

Sir Arthur Hurst introduced the “hypersthenic gastric hypothesis” investigating the position of the stomach in healthy students at Guy’s Hospital (Hur 20). In 1920 he wrote:

“Although comparative investigations of the motor functions, as ascertained with x-rays, and of the secretory functions, as determined by the fractional test meal, have not been carried out on a large scale, there is a reason to believe that the hypertonic stomach generally shows hypersecretion”.

Later, x-rays tests accounted in favour of his hypothesis. In 1929 he defined the “hypersthenic gastric condition” (Hur 29) as follows:

“This condition, though compatible with perfect digestion, is, I believe, the essential predisposing factor in the production of duodenal ulcer. On the other hand, patients with the hyposthenic gastric constitution were said to have a tendency towards hypochlorhydria, and to be predisposed to gastritis, achlorhydria, carcinoma and pernicious anaemia”.

In the same decade in the same hospital, Campbell and Conybeare found that 4 out of 6 students showing hyperacidity to a test meal were associated with high or rapidly emptying stomachs and had short wide chests. Those associated with hypochlorhydria showed low or slowly emptying stomachs and long and narrow chests (Bai 24, Cam 24).
Scientists continued their investigations in this area and in 1961 Hagarty in Australia tried to relate the position of the pylorus with duodenal ulcer (Hag 61).

In Massachusetts, at Harvard, with a large scale epidemiological cohort retrospective study another attempt by scientists to relate gastric and duodenal ulcers to the morphology of the body (body build) and masculinity was carried out (Mon 70, Pol 74, Nie 64, Dam 67).

According to Baron (Bar 78) many studies of body shape seem now to be of historical interest mainly. Some of the data suggest that patients with duodenal ulcer are more linear than normal subjects, but this may be due to the disease rather than to the cause.

3.6.2 Specific anthropomorphic factors

In particular, researchers tried to study any correlation of gastric acid secretion in relation to body weight, to lean body mass, to height, sex, age and ethnicity.

- **Gastric acid vs body weight**: Data clearly support a significant correlation between peak acid output and body weight or lean body mass in normality and yet others do not (Hum 67, Has 71, Bar 69). The fact that weight and lean body mass correlate with PAO suggests that larger individuals have a larger number of parietal cells than smaller individuals. This has been shown in persons of different ethnicity (see below).

- **Gastric acid vs height**: The PAO shows a logarithmic correlation (Fig 10.3) with height and Hobley prefers (Hob 75) to use height alone as a body criterion for gastric acid secretion ignoring completely the weight.

- **Gastric acid and sex**: All studies of gastric secretion, basal and stimulated, in normal subjects and patients with peptic ulcer, have shown higher values for men as it is displayed in Fig. 3.6 (Gro 63, Wor 65). This difference has not been found in animals of the same weight (Sha 54), nor in children or infants (Gha 65, Rod 67). The sex difference can be attributed to the higher male weight and lean body mass, since PAO is positively correlated to weight and lean body mass.
Indeed, after normalising the values of BAO and PAO over the weight or lean body mass the difference between the two sexes becomes insignificant (And 70, Bar 69, Bor 73).

![Fig. 3.6: PAO, BAO, and the % ratio BAO/PAO in 105 healthy volunteers. Closed circles for men, open for women [reprod from Joh 81, p696].](image)

**Gastric acid and age:** Scientists concluded that gastric acid secretion declines with age (Pol 53, Lev 51, Kir 55, Vak 65); but there is an uncertainty on this decline because of the changes on weight and height. A study between Indians showed an increase in PAO up to the age of 50 years and then a decline. This can be attributed to the parallel increase in weight with age which after the 50s leads to parallel decrease on weight (Vak 65).

**Gastric acid and ethnicity:** Studies of gastric acid output under stimulated conditions showed a considerable difference between the Western world and Indians, Bantus, Ghanaians and Chinese (Goy 66, Des 67, Lis 68). But also it was observed that supramaximal doses of histamine inhibit the acid output (Des 69, 67). If the dose is normalized on lean body mass then these differences are minimized between Westerners and the rest. But the Chinese, despite the normalization, showed a lower acid secretion than the Scots in Hong Kong and that was verified morphologically by actual counting of the parietal cells as the number of parietal cells in the Scots was double those in Chinese. It was also
found that the secretion per cell was similar in both ethnicities (Che 77, Fun 73, Mos 72).

Concluding, PAO and consequently parietal cell mass is a function of body weight, lean body mass, height, sex age and ethnicity. Normalizing the data over weight, lean body mass and height the differences start becoming insignificant. The number of parietal cells is correlated with the body weight and consideration should be given in not exceeding the optimum dose of the stimulants when studying different ethnicities.

![Graph showing gastric secretion profile](image)

*Time in hours*

**Fig. 3.7: Acid output after ingestion of a solid and liquid meal in healthy subjects [reprod from Mal 76].**

### 3.7 Profile and magnitude of the post-prandial gastric secretion

The gastric secretory response to an ingested stimulus shows a similar profile if the stimulus is a simple meal (crystaloid solution, water) or a complex solid and liquid meal; but the magnitude and duration of the response may be quite variable.

In particular, the gastric secretory profile is characterised by a rapid increase in the secretory rate just after the eating process starts or even on the arrival of an appetising food, and reaches a peak between 30 and 60 minutes. A decline in the secretory rate follows just after the peak towards the basal rates and a low plateau is frequently formed during the third to fifth hour after meals, with some meal still
remaining in the stomach. Thus, the gastric secretory response to a meal exhibits 2 phases, namely, initial acceleration followed by deceleration. This can be explained accepting that both stimulatory and inhibitory mechanisms operate post-prandially with the predominance of the stimulatory mechanism in the 1st phase and of the inhibitory in the 2nd phase. A balance between stimulatory and inhibitory mechanism seems to prevail during the peak time.

The gastric secretory capacity varies considerably among healthy individuals. Extensive studies of gastric acid secretion during basal conditions and in response to secretagogues (Bar 79) gave “maximal” acid secretion rates of less than 5 (probably even zero) to 60+ mEq/hr. A similar variation has been found in fewer studies in response to meals. The research showed that physiological limits for gastric secretory rates cannot be rigidly established.

3.8  **Gastric emptying**

3.8.1  **Introduction**

Gastric Emptying is the process of delivery of ingesta so as to optimize nutrient absorption in the small bowel (Win 94).

Interpretation of measurements of a single gastric function is constrained by the absence of a clear understanding of how various factors contribute to emptying. Future progress requires the study of integrative function (Win 94).

Gastric emptying is one of the most important motor functions in the gastrointestinal tract. It regulates the rate of absorption of nutrients and drugs by controlling delivery into the small intestine. The rate of delivery is modulated by feedback from nutrients and other receptors in the small intestine, by the central nervous system via the vagus and sympathetic nerves and by the release of a variety of hormones.

An overall method for assessing the effects of motor function of the stomach can be obtained by the use of gastric emptying tests that can provide the pattern of gastric emptying and the gastric emptying rates.
Most of the gastric emptying studies show an exponential pattern and the half emptying time (T50) can be calculated easily. The T50 is an easily understandable and convenient parameter reflecting gastric emptying. However, at the start of emptying and towards the end of it, there is a decline of the exponential pattern in most of the cases; thus T50 is a crude somehow measurement of the net gastric function.

The results are also usually expressed as a percentage of contents emptied from the stomach in a given time.

Attempts have been made to assess patterns rather than rates of gastric emptying; but visual examination of the gastric emptying curves is insisted always because it can provide useful information which would be missed if a simple exponential or another model would be fitted (Col 73, Ham 76). This is a consequence of the multi-factorial function of the gastric emptying.

Disorders of gastric motility are generally manifested by a rate of gastric emptying that is either too slow or too fast. This may reflect an abnormality in one or all of the major functions of the stomach and may be related to one or more motor functions of the gastrointestinal tract. As a simple example, when a stomach fails to empty properly, nausea, loss of appetite and early satiety is expected and quite often the gastric contents may be vomited.

A most common reason of impaired gastric emptying is obstruction at the gastric outlet and such disorders are known as gastric ulcer and peptic ulcer disease. Another causal factor can be the absence or disorganization of motor events in the caudad stomach and this is common in metabolic disorders such as diabetes mellitus and potassium depletion.

Vagotomy, an operation in peptic ulcer disease, to decrease acid secretion, leads to delayed gastric emptying and those patients may experience, even after a pyloroplasty, diarrhea, sweating, palpitations, cramps and a variety of other unpleasant symptoms.
Duodenal ulcer is associated with accelerated gastric emptying and this probably creates a low pH environment in the duodenum which causes the ulcer. In contrary, gastric ulcer is associated with slower gastric emptying.

Evaluation of gastric motility is usually limited to qualitative observations of gastric emptying provided by x-ray and fluoroscopy using the barium sulfate meal to fill out the stomach. Occasionally, aspiration of the stomach at specific intervals following instillation of an isotonic saline solution will give information about gastric emptying. Quantitative measurements of motility and emptying even today confined to the research laboratory (Joh 81, Rea 89, Kum 98).

3.8.2 Motility

The Gastric Motor Function (contractile motion of the stomach walls) being accomplished by the trilayered muscularis is not only associated with the emptying of the chyme into duodenum at a proper rate it can be absorbed but also compress, knead, twist and continually mix the food with gastric juice to produce the chyme. Experimental research has revealed that there are three distinctive activities, which comprise the gastric motility each one completing a different task known as Motor Function. The Motor Function activities are associated with the three different muscular layers.

For a better understanding a specific anatomical division of the stomach has been proposed, shown in Fig. 3.8 (Joh 85). The shadowed area is called caudad and the rest orad. In general the orad area exhibits less contractile activity than the caudad.
The three separate motility functions are as follows:

a) **Motor function of the proximal stomach** or adaptive (receptive) relaxation. It is responsible to accommodate the ingested volume of the food and the volume of fluid that is secreted, in response to eating. When food enters the stomach the so called adaptive relaxation takes place in the proximal stomach (fundus and upper part of the body) meaning that as a content enters into the stomach, the intragastric pressure (tone) remains relatively unchanged (pressure $<5$ mm Hg) by the relaxation of the muscular. Another anatomical division relative to gastric motor function accepts the proximal stomach, distal stomach and pylorus (Dub 83). This division is based on the muscular distinctions among the above parts of the stomach (Fig. 10). In the proximal stomach the oblique muscle layer invests the fundus and part of the gastric body, lying between the circular muscular layer and the submucosa and fusing with the circular muscle at the greater curvature and with the lower oesophageal sphincter. The middle circular muscle layer is poorly developed in the paraoesophageal region and it is thickening distally along the distal stomach to become a distinctly thick in the pylorus. The outer longitudinal muscle layer along the stomach is absent from the anterior and posterior surfaces of the stomach.walls of this region, and it is obvious that it is due mainly to the unique of this part oblique muscularis and lesser to the circular layer, since the longitudinal layer is almost absent in this location. After

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Fig. 3.8: Diagram of the divisions of the stomach with respect to secretions and motility [reprod from Joh 85, p32].

Fig 3.9: A drawing to show the distribution of the muscular layers of the stomach [reprod from Wes 98, p632].
eating has ceased, tone is gradually restored (meaning relaxation progressively stops) to press the contents into the distal stomach. During this period peristalsis (shallow contractions for 1 h) is inhibited.

b) *Motor function of the distal stomach.* The stomach has intrinsic electrical activities known as, slow waves or pacemaker potentials or electrical control activity or basic electric rhythm. The pacemaker region is located on the greater curvature of the stomach near the junction of the fundus and proximal gastric corpus (Fig. 9) (Win 93, pp291). The slow waves from the region where they are generated are propagated distally and towards the pylorus at an average rate, three per minute. The gastric slow wave itself generates little or no circular muscle layer contraction. As the slow wave approaches the distal antrum, another slow wave develops in the pacemaker region. When peristaltic contractions occur, they begin in the mid stomach and move toward to gastroduodenal junction as slow waves do, also, increasing their force and velocity. The duration of these contractions ranges between 12 and 20 seconds (3-5cpm) and it is proven that the slow waves control their timing and propagation. Electrodes placed on the serosal surface to detect activities from the smooth muscle cells showed, that slow waves begin from the mid-region of the stomach continuously at intervals of 12 to 20 seconds and are moving with increasing velocity to the caudad area (Fig. 3.10) (Joh 81, Kel 69).

*Fig. 3.10:* Diagram of the migration pattern with amplification and acceleration of gastric slow waves. P indicates a peristaltic wave [reprod from Win 93].
Mechanical and electrical activities of the smooth muscle detected by intraluminal pressure and electrical potentials showed, also, that there is a threshold on the amplitude and duration of the potential changes above which contractions appear (Fig. 3.11)) (Joh 81a).

![Fig. 3.11: Top trace on left (A) shows mechanical events and bottom trace the associated action potentials (slow waves). On right (B) action potentials with oscillations and spikes during plateau from distal antrum [reprod from Joh 85, p36].](image)

c) Migrating Motor Complex (MMC). During fasting, the pattern of electrical and motor activity is modified and cycles of motor activity migrate from the stomach to the ileum. Each cycle known as Migrating Motor Complex, is identified by three stereotype phases. It starts with a quiescent period (phase I) during which no contractions are happening nor electrical spikes occur and lasts about 30 minutes. Phase II is characterized by irregular electrical (spikes) with frequency 1-2 per minute and strong contractions and finally phase III is characterized by a burst of strong and regular contractions at a frequency 3 per minute; it lasts 2 to 10 minutes. The MMC cycles occur at intervals of about 90 min, with a velocity 5 cm/min and it originates in the fundic cardia. It seems that during the MMC cycle gastric and pancreatic secretion as well as bile flow increase and they may clear the stomach and small intestine of luminal contents in preparation for the next meal. They are completely inhibited by a meal, they resume 90 to 120 minutes after a meal and they are sometimes mildly painful, so are called hunger contractions (Gan 97, Joh 81, Win 90, How 81).
3.8.3 Methods of studying gastric emptying.

The pioneer of the stomach's emptying studies was William Beaumont observing directly the time needed for a meal to completely leave the stomach in 1825 conducting hundreds of experiments with the same subject (Bea 96).

Since then, there have been developed various clinical tests in studying the gastric emptying.

Gastric aspirations have been used in combination with various dyes and repeated sampling of the gastric and duodenal contents examined based on their color can give an estimation of gastric emptying rates.

In routine diagnostic procedures, Radiology using the barium sulfate non-digestible meal has the priority since long ago and as it is known it was the first developed diagnostic tool of the stomach. It can provide the clearest indication of disturbances in contractile activity and movement of the luminal content. The radiation dose limits the length and number of tests (studies) that can be performed. Under these circumstances radiology can demonstrate clearly but gross and persistent disturbances.

Gastroscopy, endoscopy of the gastric region, is the preference of the physicians for the stomach's clinical examination especially these days after the offer the development of the very thin tubes as gastroscopes with the capability to produce images of the gastric interior and even to extract specimens for biopsy; but the study is limited in observing an empty stomach not actual movement and it is an invasive one.

Both radiology and gastroscopy are based only on observation and they don't provide specific measurements of gastric emptying or any other numerical parameter of the gastric motility.

Gamma-ray scintigraphy is considered as the technique able to provide images of the interior of the stomach during the course of digestion and assess the gastric emptying rates. Scintigraphy can follow the digestion and emptying of either a liquid or a solid
test meal. More than one tracers for radio-active labeling can be used according to the nature of the meal (DTPA for liquid or colloid solid test meal). But even these days the gastric scintigraphy has not been yet established as a clinical routine test and not any test meal is available nor any normal emptying rates have been established. It is mostly used as an investigation (research) method than as routine test. It is costly, occupying the gamma-camera for long period and involves radiation risk to humans.

Gastrointestinal manometry can be clinically used with multi-lumen manometric probes providing the pressure at predefined sites of the stomach. It is invasive and requires highly skilled experts and again it more useful as an investigation tool.

Ultrasound and Magnetic Resonance Imaging are capable to provide emptying rates and motility but there are limits on the state (nature) of the test meals, requires high experience on interpretation of the data and long occupancy of extremely costly equipment and both are on research status. Their establishment is especially important for testing special groups.

During the last two decades two other techniques arose based on the electrical properties of the gastric area, namely Electrogastrography and Electrical Impedance Tomography (EIT) or Epigastrography (EIE).

Electrogastrography (EGG) detects and records gastric electrical activity via surface electrodes attached to the skin. (Smo 89). Transformation of the recorded electrical activity over time to frequency spectra using Fourier transformation shows the gastric contractile activity and a shift to frequency at non-normality has been shown (Koc 87, Mal 85). It is a non invasive, not any risk inducing and inexpensive technique and must be primarily considered as a research tool and it is believed by Smout and van der Schee as a potential clinical method in patients with functional upper gut symptoms.

The electrical impedance techniques are based on the gastric impedance developed when an alternating current is applied on the gastric region via surface electrodes. The current is usually of a few decades of kHz and 1 to 5 mA peak to peak.
EIT using a circle of electrodes, usually 16, around the upper abdomen forms images of the stomach and can give also emptying rates and motility information based on the changes of the epigastric impedance (Avi 87, Kil 96, Mee 96).

EIE based on the same principles is using two or three pairs of surface electrodes one of which is to inject the current. A bio-signal over time is recorded with sampling rate at least 1 Hz and with a liquid meal with specific electrical properties the gastric emptying rates it is believed can be calculated (Sut 85, Man 88, Mur 97). Both EIT and EIE assume till now that the measured impedance changes are proportional to gastric volume. They also accept an error because of the gastric secretions and try to inhibit the gastric secretions during the study by cimetidine or omeprazol (Avi 87, Man 87-88, Bax 88); or they think blocking is unnecessary if used specific test meals (McC 85, Sut 87).

Aspiration techniques have been established long ago (Hun 61;59).

EIT and EIE are simple, non-invasive, not risk inducing techniques, promising but need for their evaluation is necessary.

Recently, the so called breath tests with various tracers are in use to assess the emptying rates. They don't provide accurate emptying rates but estimates and are useful in research studies. Octanoic acid in the breath tests is the most common in use (Sch 97a, Gho 95).

Paracetamol blood concentration during the digestion of a meal is also a measure of gastric emptying after administration of known dose of paracetamol with the test meal (Hir 95, Rob 96, Hea 73).

Concluding, it seems that gastric function and specifically gastric emptying is the least studied body function since routine tests with all the available modalities haven't been established yet, except those with radiology and gastroscopy with the limitation of providing us with gastric emptying parameters. Thus, there is need to develop other methods or to investigate further capabilities of the existing ones or to re-evaluate the existing ones.

The aim of this work is to further explore the EIE modality.
3.9 Digestion according to nutrient type

3.9.1 Introduction

The food on which the body lives, with the exception of small quantities of substances such as vitamins and minerals, can be classified as carbohydrates, fats and proteins. All the three categories are characterised as fuel molecules providing the energy for ATP formation. However, these generally cannot be absorbed in their natural forms through the gastrointestinal mucosa and, for this reason, are useless as nutrients without preliminary digestion. First, these substances are digested into compounds in the stomach, small enough for the absorption, which is the second stage in the intestine.

The chemistry of digestion is really simple, because of in the case for all three major types of food, the same basic process of hydrolysis is involved. The only difference is in the enzymes required to promote the reactions for each type of food. All the digestive enzymes are proteins and secreted by different gastrointestinal glands.

3.9.2 Carbohydrates

Carbohydrate is a primary nutrient world wide and, mammalian intestine has appropriate mechanisms to enable it to be readily digested and absorbed. The daily intake of carbohydrates varies considerably from 250 to 800 g per day in the american diet for example. In the human diet, the majority of carbohydrate (60%) is ingested as polysaccharides (starch mostly in grains), but disaccharides (sucrose in cane sugar and lactose, a major constituent in milk) are also important nutrients. Rather than being assimilated as such, dietary polysaccharides and disaccharides must first be cleaved to monosaccharides (glucose, galactose and fructose) which in turn have access to specific transport mechanisms that promote final intestinal uptake. Almost all the carbohydrates of the diet are combinations of the monosaccharides bound to each other.

The carbohydrates are digested into their constituents monosaccharides through the hydrolysis process, by which enzymes combine hydrogen and hydroxyl ions with the
poly- and disaccharides and in this way separate the monosaccharides from each other. The disaccharide $R_1R_2$ par example is digested according to the following scheme:

$$R_1R_2 + H_2O + (\text{digestive enzyme}) \Rightarrow R_2 \text{OH} + R_1 \text{H}$$

The specificity of hydrolytic enzymes available to digest carbohydrates, is limited and some carbohydrates that are eaten remain indigested in the intestinal lumen for subsequent metabolism by bacteria in the lower ileum and colon. These indigestible carbohydrates belong to the group of substances, called dietary fibre.

Following a normal meal, most of the CHO ingested is digested and absorbed within the first 20% of the small intestine, and the CHO absorptive capacity is practically impossible to saturate.

It has been shown that carbohydrates in the form of glucose solutions exert an elevation of blood GIP (Mor 79, Syk 80, Cre 77). The IR-GIP response to sucrose ingestion peaked much later compared to glucose, indicating need for hydrolysis to occur.

3.9.3 Fat

The amount of fat in the diet varies from about 25 to 160 g per day. The most common fats of the diet are the neutral fats, also known as triglycerides, each molecule of which is composed of one glycerol nucleus and three fatty acids. In the usual diet are also present small quantities of phospholipids and cholesterol esters containing fatty acids and they are considered as fat, too. Cholesterol is also considered as a dietary fat, because it is derived from fats and it is similarly metabolised to fats.

Under resting conditions, approximately half of the energy used by tissues such as muscle, liver and kidney is derived from the catabolism of fatty acids.

Oral administration of fat causes the release of GIP. Studies with triglycerides have shown that release GIP that reaches a plateau more slowly than glucose does (Bro 82). Medium chain triglycerides produce a modest increase in serum GIP with a peak
at 30 min and return to base line within 120 min. Long chain fatty acids are better secretagogues for GIP release. A corn oil suspension peaked at about 120 min at levels almost five times the basal (Fal 75). These studies also show that GIP might exert an effect on the metabolism of fatty acids.

It has been proved that a significant amount of immunoreactive secretin is released by fatty acids such as oleic acid or the digestive products of fat (Wat 86).

Ingestion of fat significantly increases the levels of NT LI in human plasma, whereas isocaloric solutions of amino acids and glucose had negligible effects (Ros 79). The first biological activity of NT LI reported was the inhibition of gastric acid secretion (And 76, Ols 84), neurally mediated. Because there is a positive correlation between the inhibition of gastric acid secretion after intraluminal administration of oleic acid and the rise of the human plasma NT LI, it is possible that endogenous NT LI may have a role in the post-prandial control of acid secretion (Kih 81).

Carbohydrate and fat are inhibitory at all levels of human intestine. Protein acts as a gastric acid stimulant in the proximal 100 cm of human gut but as an inhibitor more distally (Owy 79).

3.9.4 Protein

The term “protein” comes from the greek proteios (“of the first rank”), which aptly describes their importance. The proteins account for about 50% of the organic material in the body.

About 50 g of protein per day are required by a normal adult to supply essential amino acids and replace the amino acid nitrogen converted to urea. A typical american diet consists of about 125 g of protein. In addition to the protein in the diet, a large amount of protein (mostly enzymes) is secreted into the gastrointestinal tract by the various glands or enters it via the disintegration of epithelial cells.

The dietary proteins are derived almost entirely from meat and vegetables and they are digested primarily in the stomach and upper part of the small intestine.
Protein digestion begins in the stomach with the enzyme pepsin that is splitting the proteins into proteases, peptones and large polypeptides. Pepsin is active in a highly acid environment with best action in the range of pH between 2 and 3. Therefore the gastric HCl acid is essential for the protein digestion in the stomach; but only 10% to 20% of the protein is digested in the stomach.

In contrast, protein ingestion has been shown not to stimulate IR-GIP when given either as a meat extract or as a fillet steak (Bro 74, Cle 75).

Some obese subjects displayed elevated basal plasma IR-GIP with further exaggeration after nutrient ingestion (Cre 78).

Mazzaferri et al. (Maz 84) reported that GIP secretion could be elevated by increasing dietary carbohydrate in normal weight individuals and hypothesised that alterations in intestinal endocrine cells were produced. Because many obese subjects tend to be hyperphagic, elevated IR-GIP secretion may simply be due to increased calorific intake. On the other hand, increased plasma concentration of IR-GIP may not be necessary for an increase in the pancreatic response, if increased sensitivity or responsiveness to IR-GIP existed at the level of the islet. Another study (San 81) reported high GIP basal levels in obese patients but no increase in the integrated response to a standard test meal.

3.10 Peptides involved in gastric digestion

Peptides are involved in regulating digestion and gastric emptying. Their presence in the intestinal area and their concentration in the blood send stimulatory or inhibitory messages related to gastric function and generally to digestion. The role of the peptides expected or assumed to influence the present studies is given briefly below as well as the role of the synthetic peptides used by intravenous infusion in certain studies in order to investigate their possible effect on gastric function.
3.10.1 Gastric Inhibitory Polypeptide (GIP)

Gastric inhibitory polypeptide is involved in two physiological regulatory mechanisms: the inhibition of gastric acid secretion and the stimulation of insulin release. Both are of gastrointestinal or more specifically duodenojejunal origin.

It was shown that many food substances, when introduced into the small intestine, initiate a humoral reflex that results in the inhibition of gastric acid secretion. Kosaka and Lim (Kos 30) adopted the word enterogastrone for this humoral agent with the meaning of a substance that is released from the mucosa of the small intestine by fat or the products of fat digestion. Is really GIP an enterogastrone?

GIP was isolated on the basis of the acid inhibitory properties (Bro 70). Later, studies on secretion of IR-GIP showed that GIP was elevated in response to ingestion of fat (Bro 74). Intra-duodenal administration of acid in rat and human stimulated release of GIP (Ebe 79) but not in dog (Bro 75).

Studies in humans, confirmed that with fairly high doses (2 mg/kg injected over 5 min and producing circulating GIP levels of 5ng/ml) GIP could inhibit acid secretion (Arn 78, Cle 75). Also, fat instilled in the upper intestine is a powerful inhibitor of acid secretion in the innervated stomach (Kos 30). Despite that a good correlation was found between GIP secretion and inhibition of food stimulated acid when large amounts of fat or glucose were administered intra-duodenally, significant inhibition was also obtained with amounts that did not significantly raise circulating GIP levels (Cre 83). The situation according to the outcome of the studies is complicated and lead to hypothesis that there is an indirect path for the action of GIP on the parietal cells, such as somatostatin and neuronal modulation. The inhibitory effect of GIP was most profound when the parasympathetic activity to the stomach was minimal.

Concluding, there is considerable evidence that GIP is capable of potently inhibiting acid secretion and that its action is in concert with other peptides (such as somatostatin) and / or nervous inhibitory mechanisms.
3.10.2 Cholecystokinin (CCK) and Loxiglumide.

Cholecystokinin, a gastrointestinal peptide released in response to nutrient fatty meals, is believed to have an important role in controlling the gastric motor function (Sch 91). Studies with intravenous infusion of CCK showed a slower gastric emptying (Kle 88), relaxation of the fundus (Yam 78), inhibition of antral motility (Kuw 86, Sch 83) as well as stimulation of localized pyloric contractions (Fre 93, All 89) and inhibition of gastric acid secretion (Kon 94-95).

The recently developed loxiglumide, a 5-oxo-pentanoic acid derivative, is a potent, specific, and competitive CCK-A antagonist (Rov 91, Set 87, Set 87) and suitable to be used in humans. It can also evaluate the role of endogenous CCK in the control of gastrointestinal function (Mey 89, Fri 91). It has been shown that loxiglumide accelerates gastric emptying (Sch 97) and stimulates gastric acid secretion (Kon 95;94).

3.10.3 Secretin inhibits gastric acid secretion.

The primary biological action of secretin is stimulation of the pancreas to secrete bicarbonate. Secretin stimulates the secretion of pepsin into the gastric juice and inhibits gastric acid secretion, especially during stimulation of gastrin (Hub 72). It has been shown that secretin inhibits food-stimulated gastrin release (Chi 80).

3.10.4 Gastrin

Gastrin is secreted from G-cells or gastrin cells that are housed mainly in antral mucosa and fewer in duodenum. It promotes gastric acid secretion upon its stimulation and the gastrointestinal motility. Gastrin secretion is stimulated by direct contact with amino acids especially and entering in the blood stream and finally reaches the target cells which are the gastric glands (Joh 87, Mak 89).

Gastrin secretion is inhibited when the pH of gastric juice drops below 2.0 and stimulated when pH rises. This negative feedback mechanism helps to provide the optimal low pH for the gastric digestion (Tor 00, Mar 92).
4 BIODIELECTRICS

4.1 Introduction

Since the structure of living organisms is a physical-chemical combination of the basic elements it is associated with electrical properties. Individual parts and segments of the body as well as microscopic and molecular structures may exhibit their electrical characteristics if they are exposed to electrical fields.

