BASAL GASTRIC ACID SECRETION AND MUCIN FRACTIONS IN HEALTHY SUBJECTS AND PEPTIC ULCER PATIENTS

BY

YUSUF, SADIQ ABDULKADIR
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DECLARATION

I hereby certify that I carried out the research reported in this thesis by myself in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria under the Supervision of Professor, M.A. Ali, Head, Department of Human Physiology, Dr. A.A. Ameh, Head, Department of Biochemistry, Prof. Iliyasu Muhammed, Head, Department of Surgery and Dean, Faculty of Medicine and Dr. E.M. Sheriff, Head, Department of Human Anatomy, Ahmadu Bello University, Zaria.

Where use has been made of published work of other investigators, this is acknowledged and the sources of information appropriately referred. No part of this thesis has been submitted or accepted elsewhere for higher degree or diploma.

YUSUF, S.A.
Ahmadu Bello University
Zaria, Nigeria.

October, 1996

CERTIFICATION

This thesis by YUSUF, SADIQ ABDULKADIR meets the regulation governing the award of degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

..... 3/10/96
Prof. M.A. Ali, MBBS., M.D., MPSN — Date
Major Supervisor and Head
Department of Human Physiology
Ahmadu Bello University, Zaria.

..... 3/10/96
Dr. D.A Ameh, B.Sc., M.Sc., Ph.D — Date
Supervisor and Head
Department of Biochemistry
Ahmadu Bello University, Zaria.

..... 3-10-1996.
Dr. E.M. Sheriff, B.Sc., M.Sc., Ph.D — Date
Supervisor and Head
Department of Human Anatomy
Ahmadu Bello University, Zaria.

..... 3/10/96
Prof. Ilyasu Muhammad, MBBS.,Mphil(Edin),FMCS(Nig)FWACS — Date
Supervisor and Dean
Faculty of Medicine
Ahmadu Bello University, Zaria.

..... 3/10/96
External Examiner — Date
(Prof. J.O Ibu)
Department of Physiology
University of Jos.

..... 4/6/97
Dean
Postgraduate School
Ahmadu Bello University, Zaria

DEDICATION

This thesis is dedicated to my senior brother, MUHAMMED YUSUF and his family for their patience, understanding, wisdom, guidiance, faith and support.

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ABSTRACT

Thirty patients with endoscopically proven peptic ulcer and thirty healthy adult volunteers with no history of dyspepsia, were admitted to this study. Basal gastric juice were obtained in the fasting state for measurement of basal acid output, analysis of gastric mucin fractions and pH studies in these two groups. The mean basal acid output was 3.04mmol/hr for patients with peptic ulcer disease and 1.32mmol/hr of healthy controls. The results obtained showed a significant difference ($P<0.001$) between the control group and patients with peptic ulcer. These figures are much lower than values obtained from western countries, but similar to those reported from studies in non-caucasians. The mean values obtained for pH levels in healthy controls and patients with peptic ulcer were 2.22 and 1.8 respectively. The pH of the gastric juice was significantly lower ($P<0.025$) in peptic ulcer patients when compared to controls.

The mean level of acidic mucoprotein-pepsin complex in healthy controls and peptic ulcer patients were 165.3ug/ml and 253.4ug/ml respectively. Meanwhile, the mean values obtained for neutral glycoprotein-mucoproteose complex were 211.3ug/ml (healthy controls) and 110.5ng/ml (patients with peptic ulcer). The concentration of acidic mucoprotein-pepsin complex was significantly increased ($P<0.001$) whereas neutral glycoprotein-mucoproteose complex was significantly decreased ($P<0.001$) in patients with peptic ulcer disease as compared to healthy controls. The mucoprotective index was significantly decreased ($P<0.025$) in patients with peptic ulcer disease. The mean values

were 56.1% and 30.4% for healthy controls and patients with peptic ulcer respectively. The significant increase in acid mucoprotein-pepsin complex and a marked reduction in the concentration of neutral glycoprotein-mucoproteose complex may be considered as a reduction in mucosal protective mechanism against potential ulcerogenic agents such as acid and pepsin.

Taken overall, the association between dietary intake, alcohol ingestion, smoking and ulcer is weak and these factors can best play a minor causal role in ulcer development.

The data presented above give support to the hypothesis that peptic ulceration is an heterogenous disease but it is likely that a high acid output and low mucoprotect ive index may be indicative of peptic ulceration.

It is concluded that a reduction in the neutral glycoprotein-mucoproteose complex is indicative of a reduction in mucosal protective mechanism against endogenous ulcerogenic agents such as acid and pepsin, because this fraction of the gastric mucin may contain the inhibitor found by other investigators in human gastric juice.

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CHAPTER ONE

INTRODUCTION

1.0 General Introduction

Peptic Ulcer disease is a chronic inflammatory condition of the stomach and duodenum that affects as many as 10% of people in a community at sometime in their lives (Bouchier, 1977). The disease has relatively low mortality rate, but it results in substantial human suffering and high economic costs. Although the pathogenesis of peptic ulceration remains obscure, many theories have been postulated to account for the disease but no single mechanism has found universal acceptance. It is still assumed that in most cases, the gastric and duodenal mucosa is exposed to excessive amount of acid, be it basal or in response to variety of stimulants. The explanation for the increased acid secretion in duodenal ulcer patients is not fully understood, but recognised pathophysiological defects which may contribute to the increased acid secretion include; increase in parietal cell mass (Cox, 1952), increase in parietal cell sensitivity to stimulants of acid secretion such as gastrin (Isenberg et al, 1971), increased gastrin released in response to a meal (Grossman, 1981) and decreased inhibition of acid secretion and gastrin released in the presence of acidified meal (Walsh et al, 1975). Because some patients with peptic ulcer disease tend to have reduced acid output it is suggested that the protective mechanism of the gastric mucous membrane, such as gastric mucus or mucin, is deficient or abnormal and that this may lead to lowered mucosal

resistance (Glass, 1967; Glass and Slomiany, 1977; Allen, 1979; Ali et al, 1989).

Gastric secretions have been studied for years and although many advances have been made in understanding the etiopathogenesis of peptic ulcer, the classic concept that ulcer results from imbalance between aggressive (acid and pepsin) and defensive (gastric mucus) factors of the gastroduodenal mucosa is still tenable (Dragstedht, 1956; Alphine et al, 1977; Baron, 1978;l Bittner et al, 1979; Gruslandi et al, 1980; Guth et al, 1984). An increase in acid pepsin aggressive factors or a decrease in factors responsible for mucosal protection results in the production of duodenal or gastric ulcer (Ali et al, 1989). Alphine et al, (1977) showed that increase in the intragastric acidity were parallel to the degree of ulceration. Ulcers have been produced by inducing continuous secretion of hydrochloric acid by implanting histamine in beewax under the skin (Best and Taylor, 1973). True peptic ulcer does not develop in the absence of hydrochioric acid but a small acute or subacute ulcer may sometimes develop in the absence of acid (Grossman, 1963). The difference between normal persons and peptic ulcer patients is greatest in studies on the basal or interdigestive secretion. As a result, measures directed towards the neutralization of acidity of the gastric contents are of outstanding value in encouraging the healing of the ulcer.