In particular, biological molecules are or may get polarized and the dipole moment depends on the size of the molecule and the distribution of the electrical charge throughout it. Thus, biomolecules can be considered as dielectrics or biodielectrics. On the other hand, the membranes of the cells act as capacitors with a capacitance of 0.5-2 μF/cm², so that a cell can play the role of a capacitor filled with a dielectric. A piece of soft tissue is an aggregation of cells bathed in water solutions (body fluids) where, free ions and other macromolecules are moving.

The study of the dielectric and electronic properties of biological matter has already become part of the interdisciplinary armoury of the biological sciences. These studies have provided a great deal of information on the structure and properties of molecules and tissues and by thus contributing to the total body knowledge, have eventual biological and medical application.

Indeed, dielectric properties and constants are used for the identification and quantification of various biomolecules and cells such as proteins (Kir 52), red blood cells in serum (Sch 62, Hel 63) etc. Various theoretical models for the studies have, also, been developed (Col 41).

Apart from these passive electrical charges, mechanisms exist in biological tissues for the active transport of ions, important for neural function, membrane absorption processes, feed back, etc.
4.2 External field dielectrics or Dielectrics into an electrical field

As it is known, if an external electric field is applied on a dielectric material a polarization occurs. The induced polarization \( P_i \) may be the sum of three terms, such as

\[
P_i = p_e + p_a + p_o \tag{4.1}
\]

where \( p_e \) is the electronic polarization due to the electron cloud displacement, \( p_a \) the atomic polarization due to atomic charges and \( p_o \) the orientation polarization due to the molecular orientation because of the displacement of their charges.

If the cause of the polarization (electric field) is removed the electric charges and/or the orientation of the polar molecule go back (relax) to their natural random position and orientation in a finite time \( \tau \), called, decay or relaxation time.

For electron dipoles a typical \( \tau \) is about \( 10^{-14} \) s, for small molecules as in liquids \( 10^{-11} \) s and for large macromolecules as in proteins in aqueous solutions \( 10^{-8} \) to \( 10^{-6} \) s (Gra 78).

4.3 Dielectric properties

A material can be characterized by it’s intrinsic electrical properties conductivity \( (\sigma) \) and permittivity \( (\epsilon) \).

**Conductivity**: Conductivity \( (\sigma) \) of a medium is the response to an external field due to the free electrical charges in it such as electrons, ions etc. and is a measure of the ease with which delocalized electric charge can migrate through the material under the field’s influence. It is the reciprocal of the resistivity and is expressed in S/m, where S=Siemens=Ω⁻¹.

**Permitivity**: The permitivity \( (\epsilon) \) reflects the extent to which localized charge distributions can be distorted through polarization by an external field.
If an electric field with potential $V$ is applied to a material by two electrodes of area $S$ and separated by a distance $d$, the material can be characterized by its two intrinsic properties, conductivity ($\sigma$) and permittivity ($\varepsilon$), related respectively to conductance ($G$) and capacitance ($C$) according to the following equations:

$$G = \sigma \frac{S}{d} \quad (4.2)$$
$$C = \varepsilon \varepsilon_0 \frac{S}{d} \quad (4.3)$$

As a consequence, a conduction current ($I_c$) and displacement current ($I_d$) will flow given by the following equations:

$$I_c = V / \sigma_f \quad (4.4)$$
$$I_d = \varepsilon \varepsilon_0 \frac{dV}{dt} \quad (4.5)$$

where: $\varepsilon_0$ is the permittivity of free space equal to $8.854 \times 10^{-12}$ F$m^{-1}$ and $\varepsilon$ represents the relative permittivity known, also, as the material's dielectric constant and is the ratio of the capacitances of the same capacitor first with a dielectric and then with vacuum between the two plates.

### 4.4 Debye dispersion

Materials show a smooth decrease of relative permittivity with increasing frequency. Superimposed on the smooth fall-off, at high frequencies, are anomalies, which occur at the natural resonant frequencies of internal modes of excitation of the material's microscopic constituents. Close to the resonant frequency, energy is absorbed from the incident electric wave and transferred to internal excitation. Energy can be, also, absorbed at frequencies far from resonances and dissipated as heat via frictional processes. Absorption of energy from an electromagnetic wave results in the field amplitudes becoming smaller since these amplitudes determine the wave's energy.

For a pure polar substance with one type of molecule present, the value of permittivity falls from one plateau to another as the frequency increases. This phenomenon is called dispersion and it is associated with loss of energy of the applied electrical field. In a solution, where both solute and solvent are polar two dispersions would be expected. This is because as the frequency is rising the
molecular (or biological) dipole motion becomes progressively unable to keep up with the alternating field direction; at the same time the conductivity due to the free charges increases. The dispersion phenomenon is described by the complex permittivity ($\varepsilon^*$) of the medium (Eq. 4.6). The real ($\varepsilon'$) and imaginary part ($\varepsilon''$) are sufficient to explain the loss of energy in the medium, as the frequency increases (Spy 85, Pet 79).

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \quad (4.6)$$

$$\varepsilon' = \varepsilon_m + \frac{\varepsilon_m - \varepsilon_m}{1 + \omega^2 \tau^2} \quad (4.7)$$

$$\varepsilon'' = \frac{(\varepsilon_m - \varepsilon_m) \omega \tau}{1 + \omega^2 \tau^2} \quad (4.8)$$

where $\omega$: is the angular frequency; $\tau$: the relaxation time; $\varepsilon_m$: the permittivity at the dispersion minima and $\varepsilon_m$: the permittivity at the dispersion maximal (Fig. 4.1(A)). The drop in $\varepsilon'$ is accompanied by absorption of energy, described by $\varepsilon''$. Between the real and imaginary part of permittivity, as equation (4.6) shows, there is a phase difference of 90°.

Fig 4.1: Variation of the dielectric parameters $\varepsilon'$ and $\varepsilon''$ with frequency for a Debye type relaxation of a pure polar molecule (A) and of an aqueous solution of haemoglobin (B) [reprod from Pet 79 (A), Gra 78 (B)].
Figure 4.1(A) shows the dielectric dispersion of a pure polar molecule (Pet 79); Figure 4.1(B) shows the same for an aqueous solution of hemoglobin. In figure 4.2 the general dispersion phenomenon is presented (Pet 79, pp 20) and in Fig. 4.3 the dispersion of a muscle tissue (Cas 90).

Fig 4.2: Variations of the dielectric parameters $e'$ and $e''$ with frequency in various dispersions [Pet 79, p20].

Fig. 4.3: Dielectric dispersions exhibited by a muscle tissue [reprod from Cas 90].

It is evident from Fig. 4.3 that the dielectric constant of tissues decreases rapidly as the frequency increases. At low frequencies the decrease is due to cell membranes acting as capacitors and it is known as $\alpha$ dispersion. The dispersion between 1 to 500
MHz is due to loss of energy in dipole relaxation (β-dispersion) and finally the so-called γ-dispersion is due to the relaxation of water molecules.

If the Equations 4.6 to 4.8 are modified so that the exponent can be expressed through a variable α their general form becomes:

\[ \varepsilon^* = \varepsilon_0 + \frac{\Delta \varepsilon}{(1 + j \omega \tau)^{\alpha}} \] (4.9)

which includes the Cole brothers’ modification of the Debye dispersion Equations (4.7) and (4.8). The fit to this model is generally very good, because α allows most of the data to be fitted regardless of the actual physical mechanism that is happening. Also, the virtually indistinguishable from each other distributions of relaxation times found experimentally by Schwan (Sch 57) support the use of the α variable.

Another important consideration is that since dielectric absorption is a measure of the energy dissipated in the material then processes which are related to dc (direct current) conductivity σ, also, contribute to the total dielectric absorption; thus at a frequency f the total dielectric loss \( \varepsilon'' \) can be given by

\[ \varepsilon'' = \varepsilon''_0 + \frac{\sigma}{2\pi f \varepsilon_0} \] (4.10)

from which it can be seen that the dc conductivity could contribute significantly to the absorption at low frequencies, but not so much as the frequency increases.

Experimental studies based on the dielectric dispersion concluded that:

i) 10% of tissue water is somehow bound to its surface (Coo 74) ii) membranes exhibit a capacitance of 0.5-2 μF/cm² (Gra 78)

iii) extracellular fluid has a conductivity equal to 10mS/cm (Sch 59)

iv) the intrace of erythrocytes show a σ about 5mS/cm.
4.5 Human body-tissues as dielectrics

In the body, the displacement current due to polarisation is very much less than the current carried by the free charges (ions) despite the high dielectric constants of tissues. The rich ionic environment is due to water in which electrolytes are dissolved. The human body is composed of about 70% water, with values of 90% water in blood and 30% in fatty tissue; Barnes et al, '67 (She 77) calculated the ratio of the free charge to the polarization charge based on the following relation:

\[
\frac{Q_f}{Q_p} = \frac{1}{\omega \rho (\varepsilon' - \varepsilon_0)} \quad (4.11)
\]

where \( Q_f \) is the free charge; \( Q_p \) the polarization charge; \( \omega \) the angular frequency in radians; \( \rho \) the material’s resistivity. Assuming a dielectric constant of 80 (water) and a resistivity of 1 \( \Omega \)m, the ratio is in the range of million; but for more realistic values \( 10^6 \) for \( \varepsilon' \) and 8 \( \Omega \)m for \( \rho \), the ratio is about 40, allowing us also to draw the conclusion that the free charge conductivity predominates within the body tissues.

\[\text{Fig 4.4: The changes of conduction (Ic) and displacement (Id) current in a biological tissue with frequency [reprod from Jac 83].}\]

This means that the displacement current associated with capacitance can actually be ignored. The same was concluded by Schwan and Kay (Sch 57). Figure 4.4, allows
us to compare the conduction and displacement current in a biological tissue for various frequencies (Jac 83).

Table 4.1 gives the conductivity of biological tissues and Table 4.2 the ratio of capacitive to resistive currents for various frequencies and body tissues (Plo 69).

**Table 4.1: Conductivity of biological tissues** [reprod from Plo 69, p205].

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean conductivity mhos/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.67</td>
</tr>
<tr>
<td>Lung</td>
<td>0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>0.14</td>
</tr>
<tr>
<td>Fat</td>
<td>0.04</td>
</tr>
<tr>
<td>Human trunk</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**Table 4.2: Ratio of capacitive (I_\omega) to resistive (I_s) currents of body tissues** [reprod from Plo 69, p205].

<table>
<thead>
<tr>
<th>Tissue/Frequ</th>
<th>10 Hz</th>
<th>100 Hz</th>
<th>1000 Hz</th>
<th>10000 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0.15</td>
<td>0.025</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Fatty tissue</td>
<td></td>
<td>0.01</td>
<td>0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>Liver</td>
<td>0.20</td>
<td>0.035</td>
<td>0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>0.10</td>
<td>0.04</td>
<td>0.15</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Concluding, it is emphasized that in human tissues the capacitive currents in the frequency range 10-10^5 Hz are negligible and can be ignored completely and thus soft biological tissues can be treated as pure free charge conductors.
5 ELECTRICAL IMPEDANCE EPIGASTROGRAPHY -
THE EPIGASTROGRAPH

5.1 Introduction

When an alternating potential difference \((V_i)\) is applied to a system of interest (biological system), a current \((I_i)\) flows in the system with the same frequency and pattern but with a phase difference (phase angle). If the system is linear in the sense that the current flowing is proportional to the applied voltage then in macroscopic measurements Ohm's law can be applied, as follows:

\[ V_i = Z I_i \quad (5.1) \]

where, \(Z\) is Impedance and represents the total resistance from the material to the current flow given by the following equation;

\[ Z = R + jX \quad (5.2) \]

where, \(R\) is the conductive resistance and \(X\) the reactance of the material given by the following equation

\[ X = \frac{1}{\omega C} \quad (5.3) \]

where, \(\omega\) is the angular frequency of the ac current and \(C\) the capacitance of the material. It is evident, based on the previous chapter that the intrinsic properties, conductivity \((\sigma)\) and permittivity \((\varepsilon)\), both influence the macroscopic impedance, varying in their magnitude through the frequency spectrum.

The present interest is focused on the macroscopic results of a soft tissue unit of the human body when an ac current with defined frequency is applied.

As was mentioned in Chapter 4 the conductivity due to free ionic charge predominates within the body tissues. Figure 4.6 indicates quantitatively that for frequencies below 100 kHz the conduction current \((I_c)\) predominates over the
displacement current \((I_d)\). Consequently the simpler form of Ohm's law can substitute Equation 5.1,

\[ V_{\text{eff}} = RI_{\text{eff}} \quad (5.4) \]

at the measuring electrodes. \(V_{\text{eff}}\) and \(I_{\text{eff}}\) are the effective potential difference and current intensity, respectively.

### 5.2 Electrical Impedance Epigastrography (EIE)

Electrical Impedance Epigastrography (EIE) can be considered as gastric plethysmography. Thus, it is the collection of information from the gastric area in the form of electrical impedance measurements using surface electrodes at a proper sampling rate under various conditions.

The method is suitable for studying gastric function, since the stomach being a collapsed muscular bag in the fasting state which gets inflated with the ingestion of a load, offers a medium with variable total electrical resistance (impedance) within the abdominal region. Following and monitoring the abdominal impedance over a period of time, gastric function can be studied.

More specifically, the stomach can be considered as a biological cylinder with its along axis \(l\), radius \(r\) and cross sectional area \(S\). The impedance of this biological object can be defined if an electrical field is applied along the axis \(l\) on the surface of the cylinder according to the following Equation 5.5:

\[ R_{\text{imp}} = \frac{1}{\sigma S} \quad (5.5) \quad \text{or} \quad R_{\text{imp}} = \frac{l^2}{\sigma V_{\text{vol}}} \quad (5.6) \]

where, \(\sigma\) is the conductivity of the gastric content and \(V_{\text{vol}}\) is the total gastric volume. According to Equation 5.6 it has been considered until now that the measured impedance is inversely proportional to the total volume and as the stomach is filled out with a liquid meal and as the meal leaves the stomach, the measured impedance gives the possibility to calculate emptying rates using the measured impedance differences over time. The conductivity of the gastric content is assumed to be relatively constant or if a gastric acid inhibitor is used the conductivity is said
to remain constant. So the Epigastrograph has been designed for gastric plethysmography.

Gastric emptying is strongly associated with normal or abnormal functions and motility, and until recently a simple, non-invasive nor risk-inducing technique to be used repeatedly had not been developed. Our current studies supported by BBSRC and EU, try to relate satiety aspects and obesity with gastric emptying. Gastric emptying is, also, very important in testing drugs. All these require a method which is repeatable, simple for both, subject and operator, non-invasive and not risk inducing. EIE seems to meet all these conditions in addition to being relatively inexpensive and may be used for screening and even for ambulatory measurements.

The method though is sensitive to artefacts of movements of the subject, breathing, imperfect attachment of the electrodes and even to emotional stress. The subject has to remain relaxed and preferably almost immobile for quite a long time.

EIE is still in its infancy with a lot of problems to overcome and a lot of effort to interpret the resulting data. It is true that advanced technologies provide us with data prior to their proper interpretation as happened with medical magnetic resonance imaging in mid 80s. Magnetic resonance tomographs were imaging the human body but the interpretation of the images was still a problem.

### 5.3 The Epigastrograph

Based on the mentioned dielectric properties of human tissues in respect to the frequency spectrum of an ac applied field, Dr. N.M. Spyrou and Mr. E.A. Worpe of the Medical Physics Group at the University of Surrey, designed about 15 years ago, with other collaborators a novel instrument, the Epigastrograph, manufactured by London Medical Electronics (a company whose directors comprised Dr. N.M. Spyrou, Dr. J.A. Sutton, Prof. D.L. Wingate and Mr. L. Chapman). The Epigastrograph is a generator of specific ac current but simultaneously a monitor of the potential difference between predefined electrode positions at a specific time interval. It was called Epigastrograph because of the purpose for which it was designed; to study the gastric function using surface electrodes over the abdominal region. In March 1997,
a new model, Mark IV of the Epigastrograph, was further developed by the Surrey group and built by the Medical Electronics Unit of St. Georges’ Hospital. Once commissioned this new system was used.

5.3.1 Model Mark IV

The new model is based on the same principles as the preceding instruments but it is built using technological developments and components, such as optical fibres and battery power operation (9V orthogonal), making it a light and completely portable system. It consists of two units, the patient-interphase unit and the computer-interphase unit. An optical lead connects the two units (Fig. 5.1).

![Diagram of the units of the Epigastrograph, Mark IV model.](image)

**Fig. 5.1: Diagram of the units of the Epigastrograph, Mark IV model.**

The patient-interphase unit is small (19.5 cm by 9.5 cm by 4.0 cm) and light (<0.5kg). The computer interphase-unit is simply a box of 15.0 cm by 8.0 cm by 5.0 cm and light, too. There are flashing indicators to show proper function and a switch button. A computer with specific software completes the system. A simplified description of the electronic circuit of the epigastrograph, Mark IV, is shown in Figure 5.2.

In particular, the Mark IV model, generates an alternating field of 32 kHz frequency supplying a current varying in intensity from 1 to 4 mA rms. The sampling rate either 5 or 1 Hz and 3 pairs of electrodes are used with the possibility of applying the current to any pair and measuring the generated potential difference by the other two
pairs (multiplexed system). In this way the system of interest can be studied by three or six sets of data.

![Simplified diagram of the electronic circuit of the Epigastrograph Mark IV.](image)

**Fig. 5.2: Simplified diagram of the electronic circuit of the Epigastrograph Mark IV.**

The operation of the epigastrograph is carried out by software based on Lab-View and the data are presented graphically on the screen of the accompanying computer by two windows; one presenting the data of the last minute of recording and the other offers the option to select a variety of time lengths.

The data are recorded on Excel spreadsheets for further analysis.

### 5.3.2 The older Epigastrograph (circa 1985)

The previous model was a robust portable but heavy unit (40cm by 35cm by 7 cm) with a built in printer for the graphic presentation of the data and a panel with appropriate push buttons for calibration. Liquid crystal windows were used for the digital presentation of the data. A computer with a special electronic acquisition card was connected to the epigastrograph. It operated at 42 kHz applying a peak to peak current of 2 mA; the sampling rate was 1Hz. Three pairs of electrodes were used, but one pair was fixed to apply the current and the other two to measure the generated potential difference. Thus, two sets of data were generated.
5.3.3 Sampling rate

The sampling rate of the epigastrograph of 1 Hz (or 5 Hz; an option on Mark IV) was based on the Uniform-Sampling theorem. According to that, if function $x(t)$ is bandlimited with no components at frequencies greater than $f_b$ Hz, it can be completely specified by samples taken at the uniform rate $f_s \geq 2f_b$ Hz. The minimum required sampling rate or sampling frequency ($f_s$) equal to $2f_b$ for complete specification of the continuous-time signal, is called Nyquist rate or Nyquist frequency. Another expression of this is maximum sample spacing with $T=1/f_s=1/2f_b$. Sampling a signal at a rate less or greater than the Nyquist rate is considered as undersampling or oversampling, respectively (Car 92, Pro 92).

It is known that the stomach's motility is characterised by a periodicity between 1.5 to 5 contractions per minute, equivalent to 0.025 Hz to 0.083 Hz. Thus, the sampling rate has to be $f_s \geq 2 \times 0.083$ Hz $\geq 0.17$ Hz. Although 0.2 Hz sampling frequency covers completely the phenomenon under study, the higher sampling frequency allows the investigation of any other higher frequencies (such as breathing, heart beating) which are involved at the same time and allows the examination of any relationships between them. Of course, a longer number of acquired data sets is generated and a higher memory capacity (at least 64Mb) and a higher speed (at least 200 MHz) computer is required for continuous function of the epigastrograph for a complete test; this is not a problem nowadays.

The epigastrograph has been designed to comply with the standards of safety described by British Standard BS5724 (Part one) and the International standard IBC601. In Fig. 5.3 the threshold sensation is given with respect to current injected via surface electrodes 5 mm wide. The current both epigastrographs generate and inject (sec 5.3.(1&2)) according to Figure 5.3 is in the range of values where no sensation of the current is expected to occur and the contact electrodes used have at least.16 mm diameter.
5.3.4 Description and Principles of the function of Mark IV

The function of the epigastrograph is based on a reference built-in resistance of 20 Ω, as Fig. 5.2 shows. The steps of the function are the following:

1) Automatic self-calibration of the system. It measures the battery's voltage and then switches current source and amplifier to reference resistor ($R_s$) and measures the voltage $V_s$ to be applied.

2) Applies the measured source voltage ($V_s$) to one pair of the electrodes connected to the object under study and switches on the amplifier to the other pairs of electrodes (also connected to the object) to measure the induced voltage difference in the object ($V_o$). This step is repeated so that all the pairs of electrodes can be used as injecting current electrodes.

3) Step 2 is repeated 5 times per second if the sampling rate is of 5 Hz or just once per second if the sampling rate is 1 Hz.

4) The built-in software calculates the impedance (resistance) $R_o$ according to Ohm's law as follows:

$$R_o=V_o/I \quad (5.7)$$
Since \( I=V/R_s \), Equation 5.7 is substituted by

\[
R_o=V_o(R_o/V_o) \quad (5.8)
\]

where, \( I \) is the intensity of the applied current. It is evident that Equation 5.8 allows the calculation of the impedance under investigation.

5.3.5 Circuitry in EIE

The epigastrograph applies and monitors an ac current to an object under investigation, which is the upper abdomen in the present study, via skin surface electrodes.

In Figure 5.4 are shown the equivalent electrical circuits between the epigastrograph and the abdomen of the body, depending on whether one or separate pairs of electrodes are used to apply the current and measure potential difference generated. The difference between the two circuits is evident. With only one pair of electrodes, both tissue and skin-electrode contact impedance are measured at the same time and can't be separated. If the measuring pair of electrodes is separate from the injecting current pair then the measured impedance is clearly due to the tissue impedance; thus, undoubtedly the use of separate pairs of electrodes for measuring is the choice.

![Fig. 5.4: Equivalent circuits (A): bipolar, (B): tetrapolar) of the abdomen and the external current source [reprod from Fen 94, p31].](image-url)
Ag/AgCl electrodes are self-adhesive and the ones chosen have a 16 mm diameter contact surface to the skin. The adhesive part's diameter is about 56 mm. Successful electrical contact with the skin is possible through the pre-gelled surface of the attached electrodes with 15 mm diameter. The gel is either in solid form or wet form.

It is evident in both circuits how significant is the skin-electrode contact impedance which is very high and frequency dependent. The frequency dependency of the skin-electrode impedance and the equivalent circuit are shown in Figure 5.5 (Jac 83). As Figure 5.5 shows the skin-electrode contact impedance at a frequency of 50 kHz is a few hundreds of Ohms, a value that was also measured within the Group.

It should be noted that a larger contact area of electrodes eases the flow of the current but abrasion of the skin is necessary.

![Fig. 5.5: Variation of the skin/electrode impedance with frequency (A) and a simple equivalent circuit (B) [reprod from Jac 83, p92].](image)

5.3.6 Testing of the new Epigastrograph.

The contact skin-electrode resistance at 50 kHz was measured by the Holtain Unit and was found to be between 178 and 286 Ohm, as expected (Rensen, 97). A model of resistors was built (by EA Worpe) to simulate the abdomen including the skin-electrode contact impedance, using as the pure abdominal resistance 27 Ohm and as
the skin-electrode contact resistance four different values (47, 100, 220 or 390 Ohm) (Fig. 5.6).

Fig. 5.6: Circuit of resistors to simulate the abdomen resistance and the contact skin/electrode resistances (Rc: skin / electrode contact resistance; Rab: abdomen resistance; colors refer to the pairs of electrodes).

The test was necessary to ensure that by increasing the applied current intensity changes occur according to Ohm's law and whether network theory in swapped pair electrodes is fulfilled. According to electric network theory, if the combination of applying-measuring electrodes is swapped, the same potential difference is generated. In fact there were problems on both matters and the manufacturers dealt with it successfully. In Figure 5.7 the behaviour of paired swapped electrodes with increasing intensity of the input current on a human arm is presented and the measured impedance differs by 2.1% in the two electrodes. The linear correlation test between current and impedance gave $R^2 = 0.90$.

It was, also, checked for stability and the fluctuation of the system over time was measured. The test was done by using the model of resistors simulating the abdominal region (Fig. 5.6). Linear fitting in the fluctuating impedance over time for each channel (Fig. 5.8) resulted in a gradient less than 0.01(Ohm/s) for all channels and indicates that the measured impedance per channel is stable over time.
Fig. 5.7: Test of paired electrodes of the epigastrograph Mark IV on a human arm with increasing input current. As the current intensity increases the measured impedance decreases. Swapped the pair shows the same impedance with 2.1% error.

Fig. 5.8: Impedance profile with the resistors simulating the abdomen (Fig. 5.6). The 6 channels show a stable mean value since the gradient of the fitted straight lines is less than 0.01. The reciprocity is well followed too.

5.3.7 Electrical field of a bulk volume

Figure 5.9 shows a cross section of the electrical field (B) generated by applying an ac current by a pair of electrodes, placed diametrically in the inner surface of a cylinder (A), filled with water to simulate the abdomen. It presents the experimental data by Fenlon (Fen 92). Of course the abdominal tissues are not homogeneous. It is
evident that the field is stronger close to the electrodes and weakens progressively with distance from the injecting point. According to these findings, any changes happening in the field would be more significant and more easily detectable near the electrodes. In practice this means that the injecting and measuring electrodes should be placed as close together as possible.
Fig. 5.9: (A) shows an electrically homogeneous cylindrical phantom of the hymen torso and (B) represents the equipotential lines of a cross section of (A) resulted by a current applied via diametrically opposed electrodes [reprod from Fen 94].
6 EXPERIMENTAL PROCEDURES AND METHODS

6.1 Introduction

Most of the data of this work comes from the clinical studies of the project “Satiety in man”. It is a joint programme between the Departments of Nutrition of the School of Biological Sciences and the Medical Physics Group in the School of Physical Sciences at the University of Surrey, the Clinical Investigation Pharmacological Unit of the Royal Surrey County Hospital and the Department of Food and Sciences Research at Reading University. It was funded by BBSRC for three years commencing October 1996. A European Commission TMR was also granted to support the work of the author for a period of one year.

The objective of the study was the investigation of the physiological mechanisms inducing satiety in humans in response to test meals controlled in total energy, carbohydrate, fat content and other nutrients. Gastric emptying, a relevant physiological mechanism, needs to be studied in respect to the feeling of satiety or hunger and to find out whether emptying rates vary significantly with the meal composition and calorific energy. Blood samples taken from the subjects during the tests were used for measurement of the peptides and hormones associated to digestion. The study also examined if intravenous infusion of nutrients and peptides affect satiety and gastric emptying rates.

Therefore, the gastric emptying test had to be simple, non-invasive and not risk inducing, such as to be repeatedly applied on the same person. As it was mentioned already, EIE seems to fulfil all the above requirements and despite its relative infancy was chosen as the most suitable method.

6.2 Materials and Methods

The setup of producing an epigatrogram is shown in the Fig.5.1 The epigastrograph is connected electrically to a human body via the skin-contact electrodes. A detailed
description of the components of the setup not including the epigastrograph itself and of the preparation of the subjects follows:

6.2.1 The skin-contact electrodes

The term electrode in bioelectricity usually means the electrode in galvanic contact with the body. It is evident that the transition from electronic to ionic conduction takes place at the interface of the electronic and ionic conductors. The electronic part of an electrode may be metallic or of carbon or polymer and the ionic part may be the electrolyte gel or the applied liquid or tissue liquid.

The electron-ion interface depends on a number of factors and it is critical feature especially when accuracy is required and the signal to noise ratio is low. It depends strongly on the surface properties (skin and contact electrode surface properties, etc), cross-sectional area and frequency. It is best to apply fields and pick-up signals from the body with non-galvanic contact with the tissue.

The Ag/AgCl electrode is one of the best electrodes in biology and medicine for current applications. It is made of silver covered by an AgCl layer, often electrolytically deposited. As the body fluids contain Cl⁻, AgCl forms a non-polarisable electrode capable of passing current with less overvoltage (polarisation) than most other types. A salt bridge is often considered for better bio-compatibility. In dc current the AgCl layer as anode will increase in thickness by the deposition of Cl⁻ and it will diminish as a cathode until it will be stripped off. This is not the case in ac current applications. The conductive electrolytic medium and the contact area of the electrodes are the major factors of the successful current application and detection and their detailed consideration is as follows:

i): Contact medium In a dry environment the outer surface layers of the stratum corneum of the skin has the lowest water content and evidently the highest impedance. Light abrasion effectively removes the surface layers and provides direct access to the deeper part of the stratum corneum with a higher water content. But even after skin abrasion an electrolyte contact medium is used between electronic conductor and skin.
A contact medium between the electronic conductor and the tissue is by definition an ionic conductor and represents a volume resistance in series with the polarisation and tissue impedance. The use of the interface contact material is necessary for the following:

- to form a high conductance salt bridge from the metal to the skin or tissue
- to enable the metal-electrolyte interface to be kept at a distance from the tissue
- to fill out spaces between an electrode plate and the tissue
- to moisten a poorly conducting skin with electrolytes
- to ensure small junction potentials
- to control the metal-electrolyte interface

Usual contact media are tissue fluids, tap water, saline or salt bridge electrolytes, gel or paste with wet electrolytes, hydrogels (solid gels) with electrolyte conductance with or without adhesive properties, ionic polymers, electric arcs and so on.