Though the importance of gastric acid in the production of ulcer cannot be ruled out, this factor alone cannot be the sole agent. For one thing, a good number of persons who have hyperchlorhydria do not develop ulcer. Why in these instances

is the gastric mucosa immune to action of the pepsin-hydrochloric acid? Until recently, there has been no convincing or even plausible answer to this question. One explanation offered was that the greater alkalinity of blood coursing through the vessels of gastric mucosa as a result of the loss of H^+ ions served to neutralize the effect of the acid juice (Best and Taylor, 1973). Others suggested that the protective action of the alkaline mucus secreted by the mucosa is responsible for the immunity (Allen and Garner, 1980).

Recognition of the existence of protective mechanism can be traced back over two centuries to Hunter (1772), who described autolysis of the stomach after death. Work on the protective role of mucus was undertaken during the 1950s by Florey (1955) and by Heatley (1959), who hypothesized that adherent mucus could act as an unstirred layer that would allow a pH gradient to develop at the surface of the stomach. Other early work centered on the epithelium itself, where a number of workers including Grant (1945), studied rapid repair and regeneration of the gastrointestinal tract epithelium.

The combination of both pre-epithelial and epithelial factors in mucosal protection was initially stated by Hollander (1954). He proposed the existence of a two-component barrier composed of the alkaline mucus gel, responsible for acid neutralization, and the underlying epithelial layer, which was capable of rapid regeneration and repair. Waldon-Edward and Skoryna (1970) attributed the development of peptic ulcer disease to a decrease in the secretion of gastric mucus. Allen and Garner (1980) suggested that, on the basis of physico-chemical

considerations, that mucus gel would maintain a pH gradient by preventing secreted HCO^-_3 from being rapidly neutralized by luminal H^+ ions. Recent studies have confirmed that this is indeed true and that there is a pH gradient demonstratable from a low pH on the luminal surface of the mucus to a near neutral pH on its mucosal surface (Venables, 1980). Therefore, as long as there is a continuous layer of mucus covering the surface mucosa, there is no way in which acid can damage the surface epithelial layers.

The varying prevalence of ulcer from place to place and from time to time indicates strongly that environmental factors are also responsible in the etiopathogenesis of peptic ulcer. These are poorly defined, but there are some limited evidence about possible important factors which has been drawn from broad comparisons of diets in places where ulcer is common and rare and from surveys conducted in western countries and elsewhere. In India, there is a strong association between prevalence of ulcer and the consumption of diets of low residue with poor masticating content, the contrast being between wheat-eating people who make chupatties with unrefined wheat, and those who predominantly eat rice (Cleave, 1962; Malhotra, 1964; Tovey, 1972). A correlation between low residue African diets and liability to duodenal ulcer has also been detected; thus the diet on the south coast of West Africa where ulcer is common contains a high proportion of carbohydrates as tubers with low fibrous residue (Tovey and Tunstall, 1975).

Reports available in African populations, especially Nigeria are lacking in the literature as regards to the characteristic

of gastric mucin, acid secretory studies and association of local diet intake in healthy subjects and patients with peptic ulcer disease. It would therefore be of immense clinical importance to study the characteristics of gastric mucin fractions, gastric acid secretion and the possible role of local diets in health and disease among Nigerians with regards to age and sex. An understanding of this problem may provide the knowledge upon which sound therapeutic advice may be based in patients with peptic ulcer disease. The results obtained may also provide answers to the role of acid, mucin and diet in the aetiology of peptic ulcer disease.

1.1:0 OBJECTIVES

The overall objective of this research is therefore to investigate the basal acid secretion and fractions of gastric mucin by measuring acidic mucoprotein-pepsin complex and neutral glycoprotein-mucoproteose complex in healthy subjects and patients with peptic ulcer disease among Nigerians.

Specific objectives are;

(a) To determine the influence of age and sex on basal gastric acid and mucin fractions in healthy subjects and patients with peptic ulcer disease.

(b) To determine whether or not, there exist any relationship between gastric juice, pH and peptic ulceration.

(c) To determine whether or not the variations in basal acid output were related to the changes in gastric mucin fractions and also how this relationship differs in healthy controls and patients with peptic ulcer disease.

(d) To determine the possible role of gastric mucin as a physiological mechanism for protection of the mucosal surface from acid and peptic digestion by determining the mucoprotective index from the mucin fractions.

(e) To define the association of peptic ulcer disease with smoking, various types of local diet, alcohol, milk and beverage (soft drinks, food drinks) consumption before the initial onset of ulcer symptoms.

1.2:0 JUSTIFICATION

Most students of peptic ulcer are particularly intrigued by several aspects of this disease. One is that gastric hydrochloric acid seems to be essential to the genesis and chronicity of peptic ulcer of any type. Another is the concept that some forces protect the gastric mucosa from the digestive power of its own secretion and the gelatinous mucus which tenaciously adheres to the mucosa surface naturally attracted attention as potential mechanism of gastric mucosal protection from acid induced injury. Although gastric acid has dominated thinking about peptic ulcer, appreciation of the means by which the gastric mucosa normally protects itself from damage may

suggest additional therapeutic approaches. In addition, reports on gastric mucin fractions are lacking in African populations, particularly among Nigerians where the strain and stress of modern life has its adverse effects on the body and insults on gastric mucosa resulting in peptic ulcer disease. It would therefore be of immense clinical importance to investigate the association of basal gastric acid secretion and gastric mucin fractions in health and disease among Nigerians with various dietary backgrounds since there are no extensive data on these two factors in the aetiology of peptic ulcer disease in Nigeria. In addition, it may also suggest ways upon which sound therapeutics or dietary advice may be based in patients with peptic ulcer disease.

1.3:0 ANATOMY OF THE STOMACH

1.3:1 Structure of the Stomach

This hollow viscus, the most dilated part of the gastrointestinal tract, connects the distal esophagus with the proximal small intestine and has a volume capacity in adults of 1000 to 1500ml. It is entirely within the abdominal cavity, its upper portion beneath the left diaphragm and occupies the left hypochondriac, epigastric and umbilical areas of the abdomen.

The esophagus opens into the stomach at the cardiac orifice, and the duodenum opens from the stomach through the pyloric orifice. The cardiac orifice which cuts the gastro-esophageal junction is the most fixed portion of the stomach and lies

beneath the diaphragm at the level of tenth thoracic vertebra just to the left of the midline. The pyloric orifice, at the gastro-intestinal junction, lies to the right of the midline at the level of the first lumbar vertebra in the recumbent position. Two surfaces, anterior and posterior, are united by lesser and greater curvatures. The lesser curvature is short and lies on the posterior surface of the stomach and is a continuation downwards of the posterior part of esophagus. The greater curvature is three to four times the length of the lesser curvature which may be 12cm long.