The mechanical or viscous properties of the contact medium are important and often an electrolyte is thickened with a gel substance or contained in a sponge or soft clothing. Commercially available electrodes are these days in the form of pregelled devices (electrodes) for single use. They contain preservatives to increase storage life and there is need for proper packaging and storage so as not to dry out.

Hydrogels are “solid gels” with natural or synthetic hydrocolloids. They do not wet the skin and this is advantageous in relation to wet gels, because the effective electrode area when applied to the skin is fairly constant and well defined. It has been found that hydrogels (McA 91) develop smaller parallel dc conductance and higher capacitance than the wet gels. This implies more undesirable properties for lower frequencies but more desired in higher frequencies (electro-surgery plate electrodes).

Wet electrolytes of high concentration >1% penetrate the skin actively for about 10 minutes (Tre 66, Alm 70). The penetration is stronger for higher electrolyte
concentration and it is accompanied by skin irritation. The most tolerable electrolyte by human skin is NaCl.

Skin impedance is usually much higher than electrode polarisation impedance (Gri 83) and being in series, electrode polarisation impedance can be ignored in skin applications, but not always. At higher frequencies, the series resistance of the contact medium may be a disturbance, and if the stratum corneum is highly penetrated by electrolytes the skin impedance may be so low that the electrode polarisation impedance becomes important.

Skin impedance is of great importance for both recording and current-carrying applications. The frequency band and the resolution in both space and time may depend on the skin impedance (Ged 66). Skin impedance varies on the same individual under different conditions (Alm 70, Bar 37, Gri 83, MaC 91, Ros 40). Rosell et al. after coating skin with gel found skin impedance varying from 10kΩ/cm² to 1 MΩ/cm² for a frequency of 1 Hz and about 120 kΩ/cm² for 1 MHz (Ros 83).

The conductivity of various commercially available contact mediums are given in Table 6.1 (Gri 00).

ii): Geometry The area of the electrode is quite a broad concept. There are two more specific terms: electrode area (EA) meaning the metal/electrolyte interface area, and the effective electrode area (EEA) meaning the interface area between the contact medium and the tissue. The following are related to the area of the skin-contact electrodes:

Various types of electrodes are used with different geometry configuration that is dependent on the application. There are flat skin surface electrodes (circular discs, rectangular or square plates etc.), multiple-point electrodes, concentric ring, invasive needle electrodes, microelectrodes for intracellular recordings, cellular patch clamps and so on.
Table 6.1: The conductivity of various commercially available contact electrolyte substances [Gri 00].

<table>
<thead>
<tr>
<th>Contact medium</th>
<th>Conductivity (s/m)</th>
<th>Synthesis</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode creme</td>
<td>3.3</td>
<td></td>
<td>Grass</td>
</tr>
<tr>
<td>Electrode paste</td>
<td>17.0</td>
<td></td>
<td>Beckman-Offner</td>
</tr>
<tr>
<td>NASA Flight paste</td>
<td>7.7</td>
<td>9% NaCl, 3% KCl, 3% CaCl.</td>
<td>NASA</td>
</tr>
<tr>
<td>NASA electrode creme</td>
<td>1.2</td>
<td></td>
<td>NASA</td>
</tr>
<tr>
<td>Redux creme</td>
<td>10.6</td>
<td></td>
<td>Hewlett Packard</td>
</tr>
<tr>
<td>NaCl (0.9%) by weight</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The flat surface electrodes are of interest to this work.

The most frequently used skin electrodes are the recessed types. The metal plate is at a certain distance from the tissue surface. The electrolyte solution is kept in a more or less rigid container for mechanical support and to minimise evaporation. Quite often the electrolyte solution is often supported or contained in a sponge. Streaming potentials (electrokinetics) can be disturbed if the solution is moved with respect to the metal as it can happen during the movement of the subject (human). With recessed electrodes the interface is stabilised and motion artefacts are minimised.

The effective electrode area (EEA) is the dominating factor in determining the electrode/skin impedance. It can also be much larger than the metal area in contact (EA) with the solution, which determines the polarisation impedance. The electrode is fixed with a tape ring outside the effective electrode area. If the electrolyte penetrates the tape area increases the effective electrode area but reduces the tape sticking area.

The so called solid contact gel, provides well sticky surface and electrode fixation; but it may introduce problems in high frequency applications, such as in impedance plethysmography at about 50kHz (Swa 83). The conductivity of the solid gel is lower because of the lower ionic mobility. In this situation a better solution is a thick gel.
Another feature dominated by the dimensions and relative position between the electrodes is the depth the current penetrates or gets detected. Using bipolar electrodes with the same shape and size, such as disks, the larger distance between them results to a deeper penetration or detection of the current (Fig. 6.1(B)); but given that the sensitivity is proportional to the current density, the contribution of a tissue volume in the proximity of the electrodes is more important than in deeper layers as it is shown in Figs 5.9(B) & 6.1(A,B) (Oli 95, Fen 92). The measured depth also depends on the electrode distance centres than the electrode size at the expense of course on the current density (Fig. 6.1(C)). In conclusion, the depth can be controlled with the distance between the electrodes.

The current density $J$ underneath the surface of a disk electrode with radius $\alpha$ and at a distance $r$ from the centre of the electrode is given by the Eq. 6.1:

$$J = \frac{I}{2\pi\alpha|\alpha^2 - r^2|^{1/2}} \quad (6.1) \quad \text{for applied current } I$$

It can be concluded from the above equation that the current density at the disc centre ($r = 0$) is the same as on the surface of a sphere radius $\alpha$ and at the edges of the disc ($r = \alpha$) should be very high. Figure 6.1(D) shows the diagram of the experimental data of the current density within a tissue (Gri 00).

In praxis, the sensitivity of a disk electrode as a function of the current density $J$ is higher near the circumference of the electrode.
(A): Equipotential lines in a tissue by a surface disk electrode injecting current

(B): Depth penetration of a current as a function of electrode distance.

(C): Depth penetration of a current as a function of electrode dimensions.

(D): Current density in a tissue surface layer formed by a disk electrode.

Fig. 6.1(A-D): Equipotentials in various cases in tissues [reprod Gri 2000].
6.2.2 Types of electrodes used.

In the experimental work both types of pre-gelled disposable electrodes were used: the wet pre-gelled and the solid pre-gelled (hydrogels). Details of the electrodes used and comments follow:

a) The solid pre-gelled electrodes manufactured by 3M Health Care, were the "3M Red Dot, Ag/AgCl, Micropore tape and solid gel (223950 code)" type. The geometrical configuration was: 0.9 cm radius of the contact contained hydrogel circular area (EEA), 3.0 cm radius of the whole electrode with 1.1 cm outer ring of micropore paper tape to attach the electrode on to the skin.

b) The wet pre-gelled electrodes manufactured by Niko, were the "Pregelled ECG, Ag/AgCl, 4060" type. The geometrical configuration was the same as in hydrogelled, with EEA radius of 0.85 cm, and total radius of 2.80 cm. Thus, the hydrogel electrodes were slightly wider on all aspects.

The solid gel electrodes were used because of the better defined EEA but soon showed difficulties in successful contact as the epigastrograph presented higher impedance of that expected and a few times indicated not contact at all as it was indicated automatically by the features of the epigastrograph. Thus, the wet pre-gelled electrodes were the preferred choice in subsequent measurements (Swa 83).

With the pre-gelled electrodes bad contact was also shown in 3 female subjects out of 40. After 15 minutes or so the contact was established by moving the electrode from one spot to another, because initially the problem was attributed to a mistaken position from an anatomical point of view. It can be said now that it was due to not-proper skin-electrode contact, probably because of extremely dry skin, despite the fact that the skin was treated before measurement using a non-conductive bath solution or gel which was applied by the individual.

Another incident was with a Sudanese male student, who repeatedly exhibited skin-electrode contact problems. His skin visually was extremely dry and after 15 to 20 minutes of moving around the electrodes, proper contact was achieved. The movement of the electrodes on the skin helped in establishing better blood
circulation and probably higher hydration of the skin resulting in successful skin-electrode contact as time progressed.

6.3 Electrode placement.

Based on the surface anatomy and location of the stomach (chapter 1) and in Fig. 6.2, three electrodes were placed in a triangle shape over the upper left abdominal region and the rest paired three on the back.

![Diagram of electrode placement](image)

**Fig. 6.2:** Location of the stomach and electrode placement anteriorly with respect to surface anatomy.

In detail, the desired region is the left part of the zone, formed by two planes perpendicular to the main axis of the human body. The upper plane passes through the mid-point of the costal margin and the lower lies 3 to 5 cm (as body size allows) over the umbilicus. The top front electrode touches circumferentialy the mid-point of the costal margin and it is considered to target the mid-body of the stomach (end of proximal stomach); the centre of the lower right electrode with the centre of the top electrode form an angle of 30° to 50° with the vertical and it is believed lie over the antral-pyloric region. The lower left may touch circumferentially the other two and it
is in most cases outside the region of the stomach. The back electrodes are arranged on a triangular configuration in mirroring to the mid-vertical plane of the anterior arrangement (Fig. 6.2). Given that the diameter of each electrode is 5.6 to 6.0 cm, the centres of the electrodes are at least 6.0 cm apart. The size of the electrodes for low to medium height individuals does not allow much choice the placement of the electrodes.

Despite the variety of the shape and size of the human stomach between individuals, and the different location and size within the same individual, due to posture and status of fullness of the stomach, it is believed that the placement of the electrodes mentioned above, target the stomach in most of the cases, either in a supine or semi-supine posture (proved by scintigraphy, see below).

6.4 Subjects

Subjects were healthy, young volunteers of both sexes with a Body Mass Index (BMI) preferably $\leq 25$ kg/m$^2$ with no previous gastric history. In persons with BMI $>26$ kg/m$^2$ the impedance deflection is small and it is believed that it is due to the deposited fat on the abdomen's wall. The volunteers abstain from food at least 6 hours before the test. They avoid alcohol and caffeine consumption as well as strenuous activities during the last half of the day before. Their last meal should be almost free of fat.

The subjects adopt a semi-supine position on a bed after the electrodes have been attached and are asked to remain immobile if possible during the test, because movement artefacts are induced. A base line is recorded for at least 20 min and the ingestion of the meal follows. The meal is taken using a straw in an attempt to make intake of the meal smooth and more convenient for the subject with the least movement to avoid movement artefacts.

The follow up recording of data lasts from 45 min to 2 hours according to the requirements of the specific test.
6.5 MEAL SELECTION for the gastric studies

6.5.1 Introduction

The human diet is extraordinarily varied. Therefore, it is difficult to define a physiological meal as a test meal or even to establish its limits. It is also evident that the human gastrointestinal tract handles ingested elements in different ways, adapting each time its response to the different characteristics of food consumed.

This is the question that arises: what is the proper meal (or one that is at least closest to a physiological meal) to be used as a test meal in gastric function studies? It has to fulfil a number of physical and chemical properties as well as an optimum volume in order to elicit fully the stomach’s response and simulate the physiological conditions of gastric emptying.

Generally stated, a potent test meal should be physically and chemically heterogeneous, meaning that the meal should be composed of both liquid and solid substances and should contain more than one essential nutrient, e.g. protein, fat or carbohydrate. Specific substances and ingredients in the food consumed may elicit either stimulatory or inhibitory responses. Is it feasible for an investigation to start from the most synthetic level?

The authors’ opinion is to start with the simplest conditions, and this means to keep the variables at the minimum possible number and then progressively adding variables and changing conditions to proceed to a more complicated status approaching in this way the physiological process. This means to start with simple plain meals and sequentially proceed to more complex ones. That has happened actually in the studies, each time adopting the test meal to be suitable to idio-morphic prerequisites of the investigation technique used. Thus, glucose solutions and sodium chloride solutions with various concentrations were adopted as digestible simple liquid meals, as were mashed potatoes, scrambled eggs, homogenised chicken liver or liver as solid test meals in the past.
It seems, that water (tap or de-ionised) as a test meal has been completely ignored in the past, with some exceptions, though the author believes that it should be the first to be used for study, since man can survive longer abstaining from food than from water. It is also logical to presume that water elicits the first fundamental gastric response in respect to digestion, a very significant feature for further steps. Another advantage is that water can be drunk pleasantly by everybody, not usually causing negative gastric responses.

It is only recently, that a number of gastric emptying studies with water but containing various carbohydrates with different calorific energy are being carried out with athletes (Mur 94). Generally, however it is difficult to find data on water emptying rates in the literature.

6.5.2 Properties and characterisation of the test meals in EIE

The meals should be in the form of a liquid or semi-liquid of a specific volume. The test meals controlled in total energy, carbohydrate and fat content would give more precise information about gastric function.

i): The most important additional properties with respect to EIE are conductivity and osmolarity. Meals are considered as conductive and non-conductive relatively to the soft tissue conductivity in the region of interest (i.e. abdomen/stomach). In practice meals are considered as non-conductive for $\sigma < 2.0 \text{ mS/cm}$ and as conductive for $\sigma > 8.5 \text{ mS/cm}$ at 37°C.

During the project the term "neutral" meal was introduced. According to literature and the experience gained since 1985 within the Group, the overall abdominal conductivity over the stomach's region appears to correspond to a value of $5.5\pm1.0 \text{ mS/cm}$ at 37°C. This was first proved experimentally by using two different in energy meals (chicken soup) and conductivity of about 5.5 mS/cm at 37°C.

Concluding, a meal in EIE is considered as non-conductive for $\sigma \leq 2.0 \text{ mS/cm}$, as conductive for $\sigma \geq 8.5 \text{ mS/cm}$ at 37°C, and as neutral when its conductivity is about 5.5 mS/cm at 37°C.
ii): The volume of the meal was usually 450 to 500 mL and in combination with the meal’s conductivity resulted in an impedance deflection in either direction in the range of 1.5% to 10% (Fen 94). According to the literature a volume of 700 mL of liquid test meal is considered to be the maximum acceptable introducing maximum stimulation on gastric secretion, a higher volume introduces problems.

iii): In EIE, water was chosen as a test meal since the method started (Sut 87); in addition it is distinctly non-conductive, inexpensive, and can be offered in repeatable tests even on the same day. Adding sequentially and progressively other substances, the conductivity can be manipulated leading possibly to further conclusions. By varying the quantity of one specific or more ingredients the influence of each parameter in the study, qualitatively and quantitatively, can be investigated.

In conclusion, starting with water as a test meal and formulating various meals keeping as the basic ingredient water, it becomes possible to study gastric function from its simplest form to more synthetic and complicated forms with the capability to approach physiological conditions.

According to this principle in the present studies the water test meal was followed by six milk shakes containing predefined amounts of carbohydrates and fat. Maltodextrine was used as the carbohydrate source (4 kcal/g) and double cream as source of fat (4.45 kcal/g). Glucose solutions were also used (10% in concentration) and NaCl solutions with various concentrations to achieve a specific conductivity. Towards the end, a mixture of yoghurt with maltodextrin with and without added water was also used. The composition and properties of the test meals used are presented as follows:

6.5.3 Test meals used

- Milk shakes

Six milk-shakes (A, B, C, D, E, and F) were used as test meals composed from basic and easy manipulated ingredients in order to achieve palatable taste with predefined
calorific load, CHO and fat content (Lon 00), and specific conductivity in the non-conductive area. These meals can be characterised as milk-shakes. The ingredients were maltodextrin, a simple polysaccharide, double cream as saturated fat, "Nesquick flavour" as a sweetener and for flavour, and of course water. The synthesis of the meals and physico-chemical properties of importance are given in Table 6.2. The volume of the milkshakes was kept constant to 450 mL by adding water. As Table 6.2 shows, A, B, and C milkshakes vary in CHO energy content from 36% to 78% while the absolute fat weight and energy remain constant; the D, E, F milkshakes vary on fat energy from 11% to 60% while the absolute CHO weight and energy remain constant. The conductivity of the meals varies slightly but remains well in the non-conductive region in respect to the soft tissue conductivity. The osmolarity covers a broad range from hypo- to slightly hyper-osmolar meals. In this way conclusions on gastric function may be drawn in respect to carbohydrate and fat content as well as osmolarity.

- Glucose solution: Glucose solution 10% by weight was also used as a non-conductive test meal of 450 mL volume and 755 kJ calorific energy, pH=6.27, conductivity=0.34 mS/cm and osmolality=126 mosmol/L. The glucose meal can be considered comparable to A and D meals in respect to energy.

-Saline solutions as test meals: The main properties of these meals were their conductivity and osmolarity. NaCl solutions were used of conductivity 14.0 mS/cm and osmolarity 310 mosmol/L, or alternatively the D and F milkshake with added salt and of 5.5 mS/cm conductivity and of osmolarity 520 mosmol/L and 525 mosmol/L respectively. Of course these meals were quite unpleasant for the subjects. The meal volume was kept the same (450 mL). The properties of the sterile physiological saline, 0.9% (w/v) concentration of NaCl, were measured as a reference if needed: cond:13.22 mS/cm, pH:6.11, and osmolarity:296 mosmol/L.

-Yoghurt meals: In the end it was decided to also use yoghurt based test meals in order to test the protein behaviour on epigastric impedance as well as the volume of the meal.
<table>
<thead>
<tr>
<th>MEALS</th>
<th>INGREDIENTS</th>
<th>Flavour</th>
<th>Total Energy (kJ)</th>
<th>Adjusted Energy (kJ)</th>
<th>Conductivity (mS/cm)</th>
<th>Osmolarity (mosmol/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>Double Cream</td>
<td>167</td>
<td>46</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>5.79</td>
</tr>
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<td></td>
<td></td>
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<td>46</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>5.62</td>
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<td></td>
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<td>200</td>
<td>30</td>
<td>200</td>
<td>5.87</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>200</td>
<td>30</td>
<td>200</td>
<td>5.37</td>
</tr>
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<td></td>
<td></td>
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<td>200</td>
<td>30</td>
<td>200</td>
<td>5.57</td>
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<tr>
<td></td>
<td></td>
<td>284</td>
<td>20</td>
<td>200</td>
<td>30</td>
<td>200</td>
<td>4.90</td>
</tr>
</tbody>
</table>

Table 6.2: Composition and physico-chemical properties of milk-shakes used as test meals.
One of the yoghurt meals was a mixture of 150 g of “Greek style yoghurt”, (Sainsbury’s) with 50 g of maltodextrin plus water making a total volume of 350 mL. The conductivity was in the non-conductive region (2.0±0.2 mS/cm) and a pH of 3.4 and osmolarity of 368 mosmol/L.

The other yoghurt meal was composed of the same type yoghurt (240 g) and maltodextrin (200 g) and a conductivity of 0.50 mS/cm was achieved of total energy 4577 kJ and volume of 435 mL.

-Water: Tap water of conductivity 0.45 mS/cm, pH:7.13, and osmolarity of 5 mosmol/L. Mineral (Evian) still water with cond:0.48 mS/cm, pH:7.02, and osmolarity of 7 mosmol/L.

All meals were given at room temperature (20°C to 22°C) and their physical properties were measured at room temperature too.

6.6 Presentation and interpretation of the data

The study can be considered as a dynamic study since the sampling rate is 1 Hz or 5 Hz. The study period can be separated in three phases: the fasting or pre-prandial phase, the second, very short, filling (or ingestion) phase of the meal and the post-prandial or emptying or digestion phase. The shape of the response of the epigastrograph in EIE depends on the meal's conductivity. The three distinctive responses are as follows:

6.6.1 Response to a meal

i) Non-conductive meal

If the empty stomach is filled out with a liquid load of 450 mL of conductivity considerably lower than the overall conductivity of the fasting gastric abdominal region, an increase in the region’s impedance follows, which is proportional to the ingested volume (Fen 94, McC 85, Sut 87, Baz 86, Cas 87, Rum 97, Tsa 94). As the meal is leaving the stomach and the non-conductive substance is becoming less and
less the impedance starts decreasing until it reaches the pre-prandial value and at this moment it is considered that the stomach has emptied.

Figure 6.3 based on experimental data, shows the three distinctive phases of a non-conductive meal in the stomach as described above.

ii) Conductive meal

The opposite happens if the meal is conductive, as clearly shown in Fig. 6.4.

If the same person goes through the test with both meals (conductive and non-conductive) on separate occasions, there is a remarkable symmetry in the shape of the ingestion of the meal period and later on, (graphs relatively to the pre-prandial period.

![Figure 6.3: Variation of epigastric impedance over time from the fasting state to 1 hour post-prandially with a non-conductive meal.](image)

iii) Neutral meal

For the first time the so called neutral meal was proposed by the author and tested. During the ingesting phase the meal is not visible in the sense that its conductivity is the same with the environment’s conductivity, into which it was introduced. Later a decrease in the impedance starts until a post-prandial base line is established.
Fig. 6.4: Epigastric impedance variation over time from the fasting state to 1 hour post-prandially with a conductive meal

Fig. 6.5: Variation of the epigastric impedance over time from fasting state to 1 hour post-prandially with a neutral meal.

It is the rule, that at the moment the initial quantity of a conductive and non-conductive meal reaches the stomach at the same moment an impedance deflection
starts developing and the highest value of the non-conductive meal and the lowest with the conductive meal coincide with the last portion of the meal ingested.

6.6.2 Artefacts

The measurements of the epigastric impedance are influenced considerably by various systematic artefacts due to respiration, heart motion and random artefacts due to subject’s movements, coughing, yawning, talking etc.

The width of the epigastric impedance profiles is due to breathing and it is called respiratory artefact. The upper values correspond to the end of inhalation, because the interposed layer of the inhaled air raises the impedance, and the lower values at the end of the exhalation of the air, which results to lower impedance values. Thus, it is believed that the lower impedance values represent the epigastric impedance without the breathing artefact. Applying a mathematical procedure to retain only the lower values of the data, the breathing artefact can be eliminated (Fig. 6.6). This, also, acts as a filter for the breathing frequency.

Artefacts appear, also, due to movements of the subject, due to coughing, talking (Fig. 6.7), yawning even to psychological stress or nervousness.

A drifting in the pre-prandial base line appeared several times either upwards or downwards (Fig. 6.6) and it is believed that it is due to a drifting of the skin-electrode contact impedance over time and probably to an electronic drifting.

It is possible that drifting to lower impedance value is due also to rising skin temperature at the site of skin-electrode contact. Studies showed that tissue impedance decreases linearly with temperature by 2% / °C (Ama 88; Con 87).

There is always a mathematical solution in dealing with artefacts and smoothing the data, without losing a lot of information such as running averages (Fig. 6.7), the already mentioned “detecting the minima” (Fig. 6.6 ) or fitting directly a mathematical model, exponential (Fig. 6.7) or power exponential or linear (Ela 82). Until EIE is evaluated and well established the analysis of the raw data should be given priority.
Fig. 6.6: Epigastric impedance profile using meal D including the breathing artefact (purple - raw data) or without breathing artefact (dark blue - keeping the lower envelope values).

Fig. 6.7: Post-prandial profile with meal F using raw data (blue) or smoothing the raw data with running averages (red) or using exponential fitting (green).
6.7 Data information

6.7.1 Half Emptying Time (T50)

The epigastrograph, as mentioned earlier, was designed to study gastric emptying. Thus, the objective is to calculate emptying rates and Half Emptying Time (T50). Indeed, the electrical impedance data from the EIE do allow the calculation of the T50 parameter, thought until now to represent the Half Emptying Time (HET). The T50 is a broadly used parameter, it can be easily calculated and it is well known in the medical area. It can be used to define the ranges of normality in various applications.

![Fig. 6.8: Representation of the calculation of the T50 of a non-conductive test meal (water) from the raw data based on the definition.](image)

The T50 parameter can be defined as follows: T50 is the time needed for the magnitude of a quantity to be eliminated to half of its maximum value. Based on the definition it can be calculated from the raw data (Fig. 6.8) and also from a mathematical model it follows on the curve, as it happens with non-conductive meals (Fig. 6.7).
If the model is well established and the duration of the test is long, then the T50 can be predicted from the mathematical model and the running of the experiment can be shorter.

6.7.2 Periodicity

If the recorded data are observed over short time periods, for example for a minute, two main periodical components can be seen. One has a periodicity of about 14 to 18 cycles per minute, which corresponds to breathing frequency, and the other 1.2 to 5 cycles per minute, corresponding to the stomach’s mechanical activity. Therefore by applying methods of signal analysis it is possible to study the gastric motility and how it is related with the breathing component.

In conclusion, the data EIE generates include information about gastric content (volume, conductivity) and gastric motility.

Fig. 6.9: The periodicity of the epigastric impedance of a fasting stomach. It is striking the 3 cpm periodicity and a higher is evident due to respiration.
7 EXPERIMENTAL STUDIES

7.1 Introduction

Several complete series of gastric function studies were conducted using electrical impedance measurements. They can be divided in simple gastric function tests using simply a test meal and in clinical gastric function studies. In the clinical studies blood samples were withdrawn from the subjects during the test in order peptides and hormones to be measured. The clinical studies can be divided in 3 subdivisions:

- water studies
- simple satiety-clinical studies
- comparison studies
- satiety-gastric emptying studies with intravenous infusion of peptides
- satiety-gastric emptying studies using test meal with potent substances in regulating satiety.

7.2 Water studies

7.2.1 Introduction

Water is the most abundant substance in the human body (>70%) and it is a constituent in most of foods even solid ones. Human beings can survive longer abstaining completely from food than from water, otherwise stated, life is associated with water.

In the past, water was completely ignored in gastric function studies for two main reasons: first it is the simplest form of food and it may not elicit the full stomach function response, and second because of the difficulty and limitation of the methods to view the water inside the stomach (however this is not true for scintigraphy and magnetic resonance imaging). Thus, there is a lack of water data in gastric function.
But during the last decade water has begun to be used as a control test meal in respect to calorific test drinks in studies with athletes on action, for hydration and supplementation of energy (Gis 98, Mur 94, Noa 91, Zac 91). In food and nutrition studies as well as in various pathological conditions, scientists, have also started recently investigations with water as a test meal, especially after the development and application of MRI, ultrasonography and "breath test methods" (Leo 99, Pou 97, Bor 99, Vis 94, Web 97).

7.2.2 Water as test meal in EIE:

Water, as tap or mineral, was the test meal of choice in EIE and electrical impedance tomography since they started in the early 80s and was used by the Medical Physics Group in the Physics Department of the University of Surrey, because of the lower conductivity (<0.5mS/cm) compared to that of human tissues (5.5 mS/cm) (Sut 87, Bale 94, Fen 92).

In this work, water has been used as the test meal for various purposes: for gastric emptying studies, for testing the new model Mark IV epigastrograph as well as for screening the volunteers' suitability as subjects for our investigations. Since 1996 seven separate water studies have been conducted and the details and results of these studies follow:

A typical profile of the epigastric impedance using water as test meal is presented in figure 7.1.

7.2.2.1 February 1996

In this study, thirteen healthy students (subjects), 7 males and 6 females, aged between of 22 and 26 years, were given 400 mL of tap water of conductivity 0.55±0.05 mS/cm. They were advised to have retain an empty stomach for 6 hours before the test, avoid fat in their last dinner and abstain from coffee, alcohol, smoking, aspirin or paracetamol and strenuous activities since the afternoon of the day before the test. Their posture during the test was semi-supine and a baseline was recorded for a period greater than 10 minutes. Then they were given the water and asked to drink it through a straw, continuously, if possible, with minimum movements, since the method is sensitive to motion artefacts.
Their anthropometric characteristics and calculated T50s in minutes are presented in the Table 7.1. Two of the subjects, one male (S2) and one female (S10), both of Greek nationality, failed to generate an interpretable signal over time, despite normal body parameters. Subject (S13), female with BMI almost 25 kg/m² also failed to generate interpretable data. Channel 1 and 2 resulted in average T50s (7.4±1.2 minutes and 8.4±1.4 minutes) with significant difference (2-tail t-paired test: p=0.03). The averaged value of both channels is 8.0±1.4 minutes.

Channel 1 reflects the impedance mostly from the proximal stomach and channel 2 from the distal stomach. Later an attempt was made to explain this systematic difference.