The portion of the stomach above and to the left of the cardiac orifice is called the Fundus. The main part is the body and the lower part which curves to the right is the pyloric antrum. At the distal end of the pyloric antrum, there is a sphincter, the pyloric sphincter, which guides the opening between the pyloric antrum and duodenum.

The stomach varies from a simple tubular dilation to a complex series of chambers. A common histological feature of the entire alimentary tract is four concentric layers or coats: the mucosa or mucous membrane, submucosa, muscular coat or muscularis and serosa or adventitia. Although the three outer layers are similar in diverse parts of alimentary tract, the mucosa has distinctive structural and functional features.

1.3:2 Structure of the gastric mucosa

The surface area of the gastric mucosa in man is about $800cm^3$. The superficial lining is made up of tall columnar

epithelial cells which commence abruptly at the cardiac-esophageal junction with the termination of stratified squamous epithelium of the esophagus. These cells have basally located ovoid nuclei and a large supranuclear portion filled with varying amount of mucinogen-containing granules. The epithelial layer invaginates below the surface to form pits or foveolae, which communicate with the gastric gland. Thus, the glands opens into gastric lumen via the gastric pits which forms shallow depressions occupying 25 to 50 percent of the mucosal surface. There are three specific types of glands, each lining a separate region of the stomach: the cardiac, oxyntic and pyloric glands; and an uppermost portion designated gastric pit or foveola into which several tubular glands may empty.

The cardiac glands are found in the region near the esophageal orifice. The zone occupied by cardiac glands varies from narrow rim of few millimeters or less in human stomach.

The gastric pits of cardiac gland areas are relatively short and are joined by tortuous branched, tubular glands composed of cardiac mucous cells and a few argentaffin cells. The functional significance of cardiac glands has not been well documented (Texter et al, 1968).

The pyloric glands occupy an area considerably larger than that of the cardiac gland region and may constitute one-fifth of the gastric mucosa between the oxyntic and pyloric gland areas. There is a transitional zone of gastric and pyloric glands, which may be the site of gastric ulceration.

The pyloric glands, which are simply branched or extensively coiled tubular glands, are composed of cells resembling mucous

neck cells. They may contain very few or no oxyntic cells and secrete alkaline mucous and some electrolytes such as calcium phosphate, sodium and potassium bicarbonates and chlorides (Konturek, 1979).

The oxyntic or gastric gland area occupies the fundus and body of the stomach. It occupies 75 to 80% of the total gastric mucosal area. They number about 35 million and are straight or slightly coiled tubules, closely parked and oriented in a perpendicular fashion to the gastric mucosal surface. In the human stomach, three to seven glands empty into a pit. At least five types of secretory cells have been identified as constituents of the oxyntic glands. These are; mucous neck cells; undifferentiated neck cells, parietal (oxyntic) cells; peptic (chief) cells and endocrine cells.

1.4:0 THE PHYSIOLOGY OF GASTRIC SECRETION

1.4:1 Composition of Gastric Juice

Normal gastric juice is a thin, light coloured acidic fluid which has a specific gravity of about 1.007. Gastric juice is a mixture of secretion from oxyntic, peptic and mucous cells. The composition of the juice may also be contaminated with saliva, duodenal secretions or food. In addition, some gastric ions diffuse back from the lumen into the plasma.

Gastric juice contains about 0.5% solid matter which is made up principally of water, electrolytes and macromolecules which include enzymes, dissolved mucin, blood group substances and other biological active materials (e.g. vitamin B_{12}, gastrin and

gastric intrinsic factor). Thus gastric juice is a complex solution of two primary components; (a) an acidic component containing hydrochloric acid and water produced by parietal cells; and (b) an alkaline component containing pepsinogens, produced by peptic cells and elctrolytes which originates in several types of cells.

The electrolyte composition of gastric juice closely resembles that of plasma, with the difference that it contains hydrogen ion in the place of some sodium. The electrolyte composition are: sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate and sulphate. The acidity of gastric juice is due to the free hydrochloric acid in it. The function of hydrochloric acid in the gastric juice include activation of pepsin, inhibition of salivary amylase, the swelling of collagen fibres in food masses, and stimulation of pancreatic secretions. The nature of the bacterial population of the stomach is affected by the predominant pH of gastric secretion (Hawk et al, 1954).

The principal enzyme in gastric juice is pepsin, for which the optimum pH is between 2 and 4. Pepsinogen is converted to pepsin by the loss of part of the molecule known as pepsin 'inhibitor'. In young animals the gastric juice cotains another enzyme called renin. The enzyme coagulates milk by converting the milk protein, caesinogen, to an insoluble form of casein. Many other enzymes may be detected in the gastric secretions and the lysozomal enzyme ß-glucuronidase has achieved prominence for its possible diagnostic role in patients with gastric cancer (Best and Taylor, 1973).

The gastric mucosa, like other epithelial tissue of the gastrointestinal tract is coated by a continuous film of mucin called mucus. This film is three-dimensional gel, synthesized and secreted by columnar apithelial cells and mucous neck cells in the oxyntic gland area and by the cells of the pyloric gland area. Organic compounds such as blood-group substances, intrinsic factor and gastrin are also found in the gastric juice. About 78% of normal individuals secretes blood-group substance A, B and H into the gastric juice. The individuals of blood group O do not secrete substance A and B but some of them secrete substance H which appears to be present in lesser degree in people belonging to other blood groups. Intrinsic factor, a mucoprotein, is secreted by the gastric-mucosa. It is necessary for the absorption of Vitamin B_{12} from the ileum. The exact nature of intrinsic factor is not known.

The cationic composition of gastric juice varies with the rate of secretion, perhaps because of the acidic parietal component is secreted with constant composition but at variable rates depending on the degree of stimulation, whereas, the alkaline, non-parietal component is secreted at a constant rate and constant composition, independent of the stimulation. Both primary component mix in the stomach and when the rate of secretion of the acidic component increases, the proportion of the alkaline component decreases (Konturek, 1979).

1.4:2 Mechanism of Gastric acid Secretion

Much of the background and what is at present known and understood about the process of acid secretion and the proton

pump have led to the development of new concepts of the mechanism involved in acid secretion and its regulation.

Hydrochloric acid is secreted into the gastric lumen by the parietal cells. When the gastric juice is collected either from the human stomach or from the pouch of the oxyntic gland area of the dog stomach, its composition is characteristically found to be a function of the rate of secretion. The acidic juice is frequently called "Pure parietal cell secretion", thus implying that it is secreted by the parietal or oxyntic cells. The general method used to identify the locus of acid secretion has been to treat the stomach with some kind of indicator and to look for the place in which the indicator show its acidic form. The acid form of neutral red has been shown in the canaliculi of the oxyntic cells of the pole cat. Using electron microscope, changes in the fine structure are seen in the stimulated, secreting oxyntic cells. This observation identify the oxyntic cells with the secretion of hydrochloric acid (Texter et al, 1968). A model of the function of the oxyntic gland in the fundus and corpus of the stomach is shown in Figures 1 and 2 respectively.

The hydrogen ion is derived from plasma, where its concentration is 5×10^{-5}m mol/L (Figure 1). This concentration is raised to 160mEq/L, about a million fold increase in the acid juice. The secretion of hydrogen ion into the gastric lumen requires an expenditure of energy. It involves two major intracellular steps; production and active transport of ion, both of which consume energy and substrate. A specific carrier system in the mucosal membrane transport the hydrogen ion into the

gastric juice against an extremely large concentration gradient, which is often greater than one million to one (Konturek, 1980).

The mechanism of hydrogen ion secretion against such a high gradient requires energy which is generated in the parietal cells mainly by aerobic metabolism and to a lesser degree by glycolysis. According to the `ATPase'theory, the energy available from the hydrolysis of ATP (Adenosine triphosphate) is transferred to a carrier system which moves prontons against an electrochemical gradient (Mayer et al, 1974). The gastric mucosa contains two kinds of ATPase: one activatd by anions (chloride or bicarbonate), and the other activated by potassium and inhabited by ouabain. Since the mucosa also secretes anions, the anion-activated ATPase is probably an integral part of the secretory mechanism.

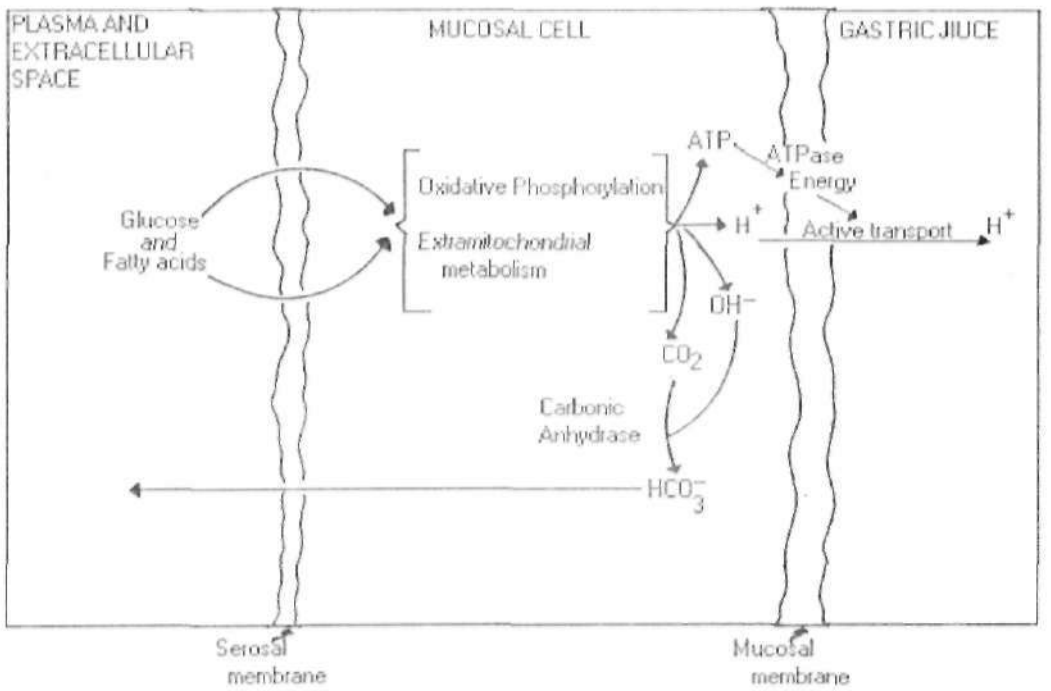


Figure 1: Production and transport of the hydrogen ion of the gastric juice (from Konturek, S.I., 1980. Scientific Foundation of Gastroenterology, 293).

The 'Redox' Mechanism

The 'redox' mechanism of acid secretion postulates the existence of an extramitochondrial oxidation-reduction process. With the oxidation of the substrate by dehydrogenase on one side of the membrane, protons are released to the other side without the participation of ATP. Results on the Cytochemical Pathways involved in acid secretion are fragmentary and derived from studies of in vitro mucosa (Konturek, 1980). They do not establish with any certainty the acceptance of either an ATP-dependent or a redox-dependent process.

The chemical mechanism by which parietal cells secrete acid is not known but it is well established that neutrality of the cell interior is maintained by replacing hydrogen ion secreted with hydrogen ion derived from carbonic acid. Carbodioxide combines with the hydroxyl radical to form bicarbonate, and with water to form unstable carbonic acid, which splits into bicarbonate, and hydrogen ion (Fig. 1). The bicarbonate then diffuses from the cell into the blood in the same molecular quantities as the hydrogen ion entering the juice. The venous blood leaving the stomach has, therefore, a high pH than the arterial blood entering the organ. The reactions involving carbodioxide are mediated by the intracellular enzyme, carbonic anhydrase, which is present in parietal cells in high concentration (Konturek, 1979).

1.4:3 Cellular Mechanisms of acid secretion

Cellular mechanism of gastric acid secretion are poorly

understood but several reports have appeared in the literature implicating adenosine 3', 5' - Cyclic adenosine monophosphate (cAMP) and prostaglandins in the final adjustment of gastric secretion resulting from the combined effects of secretory and antisecretory stimuli to oxyntic glands (Fig. 2).

Although no direct correlation has been established between the intercellular level of cAMP in parietal cells and gastric secretion, it is now well established that parietal cell possesses a histamine receptor of the H_2-type, and a cholinergic (muscarinic) receptor (Koelz et al, 1984). Thus histamine stimulates acid secretion via adenylate cyclase activation to produce cAMP, while acetylcholine and possibly also gastrin stimulates secretion via unknown mechanism which result in increased ca^{2+} permeability of the basal lateral membrane. The studies on the isolated oxyntic cells (Konturek, 1980) show that histamine causes an elevation of intracellular concentration of cAMP, which is then inactivated by enzymatic degradation by cAMP phosphodiesterase and also by nucleotide effluxes into gastric juice.

Gastric mucosa is also capable to synthesize postaglandins (Pgs) from local polyunsaturated fatty acid precursors. Pgs of E and A series are known to inhibit gastric secretion stimulated either by histamine or by other secretory stimuli (Vantrappen et al, 1982). In addition, they show a cytoprotective effect on gastric mucosa, directed against otherwise unopposed effects on the hydrochloric acid and gastric protease (Kollberg and Slezak, 1982).

Hormones such as Somatostatin (Konturek, 1980b) and

epidermal growth factor are known to inhibit acid secretion *in vivo* (Bower *et al*, 1979), but whether this is due to effects directly on the parietal cell or to indirect effects e.g. on blood flow or by release of other endogenous substances, has not been clearly established. Thus, although somatostatin binding to rat gastric mucosa has been reported, its specificity is unclear.