It should be noted that in addition to the two subjects of Greek nationality, who failed to produce interpretable bio-signals over time, three subjects, also from Greece, produced one interpretable data set, instead of two.
Table 7.1: T50 in minutes of a 450 mL water load as test meal and the anthropometric characteristics of the subjects (February 96).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Ht (cm)</th>
<th>Wt (kg)</th>
<th>Wst (cm)</th>
<th>BMI (kg/m²)</th>
<th>T50c1,i (min)</th>
<th>T50c2 (min)</th>
<th>T50 (min) ave</th>
<th>T50 (min) stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>M</td>
<td>26.0</td>
<td>178.0</td>
<td>70.0</td>
<td>22.1</td>
<td>9.7</td>
<td>8.5</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>M</td>
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</tr>
<tr>
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<td>75.0</td>
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<td>60.0</td>
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<td>8.7</td>
<td>9.8</td>
<td>9.3</td>
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</tr>
<tr>
<td>S8</td>
<td>F</td>
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<td>166.0</td>
<td>59.0</td>
<td>70.0</td>
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7.2.2.2 Summer 1997:

In this study 16 healthy volunteers participated, 12 males and 6 females (age range: 22-29 yrs, height: 160-188 cm, BMI: 24.0±4.6 kg/m²). The conditions for the tests were the same as in study 1, but they were given 450 mL of tap water (cond: 0.5±0.05 mS/cm) and they underwent through 2 tests at least at different days, one with the then existing model of the epigastrograph and the other with the new model Mark IV. The aim of this study was to compare the data obtained by the two models of the epigastrographs.

The details of the volunteers characteristics and the T50 obtained are presented in Table 7.2.
Table 7.2: The T50s and anthropometric characteristics of subjects with both models circa and Mark IV (Summer 1997)

<table>
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<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Ht (cm)</th>
<th>Wt (kg)</th>
<th>BMI (kg/m²)</th>
<th>Wst (cm)</th>
<th>Model circa</th>
<th>Model MARK IV</th>
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<td>T50 (min) - Test 2</td>
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<td>4.62</td>
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<td>2.16</td>
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</table>
As it becomes clear from the Table 7.2, three volunteers with BMI>28 kg/m² failed to produce the expected bio-signal, except for number 12, who gave the expected one with the new model. The signals of these 3 volunteers will be discussed in the chapter on *Interpretation of data*.

It is remarkable that the T50s with the 2 channels are significantly different with the Student’s paired t-test (except the second water test), but not with the rank test. As a first observation the T50s of the test water number 2 seem to be the shortest, but the t-test does not show any statistically significant difference (p values (0.18 and 0.13).

The overall conclusion is that the T50s differ significantly for the same subject in the two channels, being always longer for channel 2. This corresponds to the distal stomach. Both, the existing model and the new Mark IV resulted in T50s without any significant difference (see Table 7.2).

Subject (S16), from Greece, failed to produce any interpretable data on two occasions despite normal body parameters.

7.2.2.3 *February 1998:*

In this study twenty-three healthy students, 16 males and 7 females, went through a water test under conditions similar to those in the above described water studies. Their biological characteristics and the calculated T50s are shown in detail in Table 7.3.

According to Table 7.3, four Greek males and one Greek female failed the test, despite the fact that BMI was lower than 25 kg/m². Three males and one female with BMI>28 kg/m² failed, also. One obese male with BMI=33.6 kg/m² gave interpretable data from channel 1.

The Student’s t-test showed that there isn’t any significant difference between the two channels but the paired t-test showed a significant difference between the T50s (p=0.006) as in studies 7.2.2.(1-2) did. This supports the hypothesis that the two channels reflect two different parts of the stomach, but this is still under investigation.
Table 7.3: T50 in minutes of 450 mL water as test meal and the anthropometric characteristics of the subjects (February 98).

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<th>Sex</th>
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<th>Wt (kg)</th>
<th>Wst (cm)</th>
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7.2.2.4 December 1996 & December 1997:

The water tests in these studies were used as screening test of the suitability of the subjects for the EIE method in order to participate in the clinical tests of the project “Satiety in man” funded by the BBSRC (see earlier). Of course, the first screening was based on their anthropomorphic characteristics and specifically subjects had to have a BMI of less than 25 kg/m².

These subjects were also asked to abstain from coffee, alcohol, aspirin and strenuous activities since the afternoon the day before. They were not allowed to have water or breakfast, of course, on the morning of the day of the test. The characteristics and calculated T50s of the subjects who would participate in the Satiety project looking at gastric emptying with meals varying in carbohydrate, (CHO content) are presented in Table 7.4, and those for the study with meals varying in fat content on Table 7.5.

Table 7.4: The anthropometric characteristics of subjects and the T50s from the water screening test in the study with meals varied in CHO content.

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<th>Wt (kg)</th>
<th>Wst (cm)</th>
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Table 7.5: Anthropometric characteristics of the subjects and the T50s from the water screening test in the study with meals varied in fat content.

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<th>ch-1 (min)</th>
<th>ch-2 (min)</th>
<th>ave (min)</th>
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<td>M</td>
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<td>76.5</td>
<td>25.0</td>
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</tr>
<tr>
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<td>M</td>
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<td>76.0</td>
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<td>6.2</td>
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</tr>
<tr>
<td>S5</td>
<td>M</td>
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<td>71.5</td>
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<td>7.0</td>
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<td>F</td>
<td>168.0</td>
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<td>F</td>
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<td>2.8</td>
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<tr>
<td>S8</td>
<td>F</td>
<td>172.0</td>
<td>62.0</td>
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<td>7.8</td>
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<td>S9</td>
<td>F</td>
<td>168.0</td>
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<td>S10</td>
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<tr>
<td>ave</td>
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<tr>
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<td>1.6</td>
<td>1.0</td>
<td>1.7</td>
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</tr>
</tbody>
</table>

As it is clear from the Tables 7.4 & 7.5, the T50s for the same subject differ significantly in the two channels and again the T50 of channel 2 is longer in both studies. In both studies two subjects did not produce interpretable data and from two others the data were not recorded or partially recorded. In subsequent tests the subjects who resulted in both channels with good quality data did very well, those who produced data only in one channel of recording did not always give good quality data. Subject number 8 in Table 7.4 was excluded.

Finally, it should be noted once again that there is a significant difference between the two channels on the same subject.

7.2.2.5 June 1999:

Another water emptying study as screening for the selection of suitable subjects for the study of the Satiety project in which satiety was investigated under intravenous infusion of loxiglumide. This time, subjects were asked to have as breakfast, two hours before the test, 50g of rice krispies with 300mL of skimmed milk, which
corresponds to 0.8g of fat, 60g of CHO and 13g of protein, giving a total energy of 1233 kJ. The tests this time were conducted with the model Mark IV. Sixteen volunteers participated in this screening study of mixed sexes and the details are presented in Table 7.6 including T50s. Generally, all subjects had a successful test, except number 13. The case will be discussed later. No significant differences in the T50s from different channels of the same subject were obtained in this study.

Table 7.6: Anthropometric characteristics of the subjects and the T50s with water in the loxiglumide study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Ht(m)</th>
<th>Wt(kg)</th>
<th>BMI(kg/m²)</th>
<th>ch-1</th>
<th>ch-2</th>
<th>ch-3</th>
<th>ch-ave</th>
<th>stdev</th>
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<td>S2</td>
<td>M</td>
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<td>63.0</td>
<td>21.0</td>
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<td>5.6</td>
<td>5.9</td>
<td>0.4</td>
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<tr>
<td>S3</td>
<td>M</td>
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<td>76.0</td>
<td>24.0</td>
<td>5.9</td>
<td>6.5</td>
<td>6.5</td>
<td>0.3</td>
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</tr>
<tr>
<td>S4</td>
<td>M</td>
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<td>8.9</td>
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<td></td>
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<td>8.1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ave</td>
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<td>1.8</td>
<td>70.7</td>
<td>22.5</td>
<td>8.1</td>
<td>8.2</td>
<td>7.8</td>
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</tr>
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<td>1.7</td>
<td>1.5</td>
<td>2.5</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7.2.2.6 October 1999:

The data of water are presented in Table 7.7. This study was not a screening one, but the initiation of a study looking at the effect on emptying rates after oral ingestion of aspartame or free amino acid in a gelatine capsule before the test meal, with 450 mL
water. Volunteers were selected on the basis of BMI. In the water non-fizzy paracetamol (1.5 g) was diluted to be used for the measure of the gastric emptying on the same time. The volunteers had been asked to be prepared as in the previous studies, but they were asked to drink no more than 1/3 of a glass of water 1.5 hours before the test, in order to avoid fainting. The T50s were calculated on 25 minutes post-prandial baseline, despite that the data were recorded for one hour, in order to be comparable with the water data of the previous mentioned studies. Each subject was tested 3 times taking with the water a gelatine capsule with corn-flour as placebo or aspartame or amino-acid. The details of the volunteers, (six in total), and the T50s averaged channel, are presented in Table 7.7. Student’s t-tests, 2-tailed, 2 sample and paired, failed to show significant differences between the T50s, suggesting that the water is processed in the same way with the three different orally ingested substances.

Table 7.7: Anthropometric characteristics of subjects and T50s with 450 mL water with paracetamol and aspartame or amino acids against corn flour.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Ht (m)</th>
<th>Wt (kg)</th>
<th>BMI (kg/m²)</th>
<th>control</th>
<th>aspartame</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
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<td>S1</td>
<td>M</td>
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<td>168.0</td>
<td>62.0</td>
<td>22.0</td>
<td>12.5</td>
<td>11.3</td>
<td>11.5</td>
</tr>
<tr>
<td>S2</td>
<td>M</td>
<td>24.0</td>
<td>184.0</td>
<td>70.0</td>
<td>20.7</td>
<td>7.3</td>
<td>9.2</td>
<td>10.0</td>
</tr>
<tr>
<td>S3</td>
<td>F</td>
<td>28.0</td>
<td>171.0</td>
<td>62.0</td>
<td>21.2</td>
<td>13.2</td>
<td>10.1</td>
<td>15.1</td>
</tr>
<tr>
<td>S4</td>
<td>F</td>
<td>31.0</td>
<td>165.0</td>
<td>49.0</td>
<td>18.0</td>
<td>10.0</td>
<td>9.7</td>
<td>8.5</td>
</tr>
<tr>
<td>S5</td>
<td>F</td>
<td>31.0</td>
<td>170.0</td>
<td>57.0</td>
<td>19.7</td>
<td>8.1</td>
<td>4.3</td>
<td>6.8</td>
</tr>
<tr>
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<td>F</td>
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<td>169.0</td>
<td>54.0</td>
<td>18.9</td>
<td>12.0</td>
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<td>12.5</td>
</tr>
<tr>
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<td>27.7</td>
<td>171.2</td>
<td>59.0</td>
<td>20.1</td>
<td>10.5</td>
<td>10.8</td>
<td>10.7</td>
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<tr>
<td>stdev</td>
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<td>6.6</td>
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<td>2.4</td>
<td>5.1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

7.2.2.7 Discussion and conclusions on all water tests

A general comparison between the T50s of the presented water studies follows by combining certain studies together. The results of the combined studies are shown in Table 7.8.
Table 7.8: Averaged T50s of grouped water studies.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>7.2.2.(1-3)</th>
<th>7.2.2.4 (B)</th>
<th>7.2.2.5 (C)</th>
<th>7.2.2.6 (D)</th>
<th>7.2.2.(1-5) MALE (E)</th>
<th>7.2.2.(1-5) FEMALE (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T50 (min)</td>
<td>8.5±1.7</td>
<td>6.8±1.7</td>
<td>8.0±1.6</td>
<td>10.2±2.8</td>
<td>7.6±1.5</td>
<td>8.7±2.1</td>
</tr>
</tbody>
</table>

The T50 parameter was found significantly shorter (p=0.003, t-test) in the subjects participating in study (b) with respect to study (a). Subjects in study (b) were better prepared; they were advised to have a specific, fat free meal, the night before and the test was at 9:00 o'clock in the morning. The T50 given by study (c) was significantly longer (p=0.056) than is study (b), as well. This can be attributed to the specific breakfast the subjects were asked to have in study (c). Subjects showed same T50s in (a) and (c) studies (p=0.253).

Water study (d) resulted in longer T50s compared to all other studies (p(d,c) =0.031, p(d,b)=0.0025, p(d,a)=0.038). The 1.5g of paracetamol added in the water as well as the gelatine capsule containing 400mg of corn flour or aspartame or phenylalanine and aspartic acid, prolonged significantly the T50s. These substances do not have any calorific content but with their mass and chemical nature probably affect directly by contact the gastric mucosa (i.e. G-cells) such as to inhibit initially gastric secretions and gastric emptying.

Another thought is if paracetamole has any buffering capacity, such as to reduce the ionic gastric concentration initially and as a result to record impedance changes at a slower rate.

The averaged T50 obtained from all water studies except study (d) of male subjects (43 in number) was 7.6±1.5 minutes and the T50 of female subjects (23 in number) was 8.7±2.1 minutes; the values differ significantly (p=0.016). Thus, the gastric T50s using electrical impedance measurements, depend on sex, with longer T50s in females. The results of study (d) were excluded because of the different conditions of the test.

The T50s of the subjects who had the same light breakfast two hours before the test (c) and the T50s of the other subjects who were with empty stomach don't show significant difference (p=0.963).
In conclusion, the T50s from 66 (23 females and 43 males) healthy young volunteers with a mean age of 25.1±2.8 years over a period of 3.5 years based on epigastric impedance measurements was calculated and found to be 8.0±1.8 minutes (mean±stddev) with 2.8% error. The test lasted 25 minutes post-prandially and the calculations were based on the same time. The narrow band of the T50s with an error of 2.8% in a large number of volunteers in a period of 3.5 years, shows that impedance measurements reflect reliably the gastric changes due to gastric function when a water load of 450 mL is used as a test meal. If the conditions of the test differ it is reflected in the T50s with statistical significance. This is a reassuring conclusion, regarding the reliability of the results obtained using the epigastrograph.

Statistical tests for correlation between the T50s and the anthropomorphic characteristics of the volunteers such as weight, height, waist and BMI showed no correlation.

7.3 Simple satiety clinical studies

7.3.1 Milk-shakes varying in CHO content (A,B,C)

The study funded by BBSRC designed to investigate the effect of the meal energy and carbohydrate (CHO) content on gastric emptying (GE) and satiety while measuring glucagon-like-peptide one (GLP-1) gastric inhibitory peptide (GIP) in blood samples, taken during the study in certain time intervals.

Nine male healthy volunteers 23 to 28 years old with BMI between 20 and 25 kg/m² participated in three occasions. After an overnight fast and a standard meal the night before they were given one of the three A, B and C non-conductive (0.2 to 0.4 mS/cm) milk shakes at each occasion. The ingredients of the meals and other characteristics are given in Table 6.2.

In this study the old epigastrograph was used and a typical epigastric impedance signal is presented in Fig. 7.2.
The T-50s were calculated based on the definition from both channels of the epigastrograph. The percentages were calculated as well of the post-prandial impedance changes over to the maximum post-prandial deflection, every 15 minutes from the ingestion time of the meal. The moment the post-prandial baseline was established it was assumed that the stomach was emptied and for the calculation it was considered that 5% of the meal was still in the stomach.

The anthropomorphic characteristics of the subjects and the calculated percentages and T50s are given in Tables 7.9 & 7.10 respectively.
Table 7.9: Anthropometric characteristics of the subjects in the satiety study with meals varied in CHO content.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Wt (kg)</th>
<th>Ht (cm)</th>
<th>Wst (cm)</th>
<th>BMI (kg/m²)</th>
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</thead>
<tbody>
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<td>S1</td>
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<td>180.0</td>
<td>87.0</td>
<td>21.9</td>
</tr>
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<td>24.0</td>
<td>M</td>
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<td>172.0</td>
<td>80.0</td>
<td>20.8</td>
</tr>
<tr>
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<td>M</td>
<td>66.5</td>
<td>177.0</td>
<td>78.0</td>
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</tr>
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<td>180.0</td>
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<td>23.1</td>
</tr>
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<td>M</td>
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<td>179.0</td>
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<td>175.0</td>
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<tr>
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<td>85.0</td>
<td>25.1</td>
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Table 7.10: The T50s and percentages of the post-prandial impedance deflection from the satiety study with meals varied in CHO content.

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<th>Meal (A) -- Channel-2</th>
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</tr>
<tr>
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<td>65</td>
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<tr>
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<td>66</td>
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<td>72</td>
</tr>
<tr>
<td>S7</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>S8</td>
<td>100</td>
<td>64</td>
</tr>
<tr>
<td>S9</td>
<td>100</td>
<td>62</td>
</tr>
<tr>
<td>Ave</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>Stdev</td>
<td>0</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Using the Student’s t-test, statistical significant differences were found between the T-50s of the meals varying in CHO content and total energy. Significant difference was also found between the percentages of meal remaining in the stomach at 15min intervals. Student’s paired t-test also showed a strong significant difference.

A correlation was found between the T50s and the total area under the curve of the circulating GLP-1 (Lon 2000). The higher value of GLP-1 as the CHO content increases show inhibition in gastric emptying and gastric acid secretion (Anv 98, Gut 99, Nau 97). Thus the longer T50s and their correlation with the plasma GLP-1 as the CHO content increases, show a delay in gastric function, which is in accordance to the literature.

It can be concluded that the T50s based on epigastric impedance differ significantly if test meals varying by 850 kJ because of the CHO content are used.

<table>
<thead>
<tr>
<th>t(min) / subj</th>
<th>Meal (C) -- Channel-1</th>
<th>Meal (C) -- Channel-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  15  30  45  60  75  90</td>
<td>0.2  15  30  45  60  75  90</td>
</tr>
<tr>
<td>S1</td>
<td>100  75  55  33  22  12  8</td>
<td>33.5</td>
</tr>
<tr>
<td>S2</td>
<td>100  83  55  35  20  13  8</td>
<td>33.8</td>
</tr>
<tr>
<td>S3</td>
<td>100  71  44  31  20  14  9</td>
<td>26.8</td>
</tr>
<tr>
<td>S4</td>
<td>100  73  56  28  21  12  6</td>
<td>33.9</td>
</tr>
<tr>
<td>S5</td>
<td>100  83  58  38  22  12  9</td>
<td>35.7</td>
</tr>
<tr>
<td>S6</td>
<td>100  77  45  31  16  11  7</td>
<td>34.5</td>
</tr>
<tr>
<td>S7</td>
<td>100  77  50  25  18  12  10</td>
<td>26.5</td>
</tr>
<tr>
<td>S8</td>
<td>100  75  41  26  21  19  16</td>
<td>26.5</td>
</tr>
<tr>
<td>S9</td>
<td>100  81  61  44  30  15  3</td>
<td>40.4</td>
</tr>
<tr>
<td>Ave</td>
<td>100  77  52  32  21  13  9</td>
<td>32</td>
</tr>
<tr>
<td>Stdev</td>
<td>0  4.3  6.9  6.0  3.9  2.5  3.5  4.8</td>
<td>0  7.6  11.1  10.8  8.5  5.3  4.5  8.8</td>
</tr>
</tbody>
</table>

128
Exponential fitting on the post-prandial percentages is highly acceptable since the correlation coefficient is higher than 0.85, but the tendency is to result in shorter T-50s. Figure 7.3, represents graphically the average of the post-prandial percentages presented in Table 7.10, as well as the exponential fitting (yellow color). The calculated T50s from the exponential fitting are shorter but the regression coefficient above 0.95 (19.3 vs 18.6 min for A, 22.3 vs 27.0 min for B and 25.8 vs 33.6 min for C).

Fig. 7.3 : The averaged percentages (9 subjects) of the epigastric impedance post-prandially and the exponential fitting with A, B, and C test meals.

A significant general comment of this study is that the resulting T50s are considerably shorter than those in the literature and they may not represent half emptying times.

7.3.2 Milk-shakes varying in fat content

In this study 10 healthy volunteers (5 male and 5 female) participated following the procedure mentioned in the previous study (7.3.1). They were given the D, E and F milk-shakes varying in total energy because of varying fat content. The range of the conductivity was from 0.25 to 0.40 mS/cm (Table 6.2). The volume of the meals was the same i.e. 450 mL.
The results from the analysis of the data as in study (7.3.1) are presented in Table 7.11 and Fig. 7.4. The T50s in minutes are considerably shorter than those expected based on the existing knowledge. According to that the T50s may not represent directly gastric emptying rates.

**Table 7.11**: T50s and percentages of the post-prandial impedance deflection in the satiety study with meals varied in fat.

<table>
<thead>
<tr>
<th>Sbj / t(min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>T50 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
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<td>59.0</td>
<td>32.0</td>
<td>19.0</td>
<td>15.0</td>
<td>7.0</td>
<td>0.0</td>
<td>19.6</td>
</tr>
<tr>
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<td>46.0</td>
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<td>0.0</td>
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</tr>
<tr>
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<td>2.0</td>
<td>0.0</td>
<td>18.5</td>
</tr>
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<td>16.8</td>
</tr>
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<td>15.0</td>
<td>8.0</td>
<td>2.0</td>
<td>17.2</td>
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<td>0.0</td>
<td>18.4</td>
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<td>5.5</td>
<td>2.5</td>
<td>0.0</td>
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<td>3.5</td>
<td>0.0</td>
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<tr>
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<table>
<thead>
<tr>
<th>Sbj / t(min)</th>
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<th>60</th>
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<td>45.0</td>
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<td>19.0</td>
<td>5.0</td>
<td>2.5</td>
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continuation of Table 7.11

<table>
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<td>16.0</td>
<td>11.0</td>
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<tr>
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<td>6.0</td>
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<td>27.0</td>
<td>15.0</td>
<td>7.0</td>
<td>40.4</td>
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<td>57.0</td>
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<td>17.0</td>
<td>6.0</td>
<td>3.5</td>
<td>39.4</td>
</tr>
<tr>
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<td>79.0</td>
<td>53.0</td>
<td>34.0</td>
<td>19.0</td>
<td>7.0</td>
<td>4.0</td>
<td>38.8</td>
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<td>7.5</td>
<td>3.5</td>
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<tr>
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<td>21.0</td>
<td>10.0</td>
<td>4.5</td>
<td>42.5</td>
</tr>
<tr>
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<td>97.0</td>
<td>65.0</td>
<td>50.0</td>
<td>31.0</td>
<td>19.0</td>
<td>9.0</td>
<td>39.8</td>
</tr>
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</tr>
<tr>
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<td>11.2</td>
<td>7.6</td>
<td>7.2</td>
<td>5.0</td>
<td>2.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Fig. 7.4**: Impedance deflection (averaged %) post-prandially with the milk-shakes D, E, and F with different fat content.

A comparison of the T50s of the results of studies 7.3. (1&2) showed that the T50s depend on the total energy and on the fat content (Table 7.12.). In particular, the high energy meals showed a significant statistical difference with longer T50s on the high
fat content meal (38.7±4.8 minutes in F versus 33.5±5.9 minutes in C, p=0.04, 2-tailed t-test).

B and E meals are similar in their formulation and it was expected that similar results would be obtained as it happened indeed (p=0.85, 2-tailed t-test).

In A and D meals the effect of CHO or fat is difficult to be distinguished due to their proportionality (A with 36% CHO and 64% fat, D with 89% CHO and 11% fat). They resulted in a value of 18.8±0.9 minutes with meal A versus 18.7±1.1 minutes with meal D (p=0.94; 2-tailed t-test). The effect is somehow masked. It would be probably worth in trying the proportionality 11% in CHO and 89% in fat to study the fat effect and vice versa for the CHO effect.

Meals B and E being similar resulted to 27.0±2.9 and 27.3±4.6 minutes respectively which are similar and the two sample, 2-tailed, t-test resulted to p=0.85.

Table 7.12: Summary of the results and statistical significance of studies 7.3

<table>
<thead>
<tr>
<th>MEAL</th>
<th>ENERGY (KJ)</th>
<th>T50(MINUTES) (AVE±STDEV)</th>
<th>2-TAILED, T-TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>18.8±4.3</td>
<td>(A,B) p&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>1670</td>
<td>27.0±2.9</td>
<td>(A,C) p&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>2500</td>
<td>33.4±5.9</td>
<td>(A,D) p=0.94</td>
</tr>
<tr>
<td>D</td>
<td>985</td>
<td>18.7±1.1</td>
<td>(B,C) p&lt;0.05</td>
</tr>
<tr>
<td>E</td>
<td>1670</td>
<td>27.3±4.6</td>
<td>(B,E) p=0.85</td>
</tr>
<tr>
<td>F</td>
<td>2500</td>
<td>38.7±4.9</td>
<td>(C,F) p=0.045</td>
</tr>
</tbody>
</table>

The peptides CCK and GIP were measured in the study and showed a stepwise increase from the lower to the higher calorific meal (Lon 2000). Rise of GIP in the plasma shows delayed gastric emptying and inhibition of gastric acid secretion.

The overall conclusion from the studies 7.3 is that EIE can distinguish with statistical significance the gastric response in the form of T50s with respect to calorific content of test meals, composed of the polysaccharide maltodextrin as a CHO and the triglycerides from the double cream (commercially available) as fat. The obtained
T50s are short in order to represent half emptying time. An interpretation has to be given.

7.4 **GLP-1 infusion satiety study**

The study was funded by BBSRC and designed to investigate the role of circulating glucagon-like-peptide one (GLP-1) as a regulator of satiety (Gut 99, Flii 98). It would be also investigated any parallel role in gastric emptying.

7.4.1 **Materials and methods**

Ten male volunteers 22 to 29 years of age and with BMI in the range 20 to 27 kg/m² (mean±stdev: 23.2±2.0 kg/m²) participated in the study. The study took place in the afternoon after six hours abstaining from food. For lunch they had been given a standard meal of pasta. Subjects were infused with either 1.2 pmol/kg/min GLP-1 or saline for 40 min at 18:00 hrs. It was a randomised single-blind within subject crossover study. Twenty minutes after starting the infusion the gastric emptying of a 450 mL water load was measured by EIE.

The electrical impedance measurements of the gastric area started at 20 minutes before the infusion, and at 20 minutes since the infusion had started they were asked to drink 450 mL bottled water with conductivity 0.2 mS/cm. Twenty minutes after the ingestion of water the epigastrograph terminated the measurements and the subjects were offered an ad libitum buffet meal and their food intake was recorded.

7.4.2 **Results and discussion**

The T50s were calculated (Table 7.13) as well as the percentage of the impedance deflection over the highest post-prandial deflection (% remaining water in the stomach) every 2 minutes (Table 7.14&7.15).

The GLP-1 infusion elongated the gastric T50s from 7.0 ±1.6 minutes to 11.8 ±3.5 minutes with strong significance (p<0.001). The average percentage of the remaining water in the stomach plotted over time showed a lag phase of two minutes with GLP-1 infusion from the moment water was ingested (Fig. 7.5). This is in accordance with findings that GLP-1 slows gastric emptying (Anv 98, Nau 97) and inhibits both phases of gastric acid secretion, cephalic and gastric; it also inhibits...
pentagastrin secretion, which in turn stimulates gastric acid secretion (Sch 97-89, O’Ha 90, Wet 97;94;93, Wil 96).

Blood samples showed unaffected GIP and CCK levels though the GLP-1 level was almost twice the value normally seen following a meal (Lon 2000).

**Table 7.13:** T50s of a water load of 450 mL under iv infusion of GLP-1 against saline.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>T50 (MINUTES)-SALINE INF.</th>
<th>T50 (MINUTES)-GLP-1 INF.</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>ch-2</td>
</tr>
<tr>
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<td>7.7</td>
</tr>
<tr>
<td>S2</td>
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<tr>
<td>S3</td>
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<tr>
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</tr>
<tr>
<td>S5</td>
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</tr>
<tr>
<td>paired t-test</td>
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<td>p_p(glp-1,sal)</td>
</tr>
</tbody>
</table>

Concluding, EIE results in significantly longer T50 under GLP-1 infusion (p<0.001), which means that EIE can detect differences on T50s after intravenous infusion of GLP-1 using just a water load of 450 mL as a test meal. It has to be clarified if the longer T50s under GLP-1 infusion are due to slower gastric emptying or to inhibition of the gastric acid secretion or to both.
Table 7.14: Percentages of the impedance deflection post-prandially with 450 mL water under iv infusion of 1.2 pmol/kg/min GLP-1.

<table>
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Table 7.15: Percentages of impedance deflection post-prandially with 450 mL water under iv infusion of saline (placebo).

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Fig. 7.5: Mean and SEM of the post-prandial impedance deflection from the loxiglumide infusion study in 10 subjects and the graphical presentation of the T50s. A lag phase of 2 minutes is clear.
7.5 EIE-Scintigraphy comparison study

7.5.1 Aim

The objective of this study was the evaluation of EIE by direct comparison with Scintigraphy, since the latter is considered as the “Gold Standard” method in studying gastric emptying (Cam 98, Don 86, Rea 89).

This was required since EIE is not only a novel method, but also because EIE resulted in shorter T50s in our studies (7.3.1-2) than those expected and reported in the literature. Another reason for this comparison was the uncertainty of the proper location of the electrodes, because of the variability of the stomach’s shape and location amongst individuals and even in the same individual for different posture and status of the stomach. This study was conducted in the Medical Physics Department of the Royal Surrey County Hospital (RSCH).

7.5.2 Subjects

The approval of the proposed study was requested and given by the Ethics Committee of the Royal Surrey County Hospital. Seven healthy volunteers recruited (4 male and 3 female) over 45 years old with no previous gastric history and BMI<25 kg/m² who participated in the study in three sessions. Volunteers younger than 45 years were excluded from the study because of the ionising radiation risk involved.

They had to fast for six hours at least, before each test. They, also, were advised to avoid strenuous activities, alcohol, caffeine and a dinner rich in fat on the day before. The clinical tests were conducted regularly at 3:00 pm in the Nuclear Medicine Department at the Royal Surrey County Hospital (RSCH).

Volunteers were given a written information sheet and asked for their formal written consent.
7.5.3 Test meals

Each subject was tested on 3 separate occasions with a different radio-labelled meal.

EIE requires liquid or semi-liquid test meals. There are no restrictions, generally, on the physical state of the test meals in Scintigraphy. It was decided as test meals to use those that had been already used in EIE since it would be possible in this way to compare the results with previous studies by EIE.

The chosen test meals were the D (1310 kJ) and F (2875 kJ) milk-shakes (Table 6.2) and the glucose solution of 10% in concentration and total energy 750 kJ (chapter 6).

The volume of the meal was 450 mL as this volume was proved in resulting in an appropriate in magnitude impedance deflection (Fen 92, Tsa 94, Sut 89). The meals were given to subjects at room temperature.

The meals were chosen so as to investigate the response of the two methods to different composition and calorific content meals. Thus the next step was the choice of the radio-tracer.

7.5.4 Radio-labelling

The ideal radio-pharmaceutical should be inexpensive, non-toxic, non-absorbable, homogeneously distributed within the meal, tightly bound and having a short physical half-life as well as suitable imaging characteristics for the gamma camera. The radio-nuclide marker should be representative of the properties of the test meal so that the gastric emptying of the nuclide adequately represents the behaviour of the test meal.

In gastric studies with scintigraphy there are in use several pharmaceuticals as carriers or tracers labelled with various radionuclides and the choice depends on the physical state of the meal. With soft solid meals the radio-marker is in colloid form. Such markers are $^{99m}$Tc-sulphur colloid for fried eggs and chicken liver, $^{99m}$Tc-tin colloid for pancakes, $^{75}$Se-glycerol as a fat marker and so on. Chickens have been
also fed with $^{99m}$Tc-sulfur colloid and their radio-labelled liver was used as a solid test radio-meal for gastric emptying.

Non digestible solids such as filter paper, cellulose or microcapsules labelled with a variety of radio-nuclides have been used as solid test meals, because the emptying rate of small non digestible particles (<1 mm in size) is similar to that of digestible solids.

The proper pharmaceutical carriers for liquid meals are non-absorbable chelated forms such as DTPA (di-ethylene-triamine-penta-acetic acid). Since the test meals in the present study were liquids (glucose solution and milk-shakes) DTPA was the choice carrier. The details of the DTPA used are: DTPA, Amersham (TM) Penetate II, Nycomed-Amersham PLC.

For radio-labelling technetium-99-metastable ($^{99m}$Tc) was chosen since it is the most tested and used nuclide, it has a suitable physical half life of 6 hours, short for reducing radiation dose but long enough for a complete study lasting for several hours. It is also easily available with low cost and has high labelling efficiency. Indium-113 or -111 have been used also for radio-labelling but mostly when double labelling is necessary to study at the same time the gastric emptying of more than one phase of a meal (Cam 98, Chr 83, Col 84-83, Hea 92, Hor 85).

The physical characteristics of the meals, namely volume, conductivity, pH were unchanged by adding into them the radio-marker. There is no doubt about the representation of the glucose meal by $^{99m}$Tc-DTPA since it is a water solution. The meals were labelled with 7 MBq of $^{99m}$Tc-DTPA at about 20 minutes before the ingestion time.

The uniform distribution of the $^{99m}$Tc-DTPA throughout the milk-shake and the stability of the labelling was tested by imaging the meals in a volumetric tube with the gamma-camera, at intervals of 30 minutes for two hours. The uniform distribution and the stability of the labelling were proven. The same test was repeated as above but after adding into the meal 50 to 150 mL of HCl acid (0.8mmol, pH=1.0) in an effort to simulate the gastric environment conditions. The uniform distribution
was evident even visually and the scintigram with the gamma-camera proved again the homogeneous distribution and stability.

The above tests supported the claim that the $^{99m}\text{Tc}$-DTPA marker was suitable for our studies.

7.5.5 Test performance

It is remarkably advantageous to possess the possibility of applying both techniques, scintigraphy and EIE, simultaneously without any interference between each other. In fact, a $^{57}$Co (3.7 MBq) pen source was used to mark surface anatomical points such as xyfisternum, costal margin and umbilicus as well as the position of the skin electrodes; in this way it was possible to know precisely the location of the electrodes with respect to the stomach and gain experience about the location of the stomach in respect to surface anatomy.

However in these tests, unlike those previous mentioned, the subjects were almost in a supine position with respect to the dual headed gamma-camera (Toshiba, GCA-7200A/DI), 128*128 matrix) test bed and their head slightly raised in order to enable subjects to ingest the meal. The meals were given to subjects through a long (30 to 40 cm) flexible plastic tube. The supine position was decided to avoid imaging the overlap of the upper part of the intestine with the lower stomach and subsequently to prevent the detection in scintigraphy additional activity from the upper intestine.

After the placement of electrodes and the final setting of the subject on the gamma-camera’s bed, recording of the electrical impedance signals started for at least 20 minutes before the meal was taken and the scintigraphy collection data started the moment the subject began drinking the test meal. The study continued for 90 to 120 minutes post-prandially according to calorific energy of the meal; the longer monitoring time was used for higher calorific content meals.

Blood samples were taken at intervals of 15 minutes and the volunteers had been asked to answer orally questionnaires with respect to feelings of satiety and satisfaction regarding the meal.
7.5.6 Data

In EIE, the meals being non-conductive generated the general pattern of the non-conductive meal. Figure 7.6A presents a typical graph obtained by EIE with meal D. Three phases during the study are reflected clearly in the graph. The fasting (pre-prandial) phase, the ingestion (filling) phase and the post-prandial phase.

The width of the graph is due to breathing and it is called respiratory artefact. The upper values correspond to the end of inhalation, because of the interposed layer which raises the impedance, and the lower values at the end of the exhalation of the air, which results to lower impedance values. It is believed that the lower impedance values represent the stomach impedance without the breathing artefact. Applying a mathematical procedure to retain only the lower values of the data, the breathing artefact can be eliminated (Fig. 6.6). This, also, acts as a filter for the breathing frequency.

Artefacts appear, also, due to movements of the subject, due to coughing, yawning even to psychological stress or nervousness.

A drifting in the pre-prandial base line appeared several times either upwards or downwards (Fig. 6.6) and it is believed that it is due to the drifting of the skin-electrode contact impedance over time and probably to electronic drifting.

It is possible that drifting to lower impedance value is due also to rising skin temperature at the site of skin-electrode contact. Studies showed that tissue impedance decreases linearly with temperature by 2%/°C (Ama 88; Con 87).

There is always a mathematical solution in dealing with artefacts and smoothing the data, without losing a lot of information such as running averages (Fig. 6.7), the already mentioned “detecting the minima” or fitting directly a mathematical model (Fig. 6.7). Until EIE is evaluated and well established the analysis of the raw data should be given priority (Cam 98, Rea 89).
The T50s were calculated from the raw data based on the definition and the percentages of the post-prandial impedance deflection over the maximum deflection as well.

In scintigraphy data were collected as 60 seconds frames anteriorly and posteriorly by the two heads of the gamma-camera for the same period after the ingestion of the meal, as with EIE. It was used a general purpose low energy collimator. Using the dual headed gamma-camera it was possible with the geometric mean of the two images (anterior and posterior) to compensate the non uniform attenuation of the detected activity due to the location and anatomy of the stomach (Col 84, Chr 80;83, Jac 82).

The one-minute images shows clearly the stomach or stated more accurately the content of the stomach, since the radioactive concentration in the image progressively increases from the walls to the interior of the stomach and expands during the study.

The radioactivity concentration per image corresponding to the visualised whole stomach was extracted and the profile of the stomach’s retention radioactivity per minute was obtained in this way. A typical profile of the recorded radioactivity per image (in 60 seconds) of the whole stomach is presented in Fig 7.6B and it can be compared visually with that by EIE (Fig. 7.6A).

The peak value appears simultaneously in both methods and the duration of meal intake is represented in the same time segment.

7.5.7 Results

The T50s by EIE were calculated by applying the definition of the T50 from the raw post-prandial data of the impedance deflection. According to literature, generally, the raw data may include useful information, which by fitting or smoothing might be missed (Col 73, Ham 76). This is advisable especially in the situation of the evaluation of a method.
Fig. 7.6: Gastric profile with meal D by EIE (A) and scintigraphy (B); the T50s are presented for comparison.
The T50s in scintigraphy were based also on the raw post-prandial data and on exponential fitting too. At the end, the T50s given by the exponential fitting were used, because the time of the study especially for meal F was shorter than the resulting T50s (Table 7.16).

Calculation of T50 from the recorded data of the two methods resulted in considerably different values. The T-50s by scintigraphy are far longer from those by EIE and the discrepancy becomes higher as the meal increases in caloric value.

The mean T50 of seven subjects is given in Table 7.16. The T50s calculated from scintigraphy's data are in accordance with those from the literature. (Tro 94, Cun 88, Ela 82, Van 83, Chr 80) Statistical significant differences exist in both methods between meals D and F (p<0.05), glucose (Gl) and F (p<0.02) but not between glucose and D.

Table 7.16: T50 in minutes from EIE and scintigraphy and the statistical results

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7.5.8 Interpretation of the discrepancy by direct comparison of the data

Since the T50s by EIE are short and those by scintigraphy are in accordance with the values in the literature, a direct mathematical comparison of the data obtained by the two methods was performed thinking that it will give useful information.

In particular, the raw acquired data in the post-prandial period by both methods, were expressed as percentages (Ela 82) normalised to the peak value of the recorded radioactivity in scintigraphy and to the maximum post-prandial impedance deflection in EIE. In scintigraphy, the peak value in both methods, as already has been mentioned, occurs simultaneously in both methods. This was confirmed testing the linear correlation between the peak times for all subjects and separate meals. Table 7.18 shows the peak time ($t_{\text{peak}}$) in minutes after ingestion of the meal commenced for all subjects and meals. Figure 7.7 graphically presents table 7.18 and the linear correlation, obtained

$$y=0.93x+0.17, \quad R^2=0.96 \quad (7.1)$$

The slope of the straight line equal to 0.93 (min/min) with $R=0.98$ confirms the simultaneous occurrence of the detected maximum value in both methods.

It is accepted that scintigraphy's percentages reflect gastric emptying since scintigraphy is the "gold standard" method in gastric emptying studies (Rea 89, Cam 98). What EIE post-prandial percentages do represent? The peak value (100%) is considered at zero post-prandial time and it is the reference point of the gastric function post-prandial calculations. by both methods. In scintigraphy the 100% represents the stomach full of the ingested meal and zero percentage the stomach emptied from the ingested meal. This mathematical transformation was applied to all sets of data and the averages for all subjects for each meal and method were calculated. The percentages in the scintigraphy are the geometric mean calculated from the anterior and posterior images with the heads of the gamma camera in each test. The recorded activity per frame was corrected for the physical decay of $^{99m}\text{Tc}$.

Since the peaks (100%) were recorded simultaneously, it was possible for the two sets of data in percentages per meal given by the two methods to be superimposed.
and to be compared by direct subtraction. The pattern of the differences (residuals) over time, i.e. the residual curve, was thought would give useful information (Fig. 7.8).

The superimposition of the two graphs shows clearly the faster emptying given by EIE. if it is hypothesised that EIE also reflects gastric emptying. The data set of the residuals (residual curve) exhibits a consistent pattern over time for all tests. The residual curves ($\Delta G$), ($\Delta D$), and ($\Delta F$) rise initially until reach a peak and then start progressively decline (Fig. 7.8). This shows that may represent a parallel to gastric emptying physiological process, which lowers the impedance at a faster rate than that due to gastric emptying.

Exponential fitting was applied on the averaged post-prandial percentages for all meals and both methods and the resulted fitted graphs are presented in Fig. 7.9. The correlation coefficient of all fittings is very high ($R>0.95$) and this shows that exponential fitting is can describe the post-prandial gastric function with both methods accepted.

The T50s from the fitting tend to be shorter by both methods than those without fitting. In table 7.17 are presented the T50s from both methods and their percentages difference with respect to non-fitted data.

**Table 7.17: Difference (%) between T50s by fitting or not in EIE-scintigraphy study**

<table>
<thead>
<tr>
<th>meal</th>
<th>EIE</th>
<th></th>
<th></th>
<th>Scintigraphy</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fit</td>
<td>no fit</td>
<td>% error</td>
<td>fit</td>
<td>no fit</td>
<td>% error</td>
</tr>
<tr>
<td>Glucose (G)</td>
<td>17.3</td>
<td>17.2</td>
<td>0.6</td>
<td>41.0</td>
<td>50.7</td>
<td>-19.1</td>
</tr>
<tr>
<td>D</td>
<td>19.8</td>
<td>20.2</td>
<td>-1.9</td>
<td>53.3</td>
<td>61.2</td>
<td>-12.9</td>
</tr>
<tr>
<td>F</td>
<td>26.3</td>
<td>29.3</td>
<td>-10.2</td>
<td>147.0</td>
<td>160.0</td>
<td>-8.3</td>
</tr>
</tbody>
</table>

147
Table 7.18: Time the stomach showed the max indication ($t_{peak}$) from the initiation of the meal ingestion in both methods.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Meal</th>
<th>EIE</th>
<th>Scintigr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>glucose</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>S2</td>
<td></td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>S3</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S4</td>
<td></td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>S5</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S6</td>
<td></td>
<td>8.2</td>
<td>8.5</td>
</tr>
<tr>
<td>S7</td>
<td></td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>S1</td>
<td>D</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>S2</td>
<td>D</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>S3</td>
<td>D</td>
<td>2.4</td>
<td>3</td>
</tr>
<tr>
<td>S4</td>
<td>D</td>
<td>1.7</td>
<td>1.5</td>
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<tr>
<td>S5</td>
<td>D</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>S6</td>
<td>D</td>
<td>5.1</td>
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<tr>
<td>S1</td>
<td>F</td>
<td>1.9</td>
<td>2</td>
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<tr>
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<td>F</td>
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<td>2</td>
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<tr>
<td>S3</td>
<td>F</td>
<td>1.8</td>
<td>1.7</td>
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<tr>
<td>S4</td>
<td>F</td>
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<td>2</td>
</tr>
<tr>
<td>S5</td>
<td>F</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td>S6</td>
<td>F</td>
<td>2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Fig. 7.7: The linear correlation between the $t_{peak}$ in EIE and scintigraphy.
Fig. 7.8: Gastric post-prandial profiles resulted from EIE and scintigraphy, expressed as percentages (%) of the gastric post-prandial indications with G (glucose), D and F meals. The residual profiles ($\Delta G$, $\Delta D$, $\Delta F$) are shown, too.

It is well established and was mentioned earlier that the digestion process of a meal in the stomach involves mixing of the food and chymification of the gastric content with HCl acid secreted in high concentration by the parietal cells of the gastric mucosa. The gastric content has to be acidified down to pH of about 2, in order to be forwarded to the duodenum for the next step of the digestion. The intragastric pH measurements when a meal was ingested showed pH of 4.5 and over (Ver 95, Mal 76) depending on the pH and buffering capacity of the food ingested.
Fig. 7.9: Exponential fitting of the averaged post-prandial percentage fractions of the meals G, D, and F with EIE and scintigraphy and the corresponding T50s.

In laboratory experiments, designed and conducted by the author, the pH and the conductivity of solutions of HCl acid, HCl with NaCl, and HCl acid with meal F were measured, and the results are presented in Table 7.19. Hydrochloric acid was simulating the gastric acid of fasting stomach (60 mL), and water and saline were simulating the test meal (450 mL). In this way the ingestion phase was simulated in the lab. A much higher pH of the solutions (gastric content) resulted after the mixing (ingestion of meal).
Table 7.19: In lab measurements of pH and conductivity of solutions simulating the ingestion of a test meal in the stomach.

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Species of solutions</th>
<th>Vol of sol (ml)</th>
<th>pH</th>
<th>Conduct. (ms/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HCl</td>
<td>10</td>
<td>2.35</td>
<td>4.54</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>75</td>
<td>7.35</td>
<td>0.48</td>
</tr>
<tr>
<td>3</td>
<td>NaCl</td>
<td>75</td>
<td>7.71</td>
<td>4.54</td>
</tr>
<tr>
<td>1+2</td>
<td>HCl+water</td>
<td>85</td>
<td>6.36</td>
<td>0.50</td>
</tr>
<tr>
<td>1+3</td>
<td>HCl+NaCl</td>
<td>85</td>
<td>6.87</td>
<td>4.24</td>
</tr>
</tbody>
</table>

The changes of the pH from 2.5 (fasting stomach) to at least 4.5 (after ingestion of the meal) as studies have shown (Ver 95, Sle 98) and the simulation in laboratory measurements show parallel changes (Table 7.19). The order of magnitude difference in H⁺ concentration can be from zero to 2 (100 fold) at least after the ingestion of a meal. Conductivity, as expected, follows wide range of changes; thus it can remain unchanged or become even 9 fold lower according to Table 7.19 (4.5/0.50=9) and literature (Sle 98).

Immediately after the ingestion of the meal gastric acid starts secreted with a higher rate until the necessary acidification of the gastric content is achieved and then the gastric secretion starts declining in order simply to maintain the necessary acidic environment (Figs 3.7;7.10A). Gastric acid secretion progresses rapidly during the gastric phase for the triple task of:

- protection of the human gastrointestinal tract and body from the ingested bacteria-microorganisms via food (defensive mechanism)

- hydrolysis of the basic common constituents of the food, such as CHO, fat and protein

- elimination of the gastric content particle size, if it is necessary.
The process of acidification is expected to influence considerably the impedance measurements besides gastric emptying of the contents that occurs at the same time and may support the hypothesis that gastric impedance measurements may be controlled more by the acidification of the gastric content than the volume.

**Fig. 7.10:** Acid secretion (mean±SE) with intragastric titration to pH 5.5 in (A) and intragastric pH in (B) following a sirloin steak meal in healthy subjects [reprod from Sle 98, p595].

In conclusion, it can be said that the initial rise in impedance from the ingestion of the meals Glucose (G), D and F is due also to the change of conductivity in the gastric content because of the dilution of the existing ions. After the ingestion, as digestion starts with gastric acid secretion, the gastric ionic content increases considerably due to the $\text{H}^+$ increasing concentration and consequently the conductivity increases and lower impedance is measured for this reason besides the emptying process.

7.5.9 Secretion curve by EIE

Visual examination of the residual curves (Fig. 7.8) representing the average residual percentages for each meal of all subjects shows a shift in both axes towards higher values as the calorific content of the meal increases, as expected.
It has been shown with previous studies that gastric acid (HCl) secretion is stimulated and it is poured into the lumen when food reaches the stomach. The gastric acid secretion reaches a peak and then starts declining due to negative feedback mechanisms for protection against over-acidity. The pattern of the residual graphs (AG), (AD), and (AF) (Fig 7.8-7.9) resembles the pattern of the gastric acid output (Figs 3.7; 7.10A) of the digestion of the meal (Sle 98, Mal 76, Ver 95). Figure 7.10A shows the gastric acid output when measured using in vivo intragastric titration to pH 5.5 in six healthy subjects after a sirloin steak meal. Figure 7.10B shows the pH of the gastric content for the same meal and subjects as in Fig 7.10A, and it is clear the time delay of the peak acid output in respect to pH peak.

In terms of EIE the emptying of the stomach is represented by a decrease in impedance if a non-conductive meal is used. It is hypothesised that the additional decrease compared of that given by scintigraphy can be attributed to mixing of the meal with the gastric secretions poured into the lumen during the digestion process. Gastric secretions are rich in ions and highly acidic (pH<2) containing mainly HCl in ionic form, as mentioned earlier, and this results to an additional increase of the gastric content conductivity and a decrease in the measured gastric impedance.

The residual curve, that describes the discrepancy of the EIE's data from these of scintigraphy, is considered by the author as representing the gastric acid secretion during the gastric phase and it can be named as the "secretion" curve.

A mathematical model of the form $y=ce^{at}$ is suitable for the secretion curve. This model is a common one to describe physiological processes especially secretions, and this can be an additional supportive element of the hypothesis of representing the gastric acid secretion. The same pattern (Fig. 3.7) for gastric acid secretion is described generally by physiologists (Sle 98, Ver 95, Mal 76).

In conclusion, the change of the impedance in the stomach post-prandially, according to the results of the direct comparison between scintigraphy and EIE, may be due to the acidification of the gastric content by the gastric acid secretion rather than to the volume of the meal, as has been stated hitherto (Sut 85, Fen 94, Avi 87, Lig 96, Tsa 95).
If we consider the stomach as a cylinder with radius \( r \) and length \( l \) with a material characterised by resistivity \( \rho \) the resistance across the length is given by equation 7.2

\[
R = \frac{\rho l}{S} = \frac{\rho l^2}{V} \quad (7.2)
\]

where \( S = \pi r^2 \) and thus \( V \) the volume of the cylinder is equal to \( \pi r^2 l \). Equation 7.2 shows that both volume \( (V) \) and resistivity \( (\rho) \) affect the total resistance. Resistivity \( (\rho) \) which is the reciprocal of conductivity \( (\sigma) \) is not a constant during the digestion but a variable.

This is supported by the linear change of the conductivity when the high-fat, high energy meal (F), used in studies (7.3.2) and (7.5) was mixed by gastric aspirations of an empty stomach taken during gastroscopy on a patient of the Royal Surrey County Hospital. The pH of gastric aspirations was <1 and the conductivity 13.8 mS/cm. The conductivity of meal F was 0.9 mS/cm and the volume 125mL (Fig. 7.11).

![Figure 7.11: Meal's F (125 mL; 0.8 mS/cm) conductivity variation when mixed with human gastric aspirations (13.8 mS/cm).](image)

Figure 7.8 shows the average percentages of the data of the three meals used by EIE and scintigraphy simultaneously as well as the “residual” graphs, which resulted. The influence of the energy of the meals on the quantity of the secretions and on the peak
time is evident. The secretion seems to last longer as the energy and the fat content of the meal increases, with a shift of the peak at a later time. With the high-fat meal the secretion peak is reached at about one hour but with the low calorific meals earlier, between 30 and 40 minutes after ingestion of the meal.

7.5.10 Ingesting phase

Another aspect, which it was thought required more serious consideration after having carried out the direct comparison between scintigraphy and EIE, was the ingestion phase of the meal.

The ingesting phase of the meal in the graph was previously thought to be due exclusively to the increasing volume of the stomach, but it is proposed that it is mainly due to decrease in concentration of $H^+$ in the stomach because of dilution and/or buffering of the already existing basal gastric acid because of the ingested meal. There is always a certain amount (30 to 60 mL) of gastric acid juice in an empty stomach having a pH=2 and when the food enters the stomach dilution of the existing ions (mainly $H^+$) occurs and/or interaction of the ions starts (buffering) changing the conductivity in either direction.

If only dilution exists and no ion interactions between ions take place, according to the law of independent migration of ions (Mur 94a), the final conductivity $\sigma_f$ of a mixture of two substances in the gastric lumen is given by the following equation:

$$\sigma_f = \frac{\sigma_m v_m}{v_t} + \frac{\sigma_f v_f}{v_t} \quad (7.3)$$

where $\sigma_f$: the final conductivity of the gastric content, $\sigma_f$ and $v_f$: the conductivity and volume of the gastric juice, $\sigma_m$ and $v_m$: the meal’s conductivity and volume respectively, $v_t$: total gastric volume. Actually $\sigma_f$ and $v_f$ are variables as well as $v_m$.

This was supported in laboratory measurements by the author; the conductivity of mixtures of gastric aspirations (16.0 mS/cm) taken from a subject during gastroscopy in RSCH with meal F (0.80 mS/cm) was measured and a considerable decrease in the conductivity of the mixtures with respect to that of gastric aspirations resulted as
Figure 7.12 shows. The variation of the aspiration conductivity as the meal's volume increases follows power reduction with $R=0.99$. At the volume 56 mL of meal F the ratio “volume of aspiration/volume of meal” simulates the proportionality “volume of gastric juice/volume of ingested meal” in our experiments.

![Graph showing conductivity vs. volume of meal F](image)

**Fig. 7.12: The variation of the conductivity of gastric aspirations (8.0 mL, 16.4 mS/cm) by adding in it progressively meal F.**

7.5.11 Additional evidence

7.5.11.1 Neutral meal

After the results and conclusions from the comparison between EIE and scintigraphy, the concept of the “neutral meal” was proposed by the author and a series of tests were conducted for the first time with the MSc in Medical Physics students as subjects and operators in their laboratory practice. These tests confirmed the hypothesis that if a meal with conductivity 5.0 to 6.0 mS/cm is taken in 37°C, no initial impedance deflection is generated and the impedance starts declining shortly afterwards (Fig. 6.5.). This can be explained if one accepts that the decrease in concentration of $\text{H}^+$, because of the volume of the ingested meal, is balanced by the $\text{Na}^+$ ions which were introduced by adding a certain amount of salt to achieve a higher meal conductivity (5.5 mS/cm from 0.7 mS/cm at 37°C). Stimulation of
gastric acid secretion follows, the gastric conductivity increases and the impedance declines until it reaches the so-called post-prandial base line.

The absence of the initial impedance deflection and the decrease in impedance which follows until the post prandial baseline is reached provides evidence that the change of gastric conductivity due to gastric secretions contributes mostly to the value of the measured gastric impedance.

7.5.11.2 GLP-1 study

The findings of the GLP-1 study by EIE support also the hypothesis that epigastric impedance is affected considerably by gastric acid secretion during the digestion period. As it has been mentioned if the plasma levels of GLP-1 are raised by 3 to 4 fold of the physiological post-prandial concentration (25 pmol/L), then there is inhibition of gastric acid secretion and slowing down of gastric emptying. In our GLP-1 infusion study a significantly (p<0.001) slower T-50 was found with GLP-1 infusion and a short lag-like period followed the ingestion of the meal and the decrease of impedance started later. In the placebo tests no such lag-like period appeared. It is the author’s opinion that the lag-like period reflects a delay in the stimulation of the gastric acid secretion in response to the ingested water because of the initial inhibition caused by the plasma elevated GLP-1. Later because of the gastric distension the gastric acid secretion is stimulated. The response to the gastric distension because of the water ingestion was not immediate because of the earlier induced inhibition by GLP-1 infusion.

The simultaneous application of EIE and scintigraphy for the study of the gastric function and the direct comparison of the data lead the author to express the following as overall conclusions:

- the placement of the electrodes proved right as the marks with the $^{57}$Co pen source showed on the scintigrams

- once again was proved that EIE provides T50s of gastric function which depend on the meal energy and for energy difference by 1200 kJ strong significance exists between the T50s
- the T50s by EIE are considerably shorter than the expected and those by scintigraphy indicating that may not represent half emptying time exclusively and lead to a number of hypotheses and laboratory tests

- the introduction and usefulness of the neutral meal

- the initial impedance deflection is due mainly to the dilution of the intraluminal content by the ingested meal

- the impedance decrease post-prandially is due also to increased ionic concentration in the gastric lumen, because of the arrival of H⁺ ions during the digestion besides the gastric emptying

- the post-prandial baseline itself being formed earlier that the final emptying according to scintigraphy results, shows a gastric equilibrium and this can be attributed to the gastric conductivity rather than to gastric volume

- the time of the starting point of the post-prandial baseline may coincide to the maximum acid secretion rate.

Finally, it seems that the epigastric impedance is formed by the conductivity of the gastric content rather than the volume of the gastric content. It needs to be investigated further.
7.6 **EIE-Octanoic acid Breath test**

7.6.1 Introduction and aim

Gastric emptying is of great importance in nutritional research because it determines rates of nutrient absorption and consequently post-prandial hormonal, metabolic and satiety responses. The tests of gastric emptying apart from having to be simple, non-invasive and not inducing any risk, should also be inexpensive so to as to be used repeatedly on the same subject and in the study of wide groups of the population (age, health, condition). The application also of tests based on different principles gives the possibility of the development of complementary tests and in this way a broader spectrum of information on a particular study becomes possible. The results of the different methods may not coincide as they may reflect the same body function via different physiological procedures but it is possible always to establish correction factors or correlators.

Under these considerations the present study was designed to compare and evaluate the indices of gastric emptying using 3 methods, completely different with regards to physiological procedure. The methods are based on:

a) electrical impedance measurements (EIE)

b) paracetamol absorption (Rob 96) and

c) octanoic acid breath test (Gho 93, Sch 97a, Ver 97).