Based on available experimental evidence, it has been suggested (Dousa and Dozois, 1977) that most, possibly all, secretory stimuli in general, directly or indirectly, increase the intramucosal level of histamine on the vicinity of H_2-receptors by stimulating the synthesis of histamine or its release from local stores or both. Histamine results in the stimulation of acid via the activation of H_2-receptors and adenylate cyclase - cyclic AMP system in the oxyntic cells. Pgs are constantly generated within the gastric mucosa and stimuli which inhibit gastric secretion may positively influence the synthesis of Pgs, which in turn would have dual effects, antagonizing action of histamine on hydrochloric acid secretion and protecting the gastric mucosa against deleterious effects of hydrochloric acid. Thus histamine and Ogs, both locally produced, are functionally antagonistic systems and balance between them may be critical in the regulation and the fine adjustment of gastric secretion of acid.

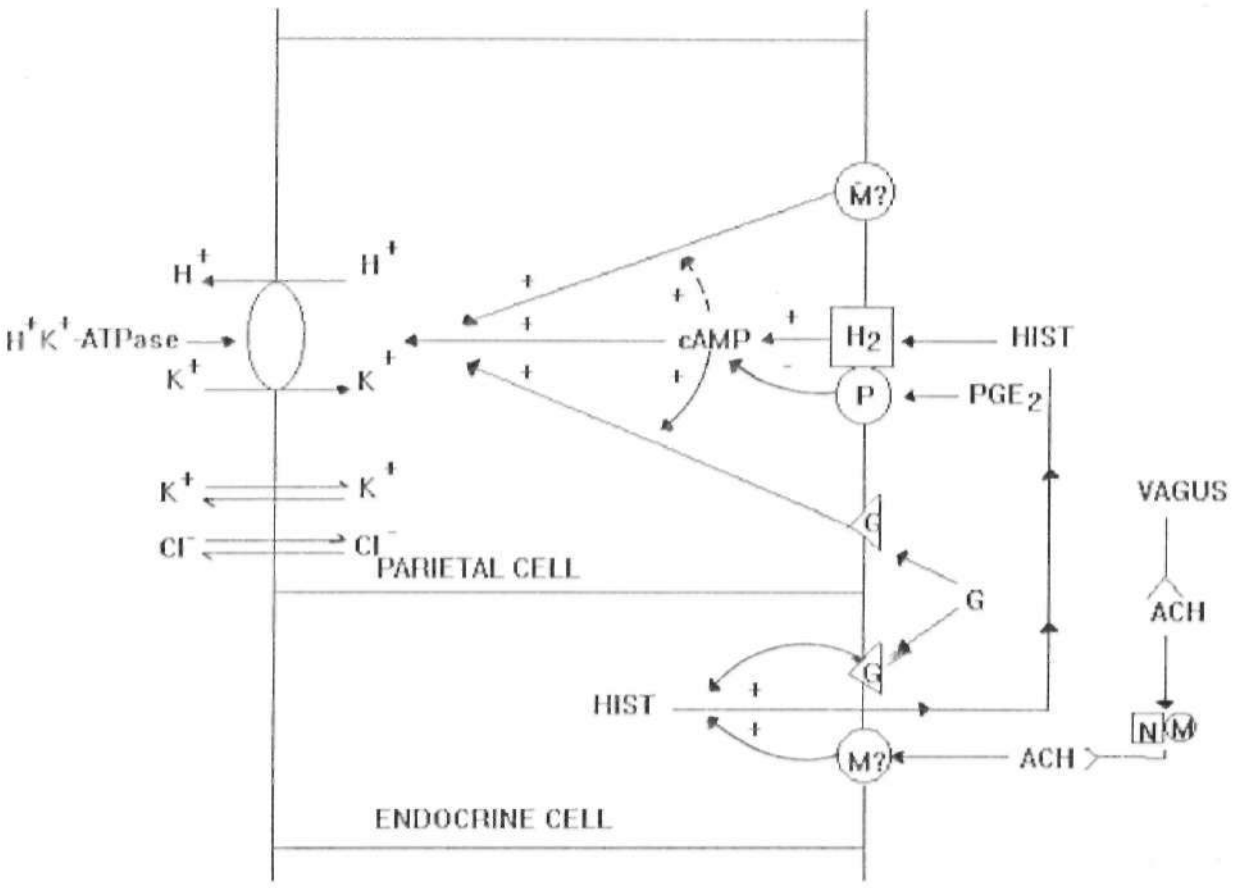


Figure 2: Regulation of acid secretion by the parietal cell. Ach = acetylcholine; G = gastrin; HIST = histamine; CAMP = Cyclic AMP; PGE_2 = Prostaglandin E_2; (+) = Stimulation; (-) = inhibition; H_2 = H_2 - histamine receptor; (G) = gastrin receptor; P prostaglandin receptor; (N) = nicotinic cholinergic receptor; (M) = muscarinic receptor [From Bruton, L.L. 1990: Agents for control of gastric acidity. In: Gullman, A.F.; Rall, T.W. and Taylor, P. (eds). The Pharmacological Basis of Therapeutics. pp. 897-913].

1.4:4 The 'Proton Pump' Mechanism

Acid secretion by the parietal cell is now known to be dependent on K^+, cl^- and ATP (Fig. 2) and due to a K^+-stimulated, proton-translocating adenosine triphosphatase, the (H^+ + K^+) ATPase or proton pump (Koelz et al, 1984). Upon stimulation, a marked morphological transition occurs in the parietal cell. The resting parietal cell's cytoplasm is packed with tubulovesicles and contains an apical membrane infolding, the secretory canaliculus, characterized by short, stubby microvilli. On stimulation, the canaliculus expands, develops many long, thin microvilli, and the tubulovesicles disappear. Currently, using monoclonal antibodies against one sub-units of the gastric (H^+ + K^+) ATPase, it has been shown that the proton pump moves from tubelovesicular location to the expanded secretory canaliculi (Smolka et al, 1983)

Early studies of the H^+ transport mechanism, performed on hog gastric microsomal vesicles which contained the (H^+ + K^+) ATPase, indicated that an electroneutral H^+ for K^+ exchange was occurring. The rate-limiting step for transport and ATP turn-over was found to be entry of KCL into the vesicle interior. Thus, either long preincubation in KCL media or addition of the K^+-selective ionopore, valinomycin, was essential (Sachs et al, 1979). The mechanism for K^+ and cl^- movement across the apical membrane - 'a kcl pathway' - seemed to be inactive or lacking. hence, it was postulated that stimulation of secretion might involve activation or appearance of this pathway in the apical membrane, thus lifting the K^+ restriction from the proton pump.

The nature of this 'Kcl pathway' has been the object of

considerable interest in the past year. An early study (Wolosin and Forte, 1981) and more recently kinetic analysis of the Kcl flux (Wolosin and Forte, 1990) suggested electroneutral cotransport of Kcl. In contrast, however, measurement of conductances of the membranes using potential-sensitive probes now strongly suggests that the pathway for both K^+ and cl^- are conductive (Cuppoletti, 1990). The K^+ conductance is apparent in vessicles isolated from both resting and stimulated tissue, while the cl^- conductance appears only evident in vessicles from stimulated tissues. These experiments first of all, points to the independence of K^+ and cl^- pathways, also supported by the report that in vesicles from stimulated tissue a cl^-/cl^- exchange occurs in the absence of K^+ (Wolsin and Forte, 1983). Secondly, these experiments suggest the novel concept that stimulation may provoke the activation or appearance of only cl^- conductance in the membrane containing a $(H^+ + K^+)$ ATPase, which always seems to contain a K^+ conductance. Thus, the activation of H^+, K^+-ATPase and its insertion onto the membrane of the apical canaliculus of the parietal cell, catalyzes the exchange of intracellular H^+ for extracellular K^+, and, with the concomitant increase in the permeability of the apical membrane to K^+ and cl^-, about 0.1N Hcl accumulates in the lumen of the canaliculus. Inhibitors of the H^+, K^+-ATPase, such as omeprazole, can virtually eliminate acid secretion.

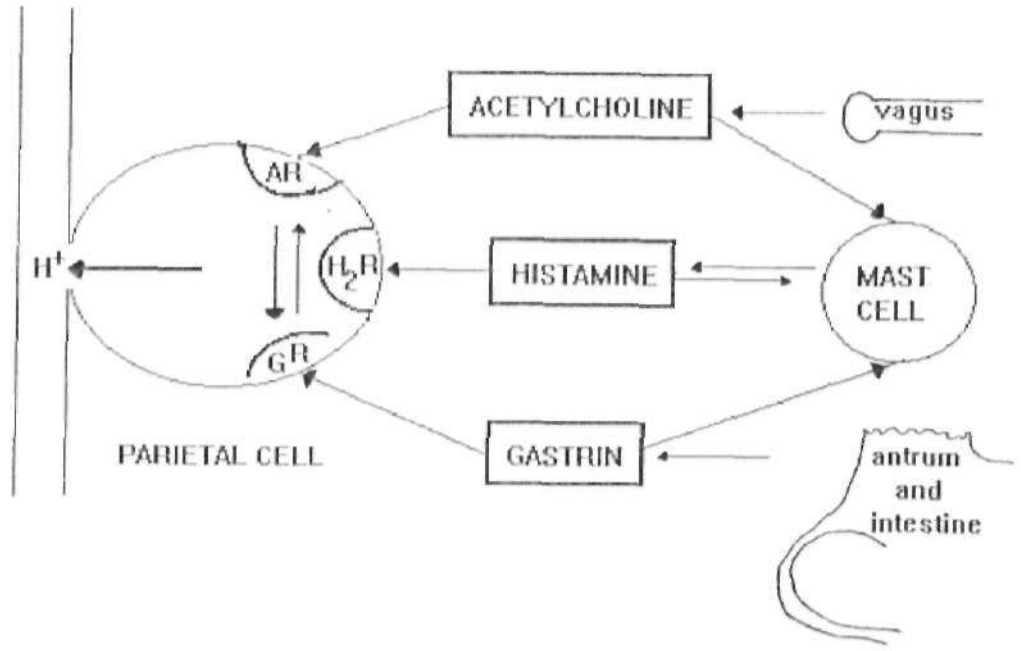


Fig. 3. A scheme demonstrating a combination of present hypotheses on metabolism regulating the actual histamine concentration on the vicinity of the parietal cell and the H_2-receptors. GR = gastrin receptor. AR = acetylcholine receptor, H_2R = H_2-receptor. (From: W. Lorentz, H. Trodl, H. Barth, H. Rohde, S. Schultz and R. Meyer. Stimulus-Secretion Coupling in the Gastrointestinal Tract. Case, R.M. and Goeball, H. Eds. 1976, pp 177-190 London. MTP Press.

1.4:5 Stimulatory Mechanisms of Gastric Secretion

The major substances in the body which stimulates gastric secretion are gastrin, acetylcholine (Ach), histamine and calcium ions. They interact and potentiate one another in the stimulation of gastric secretion (Figure 3).

Gastrin:

Gastrin (G) is a heterogenous hormone existing in several molecular forms, including, 'minigastrin' (G-13), 'Little gastrin (G-17)', 'Big gastrin (G-34)', the numbers indicating the amino acid in each molecule. The largest quantities of gastrin are present in the gastric antrum but significant amounts are also found in the upper small intestine, especially in the upper jejunum. The predominant molecular form of gastrin in the mucosa of the antrum is G-17, while in that of duodenum, it is G-34. G cells are distributed evenly throughout the normal antra mucosa. The two main forms, little gastrin (G-17) and big gastrin (G-34), have completely identical actions. The major physiological action of gastrin is the stimulation of the release of gastric acid. The mechanisms of the release of gastric acid are complex, involving numerous factors. Among these is histamine which is agonistic to gastrin and seems to be a necessary background for the maximum effect of gastrin on gastric acid secretion.

Acetylcholine:

Acetylcholine (Ach) is released in the gastric mucosa by the stimulation of pre- and post-ganglionic vagal fibres; it transmit

the excitation from pre-ganglionic to post-ganglionic neurons and from cholinergic post-ganglionic nerve endings to oxyntic or pyloric glands. The action of Ach at ganglionic junctions is called nicotinic and can be blocked by hexamethonium, whereas that at the effector gland is called muscarinic and can be inhibited by atropine. The cholinergic intramural or vago-vagal reflexes operate both in the oxyntic gland area to stimulate acid secretion by the parietal cells and in the pyloric gland area to stimulate gastrin release. The result of either type of cholinergic reflex is the release of Ach, which in turn either directly stimulates the parietal cells to secret acid or stimulate G cells in the pyloric gland area to release gastrin. The mechanism of the stimulation of the parietal cells by Ach is unknown (Konturek, 1980).

Histamine:

At least three well-known facts indicated that histamine might play some role in the mechanism of stimulation of the parietal cells; histamine is one of the most potent stimulants of gastric acid and its action seems to be confined to the oxyntic cells; histamine is formed and stored in high concentration in mast cells of gastric mucosa, particularly numerous along the course of small blood vessels and in immediate vicinity of the parietal cells, and mobilized by food, gastrin and insulin hypoglycaemia. The role of histamine in gastric secretion has been debated for decades. Code (1965) proposed that 'histamine is the final common local chemostimulator of the parietal cells of the gastric mucosa', that is, all other

secretagogues such as gastrin and acetylcholine stimulate the parietal cell via histamine (final common mediator hypothesis). An alternative hypothesis proposed that parietal cells posses interacting receptors of various stimuli such as histamine, acetylcholine and gastrin, the three receptor hypothesis (Lorentz et al, 1976). Blockage of histamine receptor by H_2-blockers decreases the affinity of the two other receptor to their agonist. This three receptor hypothesis has been expanded to the 'permissive hypothesis', according to which histamine is not released by other stimuli but is continuously generated in the mucosa and presented to parietal cells. This tonic background of histamine sensitizes the parietal cells to other stimuli and when this action of histamine is blocked by H_2-receptor antagonists, acid secretion to many stimulants is suppressed (Soll, 1978).