It was a great advantage to have the possibility to apply these three methods simultaneously without any interference between them. (The collaborating colleague undertook the responsibility of conducting the paracetamol absorption and octanoic acid breath tests).
7.6.2 Study design

The study protocol was approved by the Ethics Committee of the University of Surrey. Seven healthy subjects in this study participated and all happened to be female by coincidence. The age range was 22 to 31 years (26.±12.7(ave±stdev)) and all had a BMI<25 kg/m² (20.6±2.5 (ave±stdev)).

Subjects were requested to have a light non-fatty dinner the day before and not to take any breakfast. Subjects were provided with written information sheets and they were asked for their written consent. They could withdraw any time from the study if they decided to do so.

The tests were scheduled for the 9th hour in the morning. Gastric emptying of all subjects was tested on two separate occasions, a week at least apart, with two test meals, the already mentioned test milk-shakes D and F (Table 6.2). In the meals were added 1.5 g of non-soluble paracetamol (acetaminophen, a para-aminophenol derivative) and 50 mg of 13C sodium octanoate in the form of powder (Donation of the University of New Castle). The conductivity and pH of the meals remained the same after the addition of paracetamol and octanoic acid.

The test procedure was started by placing the skin electrodes on the subject according to the scheme already described; then a cannula was inserted in one of the arms by an appointed physician for drawing blood samples. Special tubes were used for the collection of the breath samples in predefined time intervals.

A semi-supine posture on a bed was adopted for the measurements of impedance, commencing with a baseline value of the fasting stomach for at least 15 minutes. Subjects were requested to be as immobile as possible to minimise motion artefacts. Two reference blood samples were taken as well as two reference breath samples.

Subjects were given the milk-shake at least 15 minutes after the electrical impedance measurements had started using a straw, for a smoother and more convenient ingestion of the meal and the impedance was recorded for 90 minutes post-prandially with 5 Hz sampling rate. During the post-prandial period blood samples and breath samples were collected from the subjects every so often; subjects also expressed via
questionnaires and visual analogue scales their comments on their satiety, hunger or degree of satisfaction from the test meal.

7.6.3 Data analysis and results

A profile of the impedance data is shown in Fig. 7.13. It is a typical trace of a non-conductive meal. It is clear in Fig 7.13b the lower rates of impedance changes with the higher energy meal F. The T50s and percentages of specific time intervals were calculated from the impedance data and they are presented in Table 7.20 and Fig. 7.14. The T50s of the high fat milk-shake F (28.7±3.8 minutes (ave±stddev) were longer from than that of low fat milk-shake D (20.8±5.3 min) with statistical significance (2 tailed, t-test p=0.015). Significant differences resulted between the percentages of the impedance deflection post-prandially, as it is shown in Table 7.20.

The paracetamol absorption measurements showed the maximum absorption of meal D happened significantly earlier (p<0.01) than that of meal F ((63±25) minutes vs (175±16) minutes post-prandially (mean±SEM)). This means that the high energy meal F has a considerably slower emptying rate than the lower energy meal D.

The octanoic acid breath test also showed a longer T50 for meal F than D (272±33 vs 208±26 minutes respectively (mean±SEM, p<0.05)).

Thus, all methods showed that the T50s are longer with meal F and shorter with D but with considerably different values.

7.6.4 Discussion and conclusions

If the results of this study are compared with those from the EIE-scintigraphy study (Table 7.16), the T50s given by EIE are consistent in both studies but are the shortest and considerably shorter than the other methods gave; thus it is questionable if they represent emptying rate values only.

Direct comparison of the three methods is not possible because of differences in the principles of the methods. The T50s based on the breath test are long and longer than
those resulted with scintigraphy (7.5). This can be attributed to the considerable length of time expected for the $^{13}$C-octanoic acid to be digested, absorbed and oxidised before it is expired in the breath. The paracetamol $T_{\text{max}}$ values appear to be comparable with the T50s from scintigraphy (7.5).

Fig. 7.13: Epigastric impedance signal of the same subject with meal D (a) and meal F (b) containing 50 mg $^{13}$C sodium octanoate and 1.5 g paracetamol.
Table 7.20: T50s, the percentages of the post-prandial impedance deflection and their statistical tests with meals D and F of the octanoate study.

<table>
<thead>
<tr>
<th>sbj / t(min)</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>25</th>
<th>35</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>T50(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>100.0</td>
<td>83.3</td>
<td>66.7</td>
<td>43.3</td>
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<td>13.0</td>
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<td>4.3</td>
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<td>18.9</td>
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<tr>
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<td>26.7</td>
<td>15.3</td>
<td>8.7</td>
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<td>3.5</td>
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<td>23.5</td>
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<td>6.1</td>
<td>4.0</td>
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<table>
<thead>
<tr>
<th>sbj / t(min)</th>
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<th>25</th>
<th>35</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>T50(min)</th>
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<tbody>
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<td>92.7</td>
<td>70.8</td>
<td>41.6</td>
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</tr>
<tr>
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<td>5.5</td>
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</tr>
<tr>
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<td>5.3</td>
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<td>90.0</td>
<td>65.0</td>
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<td>91.0</td>
<td>73.2</td>
<td>53.5</td>
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<td>9.5</td>
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</tr>
<tr>
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<td>91.0</td>
<td>73.2</td>
<td>53.5</td>
<td>35.4</td>
<td>23.4</td>
<td>14.9</td>
<td>9.5</td>
<td>5.4</td>
<td>28.7</td>
</tr>
<tr>
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<td>10.9</td>
<td>9.9</td>
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<table>
<thead>
<tr>
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<th>15</th>
<th>25</th>
<th>35</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>T50(D,F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
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<td>0.015</td>
<td>0.025</td>
<td>0.060</td>
<td>0.029</td>
<td>0.006</td>
<td>0.016</td>
<td>0.000</td>
<td>0.015</td>
</tr>
</tbody>
</table>
Fig. 7.14: Profile of the post-prandial impedance deflection in all subjects with meal D (a), meal F (bB) and the average with SEM of all subjects per meal (c)
Concluding, epigastric electrical impedance changes resulted in significantly different T50s but were very short to represent half-emptying time. The study shows that a causal mechanism must exist, which reduces the impedance additionally to that expected from the gastric content. Once again, as in the EIE-scintigraphy study, it is repeated by the author, that a parallel physiological mechanism exists and this is the gastric acid output during digestion which increases in concentration initially and declines later reducing additionally the measured impedance.
7.7 **Loxiglumide Study**

7.7.1 Introduction and aim

Satiation is the process that brings eating to an end and Satiety is the state of inhibition over further eating.

In 1973 Gibbs, Young and Smith discovered that peripheral administration of CCK inhibits food intake. The satiety effect was dose dependent and specific in the sense that it mimicked the satiety induced by ingested food and was not seen with other gut peptides, apart from the weaker bombesin (Gib 79;76;73a-b); but it has to be examined whether the relatively small variations in the low concentrations of plasma during meals are sufficient to elicit the satiety of the kind observed with exogenous CCK (Lie 94). However because of incomplete data of the studies about the role of endogenous CCK the loxiglumide study was designed (Lon 2000).

In particular the aim was to investigate mainly the role of the peripheral CCK in the induction of satiety by blockading CCK\(_A\) receptors with intravenous infusion of the CCK\(_A\) receptor antagonist loxiglumide (Lon 2000). In parallel possible mediator factors were to be examined such as gastric function, plasma hormone levels and metabolites. Sensation feelings of hunger or satiety or desire to eat were estimated with visual analogue scales given to subjects.

7.7.2 Study design

Ten subjects were recruited to participate in the study, 8 males and 2 female, with BMI 22.4±1.8 kg/m\(^2\) (mean±stddev), between 22 to 33 years age range without any previous history of the gastrointestinal tract. The study was approved by the Ethical Committee of the South West Surrey District and University of Surrey and all subjects were given a written information sheet and asked for their written consent prior to their participation. It was a single blind randomised within subject crossover study using saline as a control. More details can be found in the work by Long (Lon 2000).
Subjects were studied in two sessions separated by one week at least.

All subjects undertook a screening test using 450 mL water for their suitability for EIE before being chosen for the study.

Subjects were asked to have a standardised breakfast consisting of rice krispies (50g) with skimmed milk (300 mL), and one cup of tea or coffee with skimmed milk or sugar as preferred. They were advised to eat as much as they could but to eat the same amount at the same time on each test occasion.

Three hours after the standardised breakfast, the electrodes for the EIE measurements were attached to the subjects in the standard configuration. Subsequently, subjects in a semi-supine position were infused via canulae in one session with 30 mg/kg/hr loxiglumide for the first 10 minutes and then with the maintenance dose of 10 mg/kg/hr for the rest of the time. Loxiglumide was provided by Rota Research Laboratories, Milan, Italy. On the other session at least one week after the first the same subjects were infused with saline as the control at the same flow rate as in the loxiglumide study. Thirty minutes following the start of infusion, milk-shake F of 450 mL was given and the gastric function was monitored by EIE. In the test meal 1.5 g of non soluble paracetamol were added as an additional method of assessing gastric emptying. The paracetamol had no effect on the meal’s conductivity.

Electrical impedance measurements commenced 10 minutes before infusion for collection of the fasting baseline data. Blood samples were withdrawn at 15 minutes intervals and visual analogue scales were used by the subjects for the assessment of their hunger, satisfaction or desire to eat. At 60 minutes from the milk-shake (preload) the electrical impedance measurements were stopped, and an ad libitum pasta meal was offered to subjects and the food intake was recorded.

7.7.3 Data analysis and results

A typical graph of the epigastrograph’s response is shown in Fig. 7.15.

The gastric T50 of the milk-shake in minutes was calculated for all subjects using the impedance deflection measurements as in previous studies since the profile of the
impedance changes is the same (Table 7.21). The percentages also of the impedance deflection, over the peak post-prandial deflection in 15 minute intervals post-prandially were calculated, based on the raw data and are presented in Table 7.21.

![Graph A](image1)

**Fig. 7.15:** Profile of the epigastric impedance from the same subject with meal F under loxiglumide (a) or saline (b) infusion; earlier post-prandial baseline in (a).

Figure 7.17 shows the percentages of Table 7.21 of the individual subjects (a, b) and the average (c). Student's paired t-test confirmed statistical significance for shorter T50s ($p_p<0.001$) and percentages (Table 7.21) under loxiglumide infusion. The percentages start to show significance after 15 minutes post-prandially.
Plasma paracetamol concentrations showed a peak under loxiglumide infusion at 15 minutes post-prandially and at 30 minutes post-prandially under saline infusion for a value of $p=0.008$ (Lon 2000, pp248). The earlier paracetamol peak indicates faster gastric emptying.

Table 7.21: $T50$s and percentages of the post-prandial impedance with meal F under loxiglumide infusion against saline.

<table>
<thead>
<tr>
<th>Loxiglumide infusion</th>
<th>subj / t(min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>T50 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>100.0</td>
<td>68.7</td>
<td>43.7</td>
<td>21.9</td>
<td>0.1</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>100.0</td>
<td>55.3</td>
<td>37.6</td>
<td>15.0</td>
<td>0.1</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>100.0</td>
<td>66.3</td>
<td>31.6</td>
<td>7.3</td>
<td>0.1</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>100.0</td>
<td>50.5</td>
<td>28.6</td>
<td>10.8</td>
<td>0.1</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>100.0</td>
<td>46.8</td>
<td>12.3</td>
<td>8.6</td>
<td>0.1</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>100.0</td>
<td>97.4</td>
<td>48.6</td>
<td>18.4</td>
<td>0.1</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>100.0</td>
<td>63.3</td>
<td>19.1</td>
<td>6.3</td>
<td>0.1</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>S8</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S9</td>
<td>100.0</td>
<td>69.7</td>
<td>29.3</td>
<td>6.7</td>
<td>0.1</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>S10</td>
<td>100.0</td>
<td>95.2</td>
<td>28.7</td>
<td>6.6</td>
<td>0.1</td>
<td>21.7</td>
<td></td>
</tr>
<tr>
<td>ave</td>
<td>100.0</td>
<td>64.8</td>
<td>31.1</td>
<td>11.3</td>
<td>0.1</td>
<td>20.5</td>
<td></td>
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<tr>
<td>stdev</td>
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<th>30</th>
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<th>60</th>
<th>T50(min)</th>
</tr>
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<td>0.1</td>
<td>39.6</td>
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</tr>
<tr>
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<td>0.1</td>
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<td></td>
</tr>
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<td>46.4</td>
<td>26.3</td>
<td>0.1</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
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<td>43.4</td>
<td>23.2</td>
<td>0.1</td>
<td>20.8</td>
<td></td>
</tr>
<tr>
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<td>61.9</td>
<td>40.5</td>
<td>19.1</td>
<td>0.1</td>
<td>21.8</td>
<td></td>
</tr>
<tr>
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<td>96.5</td>
<td>27.6</td>
<td>0.1</td>
<td>37.5</td>
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</tr>
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<td>74.5</td>
<td>47.1</td>
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<td>0.1</td>
<td>30.0</td>
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<tr>
<td>S8</td>
<td>100.0</td>
<td>53.4</td>
<td>34.0</td>
<td>14.6</td>
<td>0.1</td>
<td>15.5</td>
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<tr>
<td>S9</td>
<td>100.0</td>
<td>76.2</td>
<td>37.7</td>
<td>22.6</td>
<td>0.1</td>
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<tr>
<td>S10</td>
<td>100.0</td>
<td>74.0</td>
<td>31.2</td>
<td>18.1</td>
<td>0.1</td>
<td>23.5</td>
<td></td>
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<tr>
<td>ave</td>
<td>100.0</td>
<td>71.3</td>
<td>48.9</td>
<td>22.2</td>
<td>0.1</td>
<td>27.1</td>
<td></td>
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<tr>
<td>stdev</td>
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<td>19.9</td>
<td>6.6</td>
<td>0.0</td>
<td>7.4</td>
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Student's 2 tail t-paired test of loxiglumide against saline

<table>
<thead>
<tr>
<th>t(min)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>T50s</th>
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<tr>
<td>$p_0$(lox,sal)</td>
<td>0.015</td>
<td>0.004</td>
<td>0.001</td>
<td>0.001</td>
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</table>
The energy intake of the pasta test meal was offered at 60 minutes after milk-shake F was significantly higher (p=0.008) under loxiglumide infusion (Lon 2000, pp238).

Two subjects (S1, S6) showed a lag phase of 25 minutes under saline infusion. With loxiglumide infusion S1 showed simply slower rate on impedance decrease and S6 a shorter of 15 minutes lag phase.(Fig. 7.17).

Subject S8 resulted in the shortest T50 under saline infusion and showed a difficult for interpretation impedance profile under loxiglumide infusion. After the expected impedance deflection upon meal ingestion, an impedance decrease occurred with a T50 of 7.0 minutes and thereafter continued increasing (Fig. 7.16). Is this a physiological process (water flow in the stomach) or a skin-electrode contact problem or an electronic fault. This is difficult to answer, with confidence. The data of this session have not been included in this study.

Fig. 7.16 : Epigastric impedance signal from subject S8 with meal F under loxiglumide infusion; the post-prandial phase is not the usual.
Fig 7.17: Post-prandial impedance profile in percentages of all subjects infused with loxiglumide (a), saline (b) and the ave (SEM) of (a) and (b).
7.7.4 Discussion and conclusions

The shorter T50s and percentages under loxiglumide infusion resulting from EIE with statistical significance are in accordance to the paracetamol results; as mentioned earlier paracetamol was used simultaneously as a second method of gastric emptying estimation. These are also consistent with the literature if it is considered that the T50s and percentages are related to gastric emptying (Sch 97, Jin 94, Kon 94). According to these studies loxiglumide, the CCK\(_A\) antagonist shortens significantly the emptying rates (Sch 97) and abolishes the delay in gastric emptying caused by adding fat in the meal which releases CCK (Kon 94).

But loxiglumide is also shown to be a potent stimulant of gastric acid secretion (Kon 94-94, Jac 93, Jin 94, Hil 91) and it has been proposed by Kontureck (Kon 95) that loxiglumide should be used as a diagnostic tool for the impaired feedback control of gastrin release and gastric acid secretion, resulting from infection with \(H\) pylori.

The question arises, whether the T50s resulting from electrical impedance measurements are shorter because they reflect the shorter emptying rates caused by loxiglumide or the additional gastric acid secreted because of loxiglumide infusion? The additional or earlier secreted acid increases the conductivity of the gastric content and as a consequence there is a drop of impedance, which results in shorter T50s. Of course the shorter T50s can be attributed to both.

If the pre-prandial baseline is observed carefully, when the loxiglumide infusion commenced, the baseline started drifting downwards and most of the times when the maintenance dose of loxiglumide started it remained stable, parallel to time axis (Fig. 7.15a). This was not observed with the saline infusion. This drifting downwards may be attributed to the secretory response of the stomach due to loxiglumide infusion. It is an indication that the gastric impedance is affected by the secretory status of the stomach.

An attempt was made to quantify these observations and Table 7.22 presents the results. According to Table 7.22, under loxiglumide infusion the pre-prandial baseline exhibited a progressively lower impedance by the start of the infusion to be
stabilised during the maintainance dose in 60% (6) of subjects; in 10% (1) of subjects the pre-prandial baseline was continuously declining from the start of infusion; thus in 70% (7 out of 10) of the subjects a declining pre-prandial impedance with an average of 2.2% impedance deflection was observed, which may be attributed to additional $H^+$ ions across the gastric mucosa because of the stimulation of the proton pump by loxiglumide infusion. Of course 2.2% impedance deflection is not a reassuring indicator that these changes are due to a physiological mechanism rather than to a statistical fluctuation. However it was thought worthy of mention since under saline infusion the percentages of the trends are almost evenly spread and do not show any specific trend.

**Table 7.22 : Number (percentages) of subjects showing a specific trend in the monitored pre-prandial impedance.**

<table>
<thead>
<tr>
<th>Trend of imped baseline/Condition</th>
<th>Lox infusion</th>
<th>Sal infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downward and stable</td>
<td>6 (60%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Downward</td>
<td>1 (10%)</td>
<td></td>
</tr>
<tr>
<td>Stable</td>
<td>2 (20%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Upward and stable</td>
<td></td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Upward</td>
<td>1 (10%)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

This trend of the pre-prandial base line after infusion of loxiglumide had commenced may indicate the effect of loxiglumide on gastric acid stimulation. The additional comment is that loxiglumide’s action by intravenous infusion is immediate since pre-prandial decline starts in the second minute after the infusion. Detailed time course studies between gastric acid output or pH and infused loxiglumide in the fasting phase are not available. Measurements usually show 15 or 30 minutes period averages and the rise starts from zero time (Kon 93).

The drifting of the pre-prandial baseline was initially thought to be due to electronic drifting or changes on the electrode-skin contact and/or to the higher temperature on the skin contact area with the electrodes as time progresses. Reconsidering the matter it is believed it may also be due to the secretory changes of the gastric mucosa, most of the times.
7.8 Aspartame study

7.8.1 Introduction and aim

Investigations have indicated that aspartame increases satiety in humans (Rog 90). This effect on satiety may be due to phenylalanine, a component of aspartame, which has been shown to affect satiety via cholecystokinin-mediated mechanisms (Bal 94). This study was designed for the same purpose; to investigate whether aspartame can affect satiety in humans and also determine possible mechanisms such as delayed gastric emptying and those that affect plasma hormonal profiles.

7.8.2 Study design

For this purpose six lean healthy volunteers (four females and two males; 24-31 years in age (27.7±2.9); BMI<25kg/m² (20.1±1.5)) were recruited for a within-subjects repeated measurement clinical study. Subjects were studied on 3 occasions and instructed to avoid alcohol, caffeine and products containing aspartame or paracetamol for 12 hours before each study day.

Gastric emptying was studied by EIE with paracetamol absorption.

After an overnight fast, subjects were given, in random order, one gelatine capsule containing 400 mg aspartame, or 176 mg aspartic acid with 224 mg phenylalanine or 400 mg cornflour (placebo) with 450 ml water. Non-fizzy paracetamol (1.5 g) was added to the water. The dispersed paracetamol in the water load had no effect on the physico-chemical properties of the meal (conductivity, pH). One hour after the treatment, meal E (1983 kJ liquid meal of 450 ml containing 22 g fat and 67 g carbohydrate) was given to subjects (see Table of meals).

Gastric emptying was measured by EIE for two conditions: for the first phase (60 minutes) the water meal was ingested and for the second phase (60 to 120 minutes) meal E was introduced. Therefore 0 to 60 minutes was the treatment and 60 to 120 minutes (meal E)). Paracetamol absorption for gastric emptying was used for the
treatment meal only, i.e. for the 60 first minutes of the study, before meal E was given.

Plasma CCK, GLP-1 and paracetamol concentrations were measured using venous blood samples taken at 15min intervals during the study. A basal blood sample was also taken before the meals were given.

7.8.3 Data analysis and Results

The gastric impedance data by EIE were treated as in the previous studies.

The paracetamol data as well as the data from the blood samples were analysed by collaborators in the School of Biological Sciences.

A representative curve generated by the electrical impedance measurements is presented in Fig. 7.18.

![Graph showing two peaks representing the two meals in the aspartame study](image)

**Fig. 7.18:** A typical profile of the two peaks of the epigastric impedance representing the two meals in the aspartame study; it is clear the longer rate of impedance reduction with the meal E.

The peak on the left is due to changes in the gastric impedance caused by the ingestion of the water in the stomach. The peak on the right is due to the ingested meal E (Fig. 7.18 ). It is easy to realise that the rate of decline of impedance is smaller with meal E than that with water, which shows a slower or an elongated
gastric activity with meal E compared with that of water. The same is observed with the other two conditions.

It is remarkable to note the steady intermediate baseline (baseline between the two meals) and this was the case with most of the subjects.

The data collected by EIE were treated in the usual way but separately for the water preload (treatment meal) and meal E in each subject. The intermediate baseline was either lower or higher or even at the same level as the pre-water baseline. The T50 in minutes was calculated for all subjects and meals based on the raw data and definition (Tables 7.23 & 7.24). The percentages also of the impedance deflection in the post-prandial period over the maximum impedance deflection were calculated for a more detailed study of the gastric post-prandial function (Tables 7.25).

The paired Student’s t-test on the data of Table 7.23 failed to show any significant difference between the water T50s under different treatments, i.e. the water showed the same behaviour (rate of impedance decrease) for all conditions in the same volunteer for all treatments. The paracetamol absorption measurements resulted also in similar emptying times between all treatments for the water pre-load.

The same statistical tests were used for the T50s of meal E and the results are given in Table 7a. There was no effect with aspartame treatment but there was a significant effect with the free amino acid treatment. The calculated T50s were shorter with the free amino acids treatment than with the placebo (cornflour) or aspartame treatment. The 2-tails paired t-test a showed significance of p < 0.01 (Table 7). The same results came out of the EIE percentages.

The hormonal investigation failed to show any significant difference for one hour post-prandially for meal E (CCK, GLP-1) with either treatment.

The desire to eat at the end of the hour was higher in the subjects when treated with the amino acid capsule at a level of p<0.05.
Table 7.23: T50s with EIE and post-prandial impedance percentages of water taken with paracetamol and amino acids (a) or aspartame (b) against corn flour (c) and significance (c) in the aspartame study.

<table>
<thead>
<tr>
<th>Amino acids (a)</th>
<th>subj/t(min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
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Table 7.24: T50s and percentages with EIE using meal E with paracetamol and aminoacids (a) or aspartame (b) against corn flour (c) and significance (c).

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|                | Aspartame (b)   |                |                |                |                |                |
| sib/t(min)     | 0               | 15             | 30             | 45             | 60             | T50(min)       |
| S1             | 100.0           | 60.4           | 38.8           | 20.0           | 8.3            | 22.2           |
| S2             | 100.0           | 69.4           | 31.2           | 13.9           | 7.3            | 23.9           |
| S3             | 100.0           | 75.2           | 40.9           | 19.9           | 7.3            | 25.7           |
| S4             | 100.0           | 78.1           | 39.6           | 17.6           | 7.7            | 23.6           |
| S5             | 100.0           | 58.4           | 24.0           | 15.1           | 6.5            | 17.9           |
| S6             | 100.0           | 72.0           | 41.0           | 17.7           | 8.1            | 25.3           |
| ave            | 100.0           | 68.9           | 35.9           | 17.4           | 7.6            | 23.1           |
| stddev         | 0.0             | 7.9            | 6.9            | 2.5            | 0.7            | 2.8            |
|SEM             | 0.0             | 3.6            | 3.1            | 1.1            | 0.3            | 1.3            |

|                | Corn flour (c)  |                |                |                |                |                |
| sib/t(min)     | 0               | 15             | 30             | 45             | 60             | T50(min)       |
| S1             | 100.0           | 80.5           | 57.0           | 26.4           | 10.0           | 33.0           |
| S2             | 100.0           | 88.8           | 31.4           | 17.1           | 8.7            | 25.1           |
| S3             | 100.0           | 73.0           | 49.3           | 25.3           | 9.6            | 24.1           |
| S4             | 100.0           | 81.1           | 32.9           | 22.5           | 7.3            | 23.1           |
| S5             | 100.0           | 58.0           | 31.4           | 14.5           | 8.1            | 17.2           |
| S6             | 100.0           | 75.9           | 46.5           | 20.3           | 8.5            | 29.0           |
| ave            | 100.0           | 76.2           | 41.4           | 21.0           | 8.7            | 25.3           |
| stddev         | 0.0             | 10.4           | 11.0           | 4.6            | 1.0            | 5.4            |
|SEM             | 0.0             | 4.7            | 4.9            | 2.1            | 0.4            | 2.4            |

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7.8.4 Discussion and conclusions

An attempt follows to explain the effect on the T50s and percentages by EIE with amino acid treatment.

Gastrin is the most potent known stimulant of gastric acid secretion (Gro 70). With radioimmunoassay it was shown that during infusion of G-17, plasma levels equivalent to postprandial values caused considerable secretory responses (Fel 78, Eys 84). Women are less sensitive than men to gastrin and 2 to 3 fold higher concentrations are needed for 50% acid output of that in men (Fel 83).

The gastrin producing cells are known as G cells. G cells are far more abundant throughout the pyloric antral mucosa in normal adult mammals and depending on the species and on the stage of development can be found in the duodenum, pancreas, vagus and pituitary gland.

The apical border of the antral G cells typically extends to the lumen and terminates there in a tuft of microvilli (Fig. 2.5); the secretory granules are concentrated in the basal portion of the cells. These characteristics provide a morphological basis for the idea that lamina stimuli might directly influence the release of gastrin into the
extracellular fluid. The predominant form of gastrin in antral G cells is the heptadecapeptide (G-17) and accounts for >95% of total COOH-terminal immunoreactivity with a concentration of 5-15 nmol/g (Mak 89, pp.317).

In the duodenal gastin cells small granules predominate and large electronlucent granules are relatively scarce (Buc 79). Intestinal gastrin concentration is 10%-50% of the antral gastrin. Over 50% of total COOH-terminal immunoreactivity is attributed to G-34 (Ber 71, Mal 76). The intestinal gastrin cells release gastrin into the circulation as antrectomy showed small but detectable mostly plasma G-34 after feeding in humans (Lam 82, Ste 73).

The apical microvilli of G cells are an indication that gastrin release is directly controlled by the luminal contents (Fig 2.5). There is also some evidence that the inhibitory effect of acid and the stimulating action of some protein digests are exerted indirectly.

Distension of the stomach (Sch 80) and luminal chemical stimuli release gastrin and it is apparent that this is related to the gastric phase. Chemical stimulation by peptone in the perfused rat stomach (Saf 80) and by intragastric instillation of amino acids in humans evokes also gastrin release by mechanisms that are reduced by low doses of atropine (Sch 82).

Nowadays it is well established that foods containing amino acids are good stimulants of gastrin secretion, whereas fat or carbohydrates are relatively poor gastrin releasers (Ric 76). Other substances that may stimulate gastrin secretion are calcium and caffeine (Wal 75).

Undigested protein is not particularly effective in stimulating release of gastrin, but the gastrin-releasing activity increases considerably after the liberation of amino acids and polypeptides by limited proteolytic digestion (Deb 74).

Earlier studies in dogs indicated that instillation into an antral pouch of various amino acids, particularly glycine and alanine, evoked acid secretion that was presumed to be mediated by gastrin (Elw 74).
Other amino acids that release gastrin are cysteine, phenylalnine and hydroxyproline (Kon 77, Str 78, Tay 82). It has been indicated that amino acids penetrate G cells to evoke release. (Lic 82).