1.4:6 Basal Gastric Secretion:

The term 'basal' is used conventionally for the fasting gastric secretion. A patient's basal secretion represent the secretion of that proportion of his parietal cell mass which is being excited under the resting condition of the test (Baron, 1978). The basal acid secretion varies considerably from person to person and in one person from time to time. Basal secretion exhibits a cicardian rhythm with highest output in the early morning and lowest output in the late evening hours.

In 1915, Calson produced the first careful data on basal interdigestive secretion. He studied patients with gastric fistulae as well as normal subjects after overnight or prolong

fasting. In 1935, Winkerstein popularized this test of overnight secretion and studied a wide range of diseases. Normal subjects produce not more than 15ml and no free acid; patients with duodenal ulcer and some gastric ulcer had hyperacidity. These results were confirmed in a later study (Cornel et al, 1944).

The factors responsible for basal secretion are probably connected with spontaneous release of small amount of gastrin and tonic vagal stimulation. Attempts to correlate fasting serum gastrin level and basal acid secretion do not show, however, a consistent relationship probably because the main form of fasting serum gastrin is big gastrin which is biological inactive (Konturek, 1980). The complete suppression of basal acid secretion requires removal of both sources of the stimulation, namely, the vagus and the antrum. All types of vagotomy, including the highly selective, reduced the basal acid output inspite of the elevated serum gastrin concentration, suggesting that direct vagal activation of parietal cells is an important determinant of basal acid secretion in vagotomized subjects probably because of reduction in the sensitivity of the parietal cells. Antrectomy in duodenal ulcer patients decreases both basal acid secretion and serum gastrin level, suggesting that the antral gastrin must be involved in the stimulatory mechanism underlying basal gastric acid secretion.

1.4:7 Phases of Gastric Secretion:

It is customary to divide gastric secretion into three phases; Cephalic, gastric and intestinal, depending on the region where the stimuli are acting. The division is artificial because

in the natural course of secretion these phases begin almost simultaneously and not only overlap in time, but also interact to great extent (Texter et al, 1968).

Cephalic Phase:

The Cephalic Phase is induced by the sight, smell, thought of food, chewing and swallowing of palatable food. The cephalic phase is mediated entirely by vagal nerves. Experimental vagal activation is probably best accomplished by sham-feeding. Besides, sham-feeding, the cephalic stimulation of gastric acid can be elicited by agents, such as insulin or 2-deoxy-D-glucose, which produce a dose dependent increase in acid output (Konturek, 1979). Gastric response to sham-feeding in man appears within 15 minutes, reaches a peak during the first 30 minutes and continues for as long from two to three hours after food has been eaten and forgotten. The gastric juice is copious in amount and high in acid and pepsin content. The vagal effects in cephalic phase of gastric secretion are attributable to three components; Direct vagal stimulation of parietal cells, vagal release of gastrin, histamine or other stimulants. Using sham-feeding, an attempt has been made to determine the relative contribution of each component of cephalic stimulation of gastric secretion, and particularly to compare the significance of direct vagal stimulation of oxyntic glands and vagal release of gastrin in normal subjects and duodenal ulcer patients (Mayer et al, 1974). On the basis of their results, it was considered that acid secretion evoked by the cephalic phase in man is due primarily to direct vagal excitation of the parietal cells. These

observations confirm in part the earlier studies of Knutson et al (1974), who investigated the effect of sham-feeding on duodenal ulcer patients before and after antrum-bulb resection.

Gastric Phase:

The gastric phase of gastric secretion may be defined as that factor of the secretory response which occurs when a food stimulus acts on receptors within the stomach, beginning as soon as food enters the stomach and continuing three to four hours after the meal. The same dual mechanism involved in the cephalic phase operates in the gastric phase. Distention of the acid-secretory portion of the stomach after resecting the gastrin-secretory portion stimulates acid secretion. That the response results from a vagal reflex is indicated by high level of pepsin in the juice. In man, distention cannot be applied exactly to any specific area of the stomach and may cause some side effects such as nausea and pain. For these reasons, little information is at present available regarding the physiological significance of distention in the stimulation of gastric secretion in man. Bergegard and Olbe (1971) distended the human corpus-fundus region and observed a marked gastric secretory response which could be completely abolished by atropine. The distention still produced acid secretion following selective proximal vagotomy, suggesting that a local intramuscular reflex was probably involved. Food stimulate gastric secretion by its chemical ingredients, particularly the products of pepsin digestion of proteins, peptone and amino acid in at least three ways; by releasing gastrin from the antral G-cells; by direct action on

the oxyntic glands and; by elevation of the gastric content pH by buffering the secreted acid and thus facilitating the release of gastrin and acid secretion (Konturek, 1979).

Intestinal Phase:

Intestinal phase is brought about through the action of protein digestion products and other food substances in the duodenum. It begins when food enters the duodenum and continues for several hours after food has left the stomach (Bell et al, 1976). The mechanism of intestinal stimulation of gastric secretion has not yet been defined. Using immunological methods for detecting gastrin, it has been found that in man the upper duodenal mucosa contains gastrin and that a steep gradient of gastrin concentration occurs in the distal duodenum, and jejunum (Mastrom et al, 1976). The mechanism of the release of duodenal and intestinal gastrin remains to be determined. Whether gastrin from the intestine is the only hormone responsible for the intestinal stimulation of gastric secretion is not known.

1.4:8 Gastric Mucin Secretion

The question of why the living stomach of warm blooded animals does not digest itself has intrigued physiologists for almost two centuries, yet it still constitutes one of the major mysteries of science. In actuality, however, the answer to this question is the key to the aetiology of peptic ulceration, because this disease process is essentially just a process of autodigestion. In normal person, digestion of the gastrointestinal mucosa by his own gastric juice is evidently

prevented by operation of some protective mechanism, but in the ulcer patient, this agent appears to have suffered impairment, at least in isolated areas. Hence the answer to our question rest entirely on the character and action of this defensive agent within the living organism.

By the early 20th century, this phenomenon of gastric mucosa resistance to autodigestion had been variously attributed to the continuous flow of blood through the mucosa (1772), to mucus protecting the mucosal surface from acid and bile (1800) and to blood's neutralization of acid diffusing through the mucosa (1853). In recent years, our knowledge of the physiology and anatomy underlying the gastric mucosa barrier has expanded to include such elements as the combination of surface mucus and epithelial HCO^-_3 secretion. Mucus is the only physical structure between gastric acid in the lumen and the surface epithelial cells of the mucosa. It is only natural then that mucus has long attracted attention as a potential mechanism of gastric mucosal protection from acid induced injury. A lubricating action that protects against mechanical damage, anti-peptic activity, and an acid neutralizing capacity have all been cited as possible mechanism by which mucus serves in the protection against autodigestion (Hollander, 1954).