As it is clear from fig 7.21 (Eys 72) the peak acid output happens between 30 to 50 minutes after the i.v. infusion of gastrins. On the other hand in fig. 7.20 the plasma gastrin reaches the peak after 10 to 20 minutes post-prandially (Doc 52). Taking into consideration the time course of the gastrin plasma after a meal as well as the time course of the peak acid output after i.v. infusion of those trials, the shorter (T50s) found in our experiments by EIE with the amino acid pre-treatment may be attributed to stimulation of additional gastrin secretion if it is considered that the release of the free amino acids in the antrum from the gelatine capsule during the water digestion process as the instillation of the free amino acids in the lumen of the stomach and specifically in the antral lumen. The chemically stimulated gastrin leads to additional secretion of gastric acid (on amino acid treatment) resulting in additional reduction of the post-prandial gastric impedance with the effect of shortening the T50 with The amount of the free amino acids in this study is lower than the amounts used in other experiments to observe a similar effect (1g to 20g by Ric 76, 30mmol by Tay 82). In the studies by Taylor (Tay 82) the meal’s pH was adjusted to 5.5 using sodium bicarbonate (0.5 M) or HCl (0.1 M) and the osmolarity was also brought to 280-300 mosmoles adding NaCl. This treatment might have had an effect on gastrin and acid stimulation in the sense that higher quantities of amino acids needed for the same result.

respect to other pre-treatments. The aspartame treatment had no effect since the two amino acids are bound to each other. Aspartame has to be hydrolysed first in order to liberate the amino acids and this takes time and if it happens before the emptying the stimulus it is logical that it would be weaker than in the free amino acid treatment.

Phenylalanine and tryptophan have been proven to be the most potent stimulants of gastric acid secretion and followed by aspartic acid. The aromatic ring may be responsible for the higher stimulation potency. In this study the time course of the response is more consistent with the stimulation on the gastric phase of acid secretion (Doc 80, Eys 84).
Amino acids also accelerate gastric emptying (Ric 76), and the shorter T50s may be attributed partly to the accelerated gastric emptying. It was also found (Tay 82) that gastric emptying of phenylalanine and tryptophan was delayed compared to saline and seven other amino acids. It is also important to mention that a 170g lean steak meal contains 12mmol phenylalanine and 3mmol tryptophan in 50g of total protein, considerably lower amounts of those used in the above experiments (Heg 64, Dav 72). All the above suggest that gastrin is the mediator of gastric acid stimulation when free amino acids enter the stomach.

There were studies (Deb 74) showing that amino acids may stimulate the parietal cells directly but this is unlikely to have happened in this study considering that the effect would be shown earlier with the water meal.

![Fig. 7.20: Time course plasma G-17 and G-34 responses to feeding in humans](reprod from Mak 89, pp320).

![Fig. 7.21: Time course of acid secretion to G-17 and G-34 in humans, in response to equimolar doses](reprod from Mak 89, pp328).
It has been shown also that duodenal or intravenous infusion of amino acids evokes stimulation of gastric acid secretion without an associated increase in plasma gastrin (Ise 78, McA 81). Since the free administered amino acids in the stomach enter the intestine, where they get absorbed and enter into the circulation, intestinal or circulating factors may contribute to the acid response. However the time course in our study does not support this idea since the peak response observed in the intestinal phase is during the second to fourth hour after administration (Ise 78).

In conclusion, the free amino acids, aspartic acid (140 g) and phenylalanine (260 g) ingested into the stomach in a gelatine capsule with 450 mL water may have stimulated further gastrin release followed by increased acid output after about an hour decreasing the intragastric impedance at a faster rate (gastric phase). This resulted in shorter T50s with statistical significance compared to the T50s of the other treatments (aspartame, and placebo). Aspartame consists of bound aspartic acid with phenylalanine and there is a reduced potency in stimulating gastrin release and if so at a later time course; this is because hydrolysis has to proceed to break the bond between the two amino acids with the consequence less potency on gastrin stimulation and delay on time for liberation of the bound amino acids. If the result was from the intestinal phase it would appear after the second hour and it would be impossible to record it.

If direct influence on the parietal cells had taken place the shorter T50s should appear on the water pre-load T50s, which was not observed, probably because of the time needed from the amino acids to be released from the gelatine capsule.

As an overall conclusion, the free amino acids, aspartic acid and phenylalanine, stimulated gastrin release during the gastric phase followed by additional gastric acid secretion in an hour, with the consequence of a faster decrease of the gastric impedance and shorter T50s. This includes an indication that gastric impedance measurements are influenced by gastric acid secretion.

As it was mentioned earlier, a faster gastric emptying might have resulted in shorter T50s as a greater desire to eat was found with the free amino acids treatment (Hal 2000).
8 SIGNAL ANALYSIS

8.1 Definitions

Most signals of practical interest, such as speech, biological signals, seismic signals etc are analogue or continuous time signals. Analogue signals can be modified to digital, represented by a sequence of finite numbers having finite precision. The procedure is called analogue to digital conversion (A/D) and the corresponding devices A/D converters. Then the digital signals can be treated as discrete time signals.

Signals may be functions of time, frequency, distance, temperature and the like. The present interest lies on the time-function signals known also as time-domain signals and on frequency-function or frequency-domain signals or simply on spectra. Frequency domain signals or spectra are usually generated from time domain signals by mathematical transformations.

A time function signal or a wave form can be represented approximately by a weighted sum (series) of basis signals. The basis signals can be expressed by sinusoidal or exponential functions exhibiting specific amplitude and phase. Thus the basis signals highlight the characteristics of the signal under study.

It is possible to approximate a continuous signal by Fourier series using complex exponential basis signals if the Dirichlet conditions (Car 92) apply to the signal. Then, Fourier transformation can be used which will result to the frequency domain corresponding signal.

All periodic signals of practical interest satisfy the three Dirichlet conditions, which are as follows:

a. The signal \( x(t) \) has a finite number of discontinuities in any period.

b. The signal \( x(t) \) contains a finite number of maxima and minima during any period.
c. The signal $x(t)$ is absolutely integrable in any period, that is:

$$\int_{-T}^{T} |x(t)| dt < \infty \quad (8.1)$$

A discrete time signal $x(n)$ can be represented as a series of basis signals using the Fourier series, as it follows (Dirichlet conditions are satisfied):

$$x(n) = \sum_{k=0}^{N-1} c_k e^{j2\pi k n/N} \quad (8.2)$$

where the $c_k$ coefficients are given by the following formula:

$$c_k = \frac{1}{N} \sum_{n=0}^{N-1} x(n) e^{-j2\pi kn/N} \quad (8.3)$$

Equation (8.2) is called the discrete-time Fourier series (DTFS) and the $c_k (k=0, 1, \ldots, N-1)$ provide the description of $x(n)$ in the frequency domain, in the sense that $c_k$ represents the amplitude and phase associated with the frequency components $e^{j2\pi kn/N}$.

Thus the spectrum of a signal $x(n)$ is a periodic sequence with period $N$.

Fourier transform of a finite energy discrete time signal $x(n)$ is defined by the following equation:

$$X(\omega) = \sum_{n=-\infty}^{\infty} x(n) e^{-j\omega n} \quad (8.4)$$

and it is proven easily that

$$x(n) = \frac{1}{2\pi} \int_{-\pi}^{\pi} X(\omega) e^{j\omega n} d\omega \quad (8.5)$$

Equations (8.4) and (8.5) consist the Fourier transform pair for the discrete time signals. The function $X(\omega)$ represents the frequency content of the signal $x(n)$.

### 8.2 Power spectrum

Energy signals and power signals:
The energy \((E)\) of a discrete time signal \(x(n)\) is defined by equation (8.6) and the power \((P)\) by equation (8.7):

\[
E = \sum_{n=-\infty}^{\infty} |x(n)|^2 \quad (8.6)
\]

\[
P = \lim_{N \to \infty} \frac{1}{2N+1} \sum_{n=-N}^{N} |x(n)|^2 \quad (8.7)
\]

The energy of a signal may be finite or infinite. If it is finite then \(x(n)\) is called an energy signal. Many signals that possess infinite energy, have a finite average power given by equation (8.7). If energy \((E)\) is finite then power \((P)\) is zero. If \((P)\) is finite and non zero the signal is called a power signal.

Signal energy and power can be used to indicate characteristics of a signal, but they are not actually measures of energy or power absorbed in or supplied by a system component when the signal passes through the component or is measured across it.

The impulse function is neither an energy nor a power signal because it has infinite signal energy and power. A periodic signal cannot be an energy signal, since its signal energy is always either zero or infinite.

The distribution of signal power as a function of frequency in a non-energy signal is of great interest and it is called power density spectrum or power spectral density (PDS). If equation (8.7) is modified in the way that it can be expressed in terms of the Fourier coefficients, it becomes evident that the average power in the signal is the sum of the powers of the individual frequency components. The mathematical procedure follows:

\[
P_x = \frac{1}{N} \sum_{n=0}^{N-1} |x(n)|^2 \quad (8.8) \quad \Rightarrow \quad P_x = \frac{1}{N} \sum_{n=0}^{N-1} x(n)x^*(n) = \frac{1}{N} \sum_{n=0}^{N-1} x(n)(\sum_{k=0}^{N-1} c_k^* e^{-j2\pi kn/N}) \quad \Rightarrow
\]

\[
P_x = \sum_{k=0}^{N-1} c_k^* \left[ \frac{1}{N} \sum_{n=0}^{N-1} x(n) e^{-j2\pi kn/N} \right] \quad \Rightarrow \quad P_x = \sum_{k=0}^{N-1} |c_k|^2 \quad (8.9).
\]

The sequence \(|c_k|^2\) for \(k=0,1,\ldots,N-1\) is the distribution of power as a function of frequency and it is called power density spectrum of the periodic signal. Equation (8.9) is known as a Parseval’s relation for discrete time periodic signals.

Another expression for PSD (power spectral density) is given by equation (8.10):

\[
P_{ds}(f) = F_d[T^R_{xt}(k)] \quad (8.10)
\]
Where \( F_d \) is the symbol indicating Fourier transformation and \( R_{xf}(k) \) is the autocorrelation function of \( x_f(n) \) given by equation (8.11):

\[
R_{xf}(k) = \lim_{N \to \infty} \frac{1}{2N+1} \sum_{n=-N}^{N} x_f(n)x_f(n+k) \quad (8.11)
\]

If \( k=0 \) then \( R_{xf}(0) \) is shown that represents the total signal power when integrated over the unambiguous frequencies in the discrete time signal, which is \(|f|\leq f_s/2=1/2T\).

This is expressed by the following:

\[
R_{xf}(0) = \frac{1}{f_s} \int_{-f_s/2}^{f_s/2} P_{da}(f) \frac{P_{da}(f)}{T} e^{i2\pi f(0)} df = \int_{-f_s/2}^{f_s/2} P_{da}(f) df \quad (8.12)
\]

and consequently the \( P_{da}(f) \) function represents the power density of the signal.

In practice, since the power density spectrum contains the autocorrelation of the original signal's function, enhances the amplitude of the periodic components relatively to non-periodic and special periodic characteristics of the signal can be extracted.

### 8.3 Motility analysis of the experimental data

As was mentioned in chapter 2 the stomach processes and empties its contents with mechanical periodic contractions. Also, the so called slow waves are running across the stomach and hunger contractions may happen in an empty stomach. The periodicity of all these activities is between 1 to 5 cycles per minute.

The sampling rate of the epigastrograph can be chosen to be either 1 or 5 Hz and it can be decided which to use according to the Nyquist theorem. Applying the principles of signal analysis based on Fourier transforms it is expected to extract gastric periodic contractions. In particular the data reflecting the time domain signal are transformed to the corresponding power density spectrum and are presented in the form of running spectra or water falls.

In detail, the time domain signal from the stomach is divided in epochs with an overlap between them and the number of the samples is always a power of 2 \((2^n)\). The power of 2 is necessary because the calculations are based on Fast Fourier Transform. Various lengths of the epochs were used and finally epochs with length 3 to 4.5 min
were chosen, because the frequency spectrum appears sufficiently clear from interference and includes the expected information. It seems that the epoch length acts as a filter and makes the power density spectrum appear quite clearly.

If the sampling rate is 1 Hz the epoch contains 256 samples (4.27 min) and the overlap is 64 samples (1.07 min). If the sampling rate is 5 Hz the epoch-length is 1024 samples (3.4 min) and the overlap 256 samples (51 s). The form of the presentation of the running spectra is the so called “water fall” and gives the possibility to observe changes in the frequency over time. The DaDisp software package is used because it is quite flexible, fast and it is not even necessary for the epoch length to be a power of two.

Fig. 8.1: Power density spectrum with meal D. The post-prandial gastric contractions appear clearly as well as rise of the peak-amplitude across the spectrum early post-prandially.

The running spectra in the “water fall” form of three different meals with the same volunteer and sampling rate 5 Hz, are presented in Fig. 8.(1,2). It is evident that post prandially the frequency of 3 to 4 cycles per min appears and it dominates for the rest of the time till the end of the test for 1 to 1.5 hrs. This is in accordance to the theory of
gastric motility and findings by other methods, mainly manometry and electrogastrography (Spy 93, Wie 81, Sma 94, Smo 94, Kil 96, Win 93).

![Fig. 8.2: Power density spectrum with meal F. The post-prandial gastric contractions of 3cpm are clear.](image)

The fasting period exhibits activity between 1.2 to 2.8 cycles per minute that is in the second phase of MMC and very often a component of 3 cpm corresponding probably to hunger contractions. The variation in the spectrum in the fasting state is probably due to long lasting multi-phase patterns in the motility to which, also, belong the so called hunger or "clearing up" contractions.

During the ingestion of the meal (and for 5 to 7 minutes later) different responses in the frequency spectrum are observed and the pattern depends on the conductivity of the ingested meal. If the ingested meal is conductive or non-conductive relatively to the gastric lumen, higher amplitude peaks across the spectrum are developed but decreasing progressively as the frequency increases for the earlier post-prandial time. With the ingestion of a neutral meal or if no impedance deflection occurs during the ingestion of the meal, this initial 5 to 7 minutes post-prandial period does not show peaks with increased amplitude across the frequency spectrum. The ingested meal has
equal volume in all situations. Before the introduction of the neutral meal, the above mentioned phenomenon in which the amplitude of the peaks is abruptly increasing

**Fig. 8.3:** Power density spectrum with water. The post-prandial contractions (2.7-3.1 cpm) are clearly visible. The fasting contractions also were detected. Disturbance across the spectrum at the very early post-prandial period is clearly shown too.

across the frequency spectrum was attributed to the sudden changes of the volume of the stomach (adaptive relaxation) in order to accommodate the meal. But since this phenomenon was not observed with the neutral meals another explanation has to be given.

If the principles of epigastrography are considered, the ionic content of the gastric mucosa and ionic gastric content form an electric circuit. An electronic impulse at a specific time at an electronic circuit can alter the frequency spectrum in the way that was described (Fre 2000). But the role of an electronic impulse, according to the author’s opinion, can play a solution that is ingested in the gastric lumen if its electrical characteristics allow it. Indeed, if the ingested meal is rich in ionic content
and considerably higher in conductivity (and consequently in conductance) than that of the gastric lumen, it behaves as an ionic impulse (positive ionic pulse decreases the resistance). The same happens if the ingested solution is considerably poorer in ionic content (i.e. water) with respect to gastric ionic content (negative impulse increases the resistance (Figs 8.4 & 5.6). In the situation of the neutral meal, the ionic impulse having the same conductivity as the gastric lumen has no effect despite the same ingested volume of the ionic substance (Figs 8.7 & 8.8). Thus, it can be said that the solutions when introduced in the gastric lumen play the role of ionic impulses and the magnitude of their effect depends on their electrical properties.

![Graph](image)

**Fig. 8.4:** Profile of the epigastric impedance of a non-conductive meal (water; 0.5 mS/cm).

This phenomenon supports the hypothesis that the volume of the meal does not affect the measurements of the epigastric impedance but only the concentration of the ions in it with respect to that of gastric content and of course the type of ions present. Ingested ions may cause interactions with those existing in the gastric lumen. If NaCl ions enter then no chemical reaction is taking place. But if a base is used, for example NaOH, then a degree of neutralisation takes place with water production.

In Figures 8.5 & 8.6 the epigastric signals using water and NaCl solutions as test meals are presented as well as the corresponding frequency spectrum. The spectrum's dependence on the conductivity, is clearly shown.
Fig. 8.5: Power density spectrum using a non-conductive meal. The sudden rise of the peak amplitude during the early post-prandial time is clear.

Fig. 8.6: Power density spectrum using a conductive meal. The sudden rise of the amplitude of the peaks during the early post-prandial period is clearly shown.
Fig. 8.7: Epigastric impedance variation with a "neutral meal (saline 4.5 mS/cm); no impedance deflection during the ingestion of the meal."

Fig. 8.8: Power density spectrum of the neutral meal (Fig. 8.) in which no sudden rise of the peak amplitude in the early post-prandial time is observed.

The peak amplitude of the spectrum during the ingestion phase and shortly after is strongly dependent on the conductivity of the ingested meal.
The total area under the peaks (E) during the ingestion and early post-ingestion period of the meals (4 epochs) in the frequency spectrum, as well as the total area under the peaks of the same frequency and equal number of epochs pre-prandially (E1) and post-prandially after the 4 first post-prandial epochs (E2) are presented in Table 8.1:

As the ratios E/E1 and E/E2 show, the total power of the spectrum for the range of frequency 2cpm to 12 cpm for the neutral meal (saline b) is almost the same in the 3 periods since the ratios are 0.91 and 1.10 respectively; as the conductivity changes to either direction of the neutral meal the ratios take values higher than 1 progressively.

The changes are shown in Fig. 8.9

**Table 8.1:** Total area under the peaks of 4 epochs in sequence (E1-pre-prandial, E-since ingestion, E2-post prandial) and their ratios of different in conductivity meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Cond (mS/cm)</th>
<th>AUC E (4 ep ing)</th>
<th>AUC E1 (4 ep pre)</th>
<th>AUC E2 (4 ep-post)</th>
<th>E/E1</th>
<th>E/E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.5</td>
<td>3.62</td>
<td>0.25</td>
<td>0.54</td>
<td>14.48</td>
<td>6.70</td>
</tr>
<tr>
<td>Saline a</td>
<td>2.2</td>
<td>2</td>
<td>1.09</td>
<td>1.82</td>
<td>1.83</td>
<td>1.10</td>
</tr>
<tr>
<td>Saline b</td>
<td>4.5</td>
<td>3.13</td>
<td>3.45</td>
<td>3.14</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td>Saline c</td>
<td>5.5</td>
<td>7.72</td>
<td>3.31</td>
<td>1.81</td>
<td>2.33</td>
<td>4.27</td>
</tr>
<tr>
<td>Soup</td>
<td>12</td>
<td>1.3</td>
<td>0.13</td>
<td>0.29</td>
<td>10.00</td>
<td>4.48</td>
</tr>
</tbody>
</table>

**Fig. 8.9:** Graphical presentation of the ratios E1/E(blue points) & E2/E (pink points) of Table 8.1. It is clear that in the neutral area of the conductivity the ratios show the lowest value. The polynomial fitting helped to show the symmetry of the ratios with respect to vertical direction in neutral area of the conductivity (4-5 mS/cm).
In the frequency spectra the breathing (respiration) frequency is always present and it can be related to the gastric function in respect to rate of respiration and power of respiration during the digestion process.

The running spectra using the model of resistors simulating the abdomen and for 27 Ω resistance of the abdomen showed a uniform distribution of peaks across the spectrum throughout the time.

The analysis of the gastric motility was not finalised. It remained in the stage of observing and recording peaks and their frequency. Peaks in the range of frequency at which corresponds the gastric motility were easily detected as well as in the range of the breathing frequency. In the post-prandial period, the frequency range corresponding to gastric motility, was from 2.5 to 3.5 cycles per minute (cpm). The results, according to the author's opinion, indicate that the frequency depends more on the individual than on the meal (milk-shakes and water); to clarify, differences on the frequency amongst individuals can be detected easier than between different test meals on the same individual. Probably for liquid meals and normal subjects, the differences (if any) in the contractile activity are small and difficult to be detected. The frequency component of the gastric contractions was always present in the analysed data but a systematic analysis with statistical consideration between different individuals and meals was not proceeded adequately. The amplitude of the peaks as well as the area under the peaks was not considered.

Quantification of the above per study i.e frequency of the detected peaks, amplitude and area under the peaks as well as statistical analysis of these to examine if there are significant differences in the power of the spectrum due to the different meal synthesis and energy load have to be explored.

Concluding, the following information about gastric motility can be provided by EIE:

a. In the post prandial period and for 7 minutes at least after the ingestion of the meal, frequencies in the range 2.5 to 3.5 cpm can be detected in the running spectra and is believed by the author that correspond to post-prandial gastric contractions.
b. In the pre-prandial period, frequencies lower than and/or equal to 3 cpm are usually detected with distinguishable high amplitude occasionally, probably due to the so called hunger contractions.

c. The ingestion phase and the early post-prandial period, show different pattern in the frequency spectrum, which depends on the electro-chemical properties of the ingested meal and specifically on the conductivity. If the meal is "neutral" with no buffering capacity (about 4.5 mS/cm at room temperature), no peaks are formed in this period, but if the meal is either conductive or non-conductive abruptly risen peaks across the spectrum appear. This shows according to the author's opinion that the meal acts as an ionic impulse in the gastric lumen.

Thus, EIE is a promising method in detecting the gastric contractile activity and studies on subjects with impaired gastric motility are necessary to investigate if there are differences on the frequency values and the amplitude of the peaks.

More sophisticated methods as wavelets and filtering may detect easier from the data differences on frequency and amplitude of the peaks amongst individuals and meals.
9 DISCUSSION AND SUMMARY OF CONCLUSIONS

The Epigastrograph was designed to study the stomach’s function, its emptying and contractile activity (motility).

The general conclusion combining the results of all studies, which were conducted, is that EIE is able to provide differences with statistical significance in the values of the T50 and percentages resulting from the post-prandial changes in impedance, if meals of the same volume are used but different in calorific and nutrient content or the same meal under intravenous infusion of various peptides (GLP-1, loxiglumide) or instilling nutrients in the gastric lumen contained in a gelatine capsule (phenylalanine, aspartame, free amino acids, corn-flour). The resulting values are generally shorter than expected according to existing knowledge, literature and other methods which were applied simultaneously (scintigraphy, paracetamol absorption, octanoic acid breath test). The discrepancy becomes larger as the colorific and nutrient content becomes higher. It is thus believed by the author, that the T50s by EIE is unlikely to represent half emptying time of the ingested meals. The question arises: what then do they represent or what physiological parameter do they reflect. Detailed consideration of the information obtained from that work follows in an attempt to clarify this matter and summarize and summarise the findings.

Plethysmography is generally understood as the measurement of volume. Dynamic plethysmography is usually associated with volume changes, which may be related, for example, to respiration or peristaltic movements of the gastrointestinal (GI) tract or as in this study the change of the volume of the gastric muscular bag as food enters into it and as food goes out of it.

In many applications the absolute volume may remain unknown with emphasis given to the relative change in volume. As has already been mentioned (section 7.5) estimation of the volume is based on the impedance which is developed if an alternating current is applied to the object assumed as a cylinder, according to the following Equations 9.1:

\[
I = \frac{1}{\sigma S} \quad (9.1a) \quad \text{or} \quad I = \frac{1}{\sigma V} \quad (9.1b)
\]
where \( I \): impedance (\( \Omega \)), \( l \) is the length of the cylinder, \( S \) is the cross sectional area of the cylinder (m\(^2\)), \( V \) is the volume (m\(^3\)) of the biological object, and \( \sigma \) its conductivity (S/m).

According to Equation 9.1a two conditions are implicated in the measured impedance, which are:

i) The geometrical fraction \( l/S \), where \( S \) is equal to \( \pi r^2 \) and \( V \) is the volume of the cylinder. If the volume of the biological regions under study increases, for example, by an increment in length \( l \), the impedance will rise; but if it is a result of a circumferential (diameter) swelling then it will decrease. This dictates a priori knowledge on the change of dimensions during the biological process under study. Yet, a volume change may be involved outside the region of the body under study, which may alter the measured impedance.

ii) The conductivity of the object under investigation. The conductivity may take different values during a process that influence inversely the measured impedance, proportionally.

Partial differentiation of equation (9.1a) after transformation to logarithmic form, gives the possibility to investigate the fractional changes of these variables:

\[
\frac{dl}{I} = -\frac{d\sigma}{\sigma} + \frac{dl}{l} - 2\frac{dr}{r} \tag{9.2}
\]

Equation 9.2 shows that the geometrical fraction factor changes in a competitive way between the two dimensions \( r \) and \( l \) of the object which develop the impedance. More specifically if the fractional change in radius \( dr/r \) is half of that in length fractional change \( d\ell/\ell \) there is no geometrical influence and the impedance depends on the changes due to conductivity.

The stomach was the organ of the body studied with impedance plethysmography since it changes its volume as the amount of contents in it changes.

Most of the work done was based on the inverse proportionality between changes in volume and impedance (Sut 89, McC 85, Fen 94). The changes in the conductivity of the gastric content because of the gastric secretions were considered as interference,
which introduced an error in the emptying rates (McC 85, Avi 87). A number of researchers, tried to overcome this interference by inhibiting the gastric acid secretion using inhibitory drugs such as cimetidine (Man 88), which results in a better repeatability, and also omeprazol (Eva 90); in the study by Evans and Wright (Eva 90) the T50s with omeprazol and cimetidine pre-treatment were shorter than with placebo (using EIT). Other researchers also claimed that it was not necessary to block the gastric acid secretion (McC 85, Eva 90) in gastric studies based on electrical impedance.

A study with omeprazol within the group (Lig 96) showed longer T50s by 10% with omeprazol pre-treatment using the non-conductive test meal, the high calorific milkshake F.

Under gastric acid inhibitor pre-treatment, delayed gastric emptying is expected since the chemical factor for disinfection and elimination of the food particle size is reduced and falls between 5% to 10% of its normal secreted amounts (Wyk 94, Ker 91). In a study (Wyk 94) the gastric emptying of liquids with omeprazol pre-treatment was delayed significantly during the first 45 minutes post-prandially (paracetamol absorption method) but not towards the end of the study. In another study (Ker 91), cimetidine pre-treatment delayed the half-emptying time of a burger meal from 146±15 min to 187±16 min (scintigraphy). But there are also contradictory results on gastric emptying rates after pre-treatment with gastric secretion inhibitors, such as accelerated gastric emptying (For 79) or unaffected gastric emptying (Hou 87, Hea 77).

In using an acid inhibitor the physiological digestion condition is modified and the observations may not reflect the true conditions. The inhibitors lessen the gastric acid output but do not inhibit completely the production. A study (Ver 95) with 24 hour ambulatory intra-gastric pH measurements in H pylori positive and negative subjects, with and without omeprazole treatment, concluded that under omeprazole treatment controls showed an elevation of pH by a half or one pH unit and a broader dispersion of acidity. It was concluded that acid secretion is not inhibited completely using omeprazole, which is considered the most effective inhibitor, but there is a shift to higher pH values within the acidic range on omeprazol treatment and differences on
pH during the 24 hours occur parallel to those without omeprazol treatment. Since EIE is based on impedance differences use of gastric secretion inhibitors may not be answer for determining unbiased emptying rates.

In another study (Bax 88) researchers tried to relate the geometry and conductivity dependence of the measured impedance by instilling liquid test meals of the same volume but different pH or the same pH with different volume as well as different conductivity. The results were expressed as percentages of the maximum indication in all situations and the conclusion was that the impedance changes were closely correlated with changes in volume ($r=0.80$), acidity ($r=0.83$) and total conductivity ($r=0.87$). They also measured the impedance changes after administration of 6μg/kg pentagastrin. The final conclusion was that gastric impedance measurements with EIT follow the acidity of the gastric contents which depends on the gastric acid secretion. An additional personal comment: it seems to the author that volume, acidity and conductivity influence equivalently the measured impedance.

There are experimental studies by EIE in which the conductivity of the gastric content was measured by aspiration during the digestion (McC 85). A change was measured from 2.23 mS/cm at the start to 2.55 mS/cm after 5 minutes to 3.4 mS/cm after 10 minutes to 6.54 mS/cm after 15 minutes and so on for 500 mL of orange squash. If the geometrical factor is ignored then the percentage change in impedance is as follows:

$$DI/I_5=(2.55-2.23)/2.23=0.32/2.23=0.14 \text{ or } 14\% \text{ in 5 minutes,}$$

$$DI/I_{10}=Ds/s_{10}, (3.40-2.23)/2.23=1.17/2.23=0.52 \text{ or } 52\% \text{ in 10 minutes etc.}$$

It is concluded that the changes in conductivity have a major contribution to impedance changes.

-Physiological consideration of changes of the stomach with the ingestion of a meal:
The mucosa of the adult human has a surface area of 800 cm$^2$ a volume of 50 cm$^3$ and a thickness of 0.06 cm (Joh 81). Eighty percent of the mucosal volume and surface area is located in the fundus. The parietal cells ($10^5$) correspond to 1/3 of the cell mass and secrete a maximum of 35 mEq/hr acid in a volume of 250 mL. The
parietal cell mass and secretory capacity of a 20 kg dog are similar to those of man. There are $10^4$ pits/cm$^2$ of gross surface area in the dog with an average diameter of 25 μm and total cross-sectional area of 0.05 cm$^2$/cm$^2$ corresponding to 5% of gross surface area. The surface area of the tubules (5 tubules per pit; average diameter 10 μm; average length 300 μm) is 5 cm$^2$/cm$^2$ of gross surface area. It is said that 80% of the luminal surface is embedded in tubules. The apical (canalicular) surface of the parietal cells is 50 cm$^2$/cm$^2$ of gross surface area in the resting state and 500 cm$^2$/cm$^2$ in the secreting state which means that the tubules expand the area of the lumen by a factor of 5; the parietal cells expand it further by a factor of 10 in the resting state and by a factor of 100 in the secreting state (Joh 81). It is evident that the changes occurring between rest and secretion statuses affect tremendously the electrical resistance of the mucosa. A fall in the mucosal resistance is associated with the expansion of the mucosal surface area, assuming constant resistance per unit area; an additional decrease of higher importance is due to the onset of acid secretion since this means appearance of H$^+$ on the mucosal surface, with progressively increasing concentration. Multiple ion channels are opening across the gastric mucosa. Is this the answer to the ambiguity as to which is the most significant contribution factor in the development of the epigastric impedance measurements? The author considers this to be the case.

Concluding, according to physiological mechanisms and observations the mucosal area expands uniformly by 100 fold from the resting state to the fully stimulated state of secretion and H$^+$ ions are generated and transferred on the expanded mucosa and flow across the mucosa with a progressive increase in concentration during the course of stimulation. This change directly affects the conductivity of a medium and consequently the measured impedance. According to Equation 9.2 this physiological change exceeds the range of changes, which may occur in the geometrical factor, which also influences the measured impedance.

In the electrical field of a medium formed by applying an electrical current across it when another medium is inserted in it alters its electrical properties, such as conductivity. If the medium introduced alters its conductivity to a considerable extent, for example, changing it from a non-conductive to a highly conductive medium with respect to the surrounding medium, then it controls the measured
impedance of that medium. According to the above mentioned undoubted changes taking place in the gastric mucosa from resting to stimulated states, the following should be noted:

a) Resting to stimulation of gastric secretion status using a stimulant: In a study by Baxter et al (Bax 88), measurements of the impedance in the resting state showed that fluctuations correlated well with the basal acid output; but after intravenous administration of pentagastrin a noticeable decrease in impedance was measured (fasting stomach) which correlated also with the gastric acid output. This can be explained by the transformation of the mucosa from a conductive medium to a higher degree conductive medium on administraton of the stimulant substance; the stimulant caused a flow of $H^+$ across the mucosal surface. Of course the electrical field is extended in the cavity of the stomach where the gastric secretions are accumulated.

b) Stimulation by a meal filling the stomach (post-prandially): As it has already been mentioned the impedance of a liquid non-conductive meal post-prandially decreases. The distension of the gastric mucosa because of the volume of the ingested liquid meal (450 mL in our experiments) is a stimulus for gastric acid secretion (section 3.3). As $H^+$ ions start to flow across the mucosa the gastric impedance decreases; the $H^+$ ions enter into the gastric content and mix with it until the concentration of the $H^+$ in the gastric content approaches that of the gastric mucosa. After that equilibrium, the epigastric impedance in most situations remains the same for a period of time and the expression post-prandial base-line is frequently used. It is expected that later the impedance level will be at about the same as the level in the fasting state. It was considered that stomach emptying had been complete at the start of the post-prandial baseline, based on the impedance decrease due to changes of the volume of the non-conductive gastric content. The calculated emptying rates based on the impedance measurements in the present work were systematically shorter than those expected from the literature and those given by other methods. This was also illustrated in our comparison study with scintigraphy. This discrepancy further supports the hypothesis that the impedance is altered by the gastric conductivity controlled by the acid secretion and that the post-prandial baseline shows an
equilibrium between the flow rate of the H\(^+\) ions across the gastric mucosa and the H\(^+\) ions concentration in the gastric content. The emptying is not complete by the onset of the post-prandial baseline but is still in progress and the acidity at this stage has reached the necessary upper limit required for disinfecting and hydrolysing the gastric content. From this time and onwards, a maintaining flow of H\(^+\) ions is retained and the gastric acid output starts declining.

Statistically significant differences in the T50s were found with longer T50s for the more complex and higher calorific meals under the same conditions (CHO study, Fat study, EIE-Scint study). This can be attributed to the prolonged time of the gastric acid secretion needed for the hydrolysis of the additional nutrients, carbohydrates and fat, since CHO and fat are inhibitors of gastric acid secretion and only their volume is involved as a stimulant. It is unlikely that the decrease in impedance represents decrease in volume entirely. Of course it can be argued that the longer T50s with the higher content of CHO and fat are associated to longer emptying rates (as shown already by other studies). The direct comparison of the results obtained, applying simultaneously EIE and scintigraphy, lead to the conclusion that the shorter emptying rates are due to the gastric acid secretion as a parallel simultaneous physiological process to gastric emptying. Thus, it is believed, that the T50s based on impedance measurements represent the half time needed for the acidity of the gastric content to reach the value needed for proper digestion.

In the study with the infusion of GLP-1, a longer average T50 resulted. This may be attributed to the inhibition of gastric acid secretion, which has been shown to be associated with infused GLP-1 by other studies. Again GLP-1 infusion is associated with delayed gastric emptying and it can be argued also that the larger remaining volume under GLP-1 infusion compared with the controls, results in a higher impedance and therefore longer emptying rates.

Loxiglumide being a CCK antagonist stimulates gastric acid secretion and accelerates gastric emptying. In the loxiglumide study the statistically significant shorter T50s and post-prandial percentages obtained can be associated with the stimulation of gastric acid secretion or the shorter emptying rates shown by other
studies. The decrease in impedance with loxiglumide infusion during the pre-prandial period indicates that the measured impedance depends on the gastric secretions.

The drifting of the pre-prandial baseline was initially thought to be due to electronic drifting but reconsidering the matter, it is now believed that it may also be due to the secretory changes of the secretory status of the gastric mucosa, during the pre-prandial period too.

Another possible reason of downward drifting of the pre-prandial baseline may be due to better skin-electrode contact as time progresses and to the rising temperature depending on the skin of the individual (Ama 88, Con 87).

The aspartame study resulted in shorter T50s with the free amino acid ingested capsule one hour before the medium calorific milk-shake (E) was given. This can be attributed to further chemically stimulated gastric acid secretion by the action of the released capsule content on the gastrin cells of the antral area. The time course from the release of the amino acids to stimulation of the gastrin cells to elevated gastrin plasma levels and stimulation of gastric acid secretion by other studies (section 7.8) is comparable with the time course in the present study. In the placebo study, of course, there were no chemical stimuli and with the aspartame capsule the time course is expected to be longer if it is considered that absorption of the hydrolysed aspartame takes place in the intestine. The stimulus, if any, is expected to occur between the 2nd and 4th hour after ingestion, and it is therefore not possible to detect in the shorter duration of our experiments. The quantity of the free amino acids used is much less than that used by other methods and it can be argued that it should not cause any effect; but it is believed that the 400 mg released in the antral area do provide a stimulus to gastrin cells.

The ingesting phase of the meal controls the response of the epigastrograph and the profile of the digestion. The condition of measurement is a fasting stomach for at least 6 hours. Until now researchers have not considered the physico-chemical changes in the gastric lumen environment during the ingestion of a meal because of the existing content in the stomach but only the volume of the ingested test meal. It is really a tricky situation. It was considered until now that if a non-conductive meal with respect to the surrounding tissues enters the stomach it shows a higher epigastric
impedance dependent on the meal’s volume than in the fasting phase; if a conductive load enters into the stomach a lower epigastric impedance results. The following are considered important by the author for the ingestion phase:

When a quantity of a liquid is swallowed by a healthy person in a semi-supine position, the liquid bathes the mucosa. The luminal surface of the mucosa is acidified even in a fasting stomach (pH: 2-2.5). Of course other ions are also secreted in the gastric cavity (Na⁺, K⁺, HCO₃⁻, Cl⁻) but because the mobility of the H⁺ ions is higher than the rest these ions occupy the outer layer. If a non-ionic liquid (non-conductive) is swallowed then the H⁺ ion concentration decreases and a higher epigastric impedance is measured. With each swallow the dilution becomes greater and the impedance increases. It is the interaction between the ingested liquid and the gastric juice probably already present that controls the epigastric impedance.

If the ingested liquid is ionic with conductivity higher than that of the gastric mucosal surface and the secreted acid probably present, the swallowing of this liquid test meal enhances the gastric lumen’s conductivity since the electrolyte (ionic) concentration becomes higher and as a result a lower impedance than in the fasting state is measured.

The gastric acid existing already in the fasting stomach, which alters its ionic concentration with the ingestion of a liquid meal had not been taken into consideration in any previous studies. Washing the stomach with saline before ingestion of a liquid test meal through a tube is equivalent to mechanical pressure on the gastric lumen and this causes gastric secretion (Bea 1833). The distension also of the stomach because of the meal causes immediately gastric acid secretion. Thus, in the study by Baxter et al (Bax 88) the strong correlation (R=0.83) between the changes in impedance with respect to ingested volume can be attributed, according to this author’s opinion, to the changes of the gastric conductivity because of ionic concentration dilution by the ingestion of different volumes of the meal but not of the volume itself. If the same volume of a solution with different pH is instilled at different times of course it is of course expected different impedance is measured since this involves different conductivity.
The conclusions from the comparison study between EIE and scintigraphy led the author to introduce the concept of the "neutral" meal. The neutral meal and the gastric content are solutions equivalent in conductivity. As has been described before the null initial impedance deflection due to ingestion of the meal and the decrease in impedance thereafter shows that the role of the volume in the measured impedance is insignificant and that the changing ionic (H\(^+\)) concentration dominates the impedance. This is in accordance with conclusions reached from electro-physiology about the alteration in the morphology of the gastric mucosa and changes in its electrical resistance from the resting state to secretion (stimulated) state. As was mentioned the conductivity of the neutral meal is about 5.5 mS/cm at 37°C. It is recommended that the composition of the neutral meal should not include buffering components, so that the dilution is the main process and not a chemical interaction which will complicate the situation.

It has been clarified that after the ingestion of the meal, gastric acid secretion is stimulated because of the gastric distension and of the chemical and hormonal nature of stimulation depending on the constituents of the meal and the presence or absence of nutrients in the intestine. The flow of the hydrogen ions across the distended mucosa enhances the mucosal conductance and the gastric content's conductivity. This results in lower measured impedance until an optimum concentration of H\(^+\) is reached and then the post-prandial baseline is established. This is the situation with non-conductive and neutral meals.

In the case of conductive meals, just after the ingestion phase higher impedance is measured progressively. This was attributed to the decrease of the volume of the conductive gastric content relatively to the surrounding tissues because of gastric emptying. But after a certain point the impedance starts to decrease. It can be argued that the initial rise in impedance after meal ingestion is due to water flow into the gastric lumen causing dilution of the existing ions. This is supported by the fact that the conductive test meals used were hyper-osmotic as the measurement of their osmolarity showed. The hyperosmolarity is due to the salt (NaCl) used in order to achieve the required conductivity for the meal. It has been proved that hyper-osmotic gastric contents cause a stream of water to flow into the gastric lumen in order to decrease osmolarity and approach isotonicity. Of course, the flowing water does
contain hydrogen ions but not initially in high enough concentrations to exceed that of the hyper-osmolar gastric load. In this way it dilutes the existing concentration and a rise in impedance is measured. As the osmolarity becomes lower less water flows and the concentration in H⁺ becomes higher the impedance starts declining again (Fig. 6.4). This is another indication of ionic influence.

The use of the neutral meals may provide a useful application as explained below. The simplest test meal after water is a sodium chloride solution (NaCl). It is a non-nutrient meal, inert chemically, compatible to life for a range of concentrations and necessary for life. The additional advantage of NaCl solutions in EIE is that the selected conductivity can be achieved based on its concentration.

A NaCl solution with conductivity in the “neutral range” ingested as a meal shows no change in the measured impedance, meaning that the gastric luminal environment possesses the same conductivity as the NaCl solution used. The gastric lumen’s conductivity depends mainly on the hydrogen ions since an acidic environment exists and because of the higher mobility of the hydrogen ions with respect to the rest. As the ions flow across the mucosa, hydrogen ions because of their higher mobility occupy the outer layer with respect to the mucosal surface and first come in direct contact with the ingested test solution. Under these conditions the molar conductivities of the NaCl and HCl molecules can be used and the calculation of the concentration of the HCl acid in the stomach is possible.

The molar conductivity (equivalent conductance) Λ is the conductivity per mole of solute per volume according to Equation 9.3:

$$Λ = \sigma / c \quad \text{or} \quad \sigma = Λ c \quad (9.3)$$

where: c is the concentration of the solute in mol/L, and σ the measured conductivity.

Equation (9.3) can be applied to the ingested NaCl solution and for the gastric HCl solution. The values of the molar conductivities are given by tables.

According to Equation 9.3 the calculation of the HCl gastric acid concentration in the fasting stomach becomes possible if a NaCl iso-conductive solution is ingested. In
practice the ingested NaCl solution does not cause an impedance deflection in which case it is concluded that the conductivity of the gastric lumen is equal to the conductivity of the ingested sodium solution.

The conductivity of the neutral meal for an individual can be found if the individual undertakes at least 3 tests with NaCl solutions differing in conductivity. The ingestion phase of the tests is of importance. The results of a such study are briefly presented below:

The measured epigastric impedance deflection was obtained from a study with a healthy, young, student volunteer on different occasions with various test meals ranging from non-conductive to conductive and given in Table 9.1. According to Table 9.1 impedance changes depend strongly on the conductivity of the meal even if the meal consists of different ingredients but has the same volume (450 mL). As Fig. 9.1 shows there is a strong linear correlation between the percentage impedance deflection just after the ingestion of the test meals over the pre-prandial impedance and the meal conductivity ($R^2=0.93$). The conductivity of the saline test meal to cause zero deflection according to the linear fitting for this subject has to be about 4.2 mS/cm.

**Table 9.1: Impedance deflection by the ingestion of meals with varied conductivity but the same volume (450 mL with the same subject)**

<table>
<thead>
<tr>
<th>Meal</th>
<th>Conductivity (mS/cm)</th>
<th>Absolute imped deflection (Ω)</th>
<th>Pre-pra imped (Ω)</th>
<th>% imped deflection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water (1)</td>
<td>0.45</td>
<td>1.6</td>
<td>17.4</td>
<td>9.2</td>
</tr>
<tr>
<td>Tap water (2)</td>
<td>0.45</td>
<td>2.7</td>
<td>21.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Saline water</td>
<td>2.12</td>
<td>0.6</td>
<td>14.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Saline water</td>
<td>4.5</td>
<td>-0.26</td>
<td>17.6</td>
<td>-1.5</td>
</tr>
<tr>
<td>Saline water</td>
<td>5.5</td>
<td>-0.46</td>
<td>21.1</td>
<td>-2.2</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>0.6</td>
<td>1.32</td>
<td>17.9</td>
<td>7.4</td>
</tr>
</tbody>
</table>
As was mentioned before, the gastric lumen is iso-conductive with the ingested meal if no deflection is caused. The predominant ions are hydrogen ions and chloride ions as was explained earlier (hydrogen higher mobility, profile of ions Na⁺/H⁺ etc.) If equation 9.1 defining molar conductivity is applied and the resulting value of the conductivity from the fitting (4.2 mS/cm) is used, then the concentration of the gastric acid can be calculated. The molar conductivity of electrolytes and ions has been measured and given by tables. There is a change of the molar conductivity due to concentration of the solution but for strong electrolytes such as HCl and NaCl the change is negligible (Table 9.2). The HCl concentration in the stomach is in the range 5 to 100 mmol/L; thus it is acceptable to use the molar conductivity of the concentration 10 mmol/L which is 0.0412 mS m² mol⁻¹ (Table 9.2). Using the Equation 9.3, the following resulted:

\[ c_{HCl} = \frac{\sigma}{\Lambda_{HCl}} \Rightarrow c_{HCl} = \frac{4.2 \text{ mS/cm}}{0.0412 \text{ mS m}^2 \text{ mol}^{-1}} \Rightarrow \]

\[ c_{HCl} = \frac{0.42 \text{ S/m}}{0.0412 \text{ mS m}^2 \text{ mol}^{-1}} \Rightarrow c_{HCl} = \frac{0.42}{0.0412} \text{ mol/m}^3 = 10.2 \text{ mol/m}^3 \Rightarrow c_{HCl} = 10.2 \text{ mmol/L} \]
This value of a fasting stomach of a healthy subject is consistent with the literature values (Bar 78, Rea 89).

It is concluded, therefore, that the epigastrograph with the stomach under certain conditions may be composed of a biological conductivity cell that can be calibrated with NaCl solutions and sequentially to estimate the concentration of the HCl gastric acid of an empty stomach.

**Table 9.2: Molar conductance (λ) in S m²/mol in aqueous solution at 25°C**

<table>
<thead>
<tr>
<th>c 10^{-3} (mol/L)</th>
<th>HCl</th>
<th>NaCl</th>
<th>KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0426</td>
<td>0.01264</td>
<td>0.01498</td>
</tr>
<tr>
<td>0.5</td>
<td>0.0423</td>
<td>0.01245</td>
<td>0.01478</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0421</td>
<td>0.01237</td>
<td>0.01469</td>
</tr>
<tr>
<td>10.0</td>
<td>0.0412</td>
<td>0.01185</td>
<td>0.01413</td>
</tr>
<tr>
<td>100.0</td>
<td>0.0391</td>
<td>0.01067</td>
<td>0.01289</td>
</tr>
<tr>
<td>1000.0</td>
<td>0.0333</td>
<td></td>
<td>0.01119</td>
</tr>
</tbody>
</table>

It is important to give emphasis once more to the absence of ionic migration (impedance deflection zero) for a specific conductivity indicating that the volume of the ingested meal does not affect the measured epigastric impedance. Volume is involved in the measured impedance indirectly by altering the ionic concentration in the gastric lumen.

Consequently, the T50 parameter based on EIE is related to ionic content in the gastric lumen and since the hydrogen ions (HCl acid) are the predominant feature, as it was made clear throughout this work, the changes in the impedance are due to the acidic concentration of the stomach; according to the author's estimation the T50 given by epigastrography, is the time needed for the pH of the gastric load to drop to half of its peak value. The measurement of the gastric pH after a steak meal in healthy subjects (Fig.7.10B, Sle 98) support this hypothesis.

Simple laboratory experiments were carried out simulating the condition of the ingestion of a “neutral” saline solution into the stomach. For this purpose HCl acid solution and NaCl solution were prepared having the same conductivity equal to
4.54± 0.3 mS/cm. Then, their pH was measured and found for HCl to be equal to 2.35 and for the NaCl solution 7.71. The pH of the HCl acid in the range of the fasting normal stomach (Bar 78, Rea 89) indicating that the results from the healthy young volunteer closely follow reality.

Mixtures of the two electrolytes were formed and their conductivity was measured. Solution (A) was formed by mixing water (10 mL; 0.45 mS/cm) and HCl (4.5 mS/cm) and solution (B) by mixing NaCl (10 mL; 4.5 mS/cm) and HCl (4.5 mS/cm). The results are presented in Table 9.3 and Fig. 9.2. It becomes clear that there is a stable conductivity with solution (B) and evident changes in (A) for the same proportionality of the HCl in both. The final conductivity of solution (A) was 3 fold that of the initial (1.50/0.50) and in (B) remains almost unchanged.

Table 9.3: Synthesis of solutions A and B and their conductivity.

<table>
<thead>
<tr>
<th>Vol of HCl (mL)</th>
<th>Conduct of A (mS/cm)</th>
<th>Vol of HCl (mL)</th>
<th>Conduct of B (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.48</td>
<td>0</td>
<td>4.13</td>
</tr>
<tr>
<td>2</td>
<td>0.49</td>
<td>2</td>
<td>4.02</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>4</td>
<td>3.94</td>
</tr>
<tr>
<td>6</td>
<td>0.51</td>
<td>6</td>
<td>3.84</td>
</tr>
<tr>
<td>11</td>
<td>0.75</td>
<td>11</td>
<td>3.87</td>
</tr>
<tr>
<td>16</td>
<td>0.98</td>
<td>16</td>
<td>3.95</td>
</tr>
<tr>
<td>21</td>
<td>1.17</td>
<td>21</td>
<td>4.01</td>
</tr>
<tr>
<td>26</td>
<td>1.34</td>
<td>26</td>
<td>4.04</td>
</tr>
<tr>
<td>31</td>
<td>1.51</td>
<td>31</td>
<td>4.08</td>
</tr>
<tr>
<td>36</td>
<td>1.66</td>
<td>36</td>
<td>4.12</td>
</tr>
</tbody>
</table>
Water is suitable as a test meal for gastric function studies. With its volume causing distension of the stomach can stimulate gastric acid secretion. Water is not a chemical stimulant, and it is not a nutrient meal; but it will cause a degree of additional stimulation of gastric acid secretion because of the dilution of the gastric acid that causes. It will be passed to the duodenum after it becomes acidified, so it may stimulate additional secretion than that by distension only. It may not elicit the gastric function in full but it can show at least if stimulation by distension is possible.

Water tests with a number of young Greek subjects (see water studies), obese subjects and diabetics showed no deflection at all. This can be explained if the intestinal involvement into gastric acid secretion is taken into consideration. If fat is present in the intestine because of a fatty previous meal there is an intestinal inhibition message for gastric acid secretion. People of Greek nationality tend to have olive oil in their meals every day either cooked or uncooked with fresh vegetables; they also eat their dinner later at night than is the norm in the UK. In addition glucose in the form of table sugar (sucrose) is generally also consumed in quite large quantities. There is probably a constant intestinal inhibitory message for gastric acid secretion in the fasting state since all the meals contain oil; the intestine is not therefore ready to accept more food. So the gastric lumen is not acidified, and

Fig. 9.2: The conductivity of solutions made by water and HCl acid (A) or NaCl and HCl (B) in mS/cm.
a non-ionic test meal as tap water seems to be iso-conductive to the gastric lumen and does not cause further migration.

As far as obese people their intestine probably retains fat longer and have the usual intestinal inhibitory messages. Of course, after ingestion of the meal the impedance in most cases starts to decrease showing that after distension gastric acid secretion has commenced. The causal factor of no impedance deflection is the lack of acidic environment at the time of ingestion and not due to the reduced conductivity of the surrounding tissues because of deposited fat. If the less conductive tissues were the cause then it would not be possible to observe the post-prandial progressive reduction of the impedance. In a number of cases with subjects having a BMI>28kg/m² a slight deflection downwards is noticed, which is then leveled with the pre-prandial value and later on starts to decline as shown in Figure 7.15.

As a final conclusion, water tests can give useful information about the ability of the stomach to secrete acid and indicate if there has been any basal acidification. EIE-water tests are not only simple but water is pleasantly acceptable as a test meal.

Another interesting phenomenon was observed with the conductive meals. Their high conductivity was achieved by adding salt up to a value of 12-16 mS/cm. The test meals pass in this way into the range of hyper-osmolarity (>350 mosmol/L). As already mentioned, impedance deflection is caused to go to lower impedance values during the ingestion of the meal and immediately after starts to rise before it starts to decrease again as shown in Fig. 6.4. It is believed that the rise of impedance after the initial downwards deflection, is due to water flow across the mucosa mainly due to the osmotic difference across the mucosa. It may also be due to hydrostatic pressure. Water flow always exists as mentioned before but in response to the needs. The favoured gastric environment is isotonic; thus when hypertonicity exists a considerable water flow rate starts (Moo 69) which dilutes the ionic gastric environment, resulting in increasing impedance. The concentration of the water in HCl acid during the water flow onset is low, and the resulting conductivity lower then that induced by the ingestion of the NaCl solution. As the osmolarity decreases the water flow also decreases and the gastric acid takes over. The total ionic gastric content starts increasing with the gastric impedance becoming lower until the

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optimum acidity is achieved and then the baseline is established. There are cases where no rise occurs in the impedance after the initial down-wards deflection, which means that there was no sudden onset of water flow. The decrease of the impedance continues but at a different rate of decrease.

It is therefore believed that the onset of water flow across the gastric mucosa can be detected with a hyperosmolar salty meal.

Water flow was also shown, it is believed, when a study with yoghurt meals (150 g yoghurt and 50 g maltodextrin with water so the total volume was 350 mL) was undertaken. The meal with a conductivity 2.2 mS/cm behaved as in the transitional zone of the “neutral” meal. After the ingestion in a number of subjects a rise in the post ingestion impedance was observed and it was attributed to water flow since the meal was hyper-osmolar too (348 mosmol/L).

With the non-conductive meals, despite the fact that the osmolarity was measured as 323 mosmol/L, phenomena interpreted by water flow were not observed in a very clear way and this is because the conductivity of the gastric content progressively increases due to higher conductivity of the flowing water with respect to conductivity of the ingested meal.

Observing the scintigraphic images of the stomach, which are reproduced from the EIE-scintigraphy study for further investigation and study (Nadiyah Hadi; PhD work in progress, Dept of Physics, University of Surrey), it was realized by the author that the volume of the stomach can be calculated as well as the volume changes. Surface anatomical marks and the distance between these were measured as well as the position of the electrodes on the sternum in respect to the xifisternum-umbilicus direction during the study. With a light radioactive $^{57}$Co pen source the same anatomical markers and electrode positions were designated on the scintigram. Thus, based on geometry an index can be calculated and the real dimensions of the stomach can be calculated using the dimensions of the scintigrams. The stomach can be considered as a pile up of slices of cylinders each one with different radius according to the scintigram. It is hypothesised that the stomach expands uniformly towards all directions when a meal is ingested. It needs a lot of computational work but it is
believed it will provide useful information. The thickness of the slice will be decided according to the shape of the stomach in the scintigram.

It is also believed that modified sham feeding will cause impedance changes. Such a study is worth to design since it is quite simple and inexpensive although the rate of success might be quite low.

It is suggested that a study with water as a test meal should be carried out simultaneously with scintigraphy, under the same conditions as in the EIE-scintigraphy study. In this way a complete study with non-conductive meals from non-nutrient to nutrient meals will exist and the role of water in the function of the stomach with respect to gastric acid secretion may be explored. Also, from this study the volume of the stomach and how it changes can be calculated and compared with those when nutrient meals are used.

Studies using carbonated water as well as carbonated calorific meals are recommended. The carbonation (dilution of sodium bicarbonate) will give the test meals of higher conductivity. The carbonation of the meals is suggested until the "neutral" point is reached. By ingestion of the meal interaction between ions is expected with the production of water and CO₂. It is interesting to observe the phenomena and compare these with the NaCl meals of the same conductivity. Sodium bicarbonate is used as an antacid and its action should present differently from NaCl.

The use of NaOH is also another solution that should be tested.

Women are characterised with lower basal acid output than men (Bar 78, Rea 89). Females through all these studies showed more difficulties in achieving a good impedance deflection. It can be attributed to the lower basal acid output so a lower to null impedance deflection is expected more in female subjects than male subjects. There were at least 3 female subjects who underwent more than one test with non-conductive meals and the response was as "neutral" or non-conductive on the separate occasions. Initially, the non-conductive response as "neutral" was attributed to the wrong site of placement of the electrodes. That was before the reassurance about the right placement of the electrodes by the simultaneous scintigraphy
experiments and before the introduction of the “neutral” test meal, taking into consideration the presence and secretion of the gastric acid in the gastric lumen.

It is recommended that the calculation of the T50s from neutral meal studies and comparison with the same meals, but non-conductive, in nature to be carried out.

The shorter T50s show that less HCl acid was secreted or a higher output rate so that the impedance was reduced earlier. In the longer T50s, more acid was secreted in total or the rate of acid output was low so a longer time was needed for the necessary acidification. It is believed that shorter T50s mean a higher rate of acid output and longer T50s a slower rate of acid output.

There are situations after the ingestion of a meal where a lag phase occurs varying from one minute up to 15 minutes. In the study of loxiglumide infusion 2 out of 10 showed a long lag phase in both sessions but under loxiglumide infusion the lag phase became shorter. This, in the author's opinion shows a delay on the initiation of gastric acid secretion. The lag phase in the GLP-1 infusion study shows also delay in the initiation of gastric acid secretion.

Consideration should also be given to development of fitting algorithms on postprandial data, using exponential or power exponential, for the cases where lag phase appears and the establishment of the range of the T50s in normal subjects studied with various meals.

It should also be possible to eliminate artefacts due to movement, talking, electronic faults either simply by subtraction or linear interpolation according to each case, or more complex interpolation.

A study with subjects suffering from achlorydria should also be designed; in order to investigate whether impedance profiles yield further information regarding gastric secretion.

Finally, it is believed by the author that epigastric impedance can form the basis for the development of an external monitoring technique in determining gastric acidity and motility.
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