There has been no clear chemical definition of mucus, the term 'mucus' having physiological connotations relevant to its physical properties. The term 'mucin' or 'mucosubstance' is often used synonymously with mucus. Chemical terms used include glycoproteins, mucopolysaccharide and mucoprotein. Whatever term may be used, they are a group of carbohydrate and amino acid

containing compounds (Piper, 1980).

Gastric mucin refers to all the viscous material secreted by the mucous cells of the gastric mucosa and it is derived from several sources: surface epithelial columnar cells; mucous neck cells of the fundic glands of the body; mucous cells of the cardiac glands, and mucous cells of the pyloric glands. Dissolved mucin is therefore, a constituent of pure gastric juice. It forms part of the non-parietal gastric secretions (Glass and Boyd, 1952) and as such, is part of Hollander's alkaline component. Dissolved mucin is a complex of glycoproteins, that is a sugar-acid-protein like substance and it is a chief component of gastric juice. By virtue of its viscosity, dissolved mucin effectively lubricates and protect gastric mucosal from mechanical injury or abrasion (Glass and Boyd, 1949a). It is 12 to 20 percent amino acids and most of the remainder is a basic carbohydrate structure Of galactose, glucosamine and galactosamine with superimposed terminal sugar residues (Schrager, 1970).

Dissolved mucin is composed of low molecular weight glycoprotein subunits. All the molecules of the subunits are linked by primary valency bonds and have stable structure which is not affected by the other components of gastric secretion and is not attacked by pepsin (Blair, 1974). This organized structure forms a filter through which solvent flows with difficulty and from which molecules the size of pepsin tend to be excluded. The forces which maintain the tertiary structure of mucin are decreased by high salt concentrations, by drugs with reducing groups and by a low pH (Schrager, 1970; Allen et al,

1993). The proportion of mucin found in the dissolved form is therefore affected by pH, the lower the pH, the greater the amount of soluble mucus; at neutral or near neutral pH the mucin is all in a semi-gel form (Schrager, 1970).

The secretion of mucin is spontaneous in the sense that it occurs in the resting stomach. Its secretion is stimulated by surface irritation, both chemical and physical, and it is important that its rate of production should not lag behind its destruction (Blair, 1974). The factors controlling mucin synthesis and secretion from the surface epithelial cells and the factors influencing mucin release from the mucus gel are poorly understood. It is generally accepted that in healthy man, mucin is secreted continuously even during resting conditions (Konturek, 1979). The major stimuli for mucin secretion are vagal or cholinergic stimulation, acidification of the gastric mucosa and irritable substances acting on the mucosa. It has been suggested that it is possible that the deficiency of mucin secretion renders the gastric mucosa susceptible to acid-pepsin digestion, in turn contributing to the mucosa damage (Glass, 1976).

1.4:9 Dissolved Mucin and its Heterogenecity

Dissolved mucin has been separated from gastric juice by various means; precipitation with acetic acid and acetone (Glass, 1967), sulfosalicylic acid and acetone, sulfosalicylic acid and alcohol, tungastic acid, ethanol or methanol at low pH and trichloroacetic acid and acetone (Glass and Boyd, 1949b). Indication of heterogenecity of dissolved mucin became apparent

in the early thirties but it wasn't until 1948 to 1949 that Glass and his associates found a marked difference in the physical characteristics and composition of dissolved mucin properties depending upon the stimuli applied (Glass and Boyd, 1949a; 1949b). These differences include external appearance, degree of hydration and variations in the content of tyrosine, nitrogen and reducing substances. Thus it became quite probable that dissolved mucin represent a complex of two or more different materials which under the effect of various stimuli, varied independently from each other.

Strong support for the dualistic concept of gastric mucin came from Grossberg et al, (1950). They noted that there were variations in the ratio of hexosamine and uronic acid in the canine gastric juice and in the gastric mucus collected after histamine stimulation and sham-feeding. They concluded that at least two different mucous material existed in these secretions; one containing more uronic acid and less hexosamine; and the other containing more hexosamine and less uronic acid. The concentration of both mucosubstances are found to vary independently from one another.

In view of the heterogenicity of dissolved mucin, Glass and Boyd (1949), attempted to fractionate the material by chemical means. The mucin fraction which precipitated as a fluffy greyish-white precipitate at pH 3.5 was called "dissolved mucoprotein". It was later renamed "glandular mucoprotein" or "acidic mucoprotein-pepsin complex" because of its presumed derivation from the gastric fundic glands. The other material which remained in solution under these conditions and precipitate

only after the addition of acetone and incubation in form of gum-like mass adhering strongly to the test-tube wall was called "dissolved mucoproteose". It too, was later renamed "neutral glycoprotein-mucoproteose complex" because it represent a complex of several material derived from fundic glands and include neutral glycoprotein and proteose like materials of gamma globulin electrophoretic mobility. Thus the acidic mucoprotein-pepsin complex and neutral glycoprotein-mucoproteose complex fractions of gastric juice is considered as two groups of macromolecular materials. The first representing the acidic complex and the second, the neutral complex of mucosubstance with other proteins of gastric juice.

The acidic mucoprotein-pepsin complex contains; acidic mucoprotein-pepsin complex and amino-polysaccharide; pepsin and some other as yet unidentified protein; and a degraded form of intrinsic factor. The neutral glycoprotein-mucoproteose complex includes; neutral glycoprotein; proteose like materials of gamma-globulin mobility; and a tertiary B12 binder, not related to intrinsic factor (Glass, 1967). Neither of the two fractions shows an even approximate constant composition. However, it is true that the composition of the two fractions, like that of other heterogenous material is subject to some variations, but this variations does not affect the basic underlying compositional features of the two complexes which are retained within a reasonal limit in the acidic gastric juice.

1.4:10 Gastric Mucus Barrier

A key element in understanding the gastric mucosa's unique

ability to withstand its acidic environment is the concept that the gastric mucosa acts as a semipermeable membrane or barrier that severely limits the passive diffusion of ions such as H^+, Na^+ and K^+ along their concentration gradients. By maintaining relatively steep gradients of these ions on either side of the mucosal epithelium, this barrier allows the stomach to function as a reservoir for gastric acid while controlling intramural acidity.

The concept of a gastric mucosal barrier did not attain clinical significance as an important mechanism of gastric mucosal protection until the landmark work of Davenport (1964a, 1964b, 1965, 1967 and 1972), who evaluated the physiologic consequences of gastric mucosal exposure to topical gastric irritants such as aspirin, ethanol and bile salts. Davenport's studies and insight established the basic concepts and the pathogenesis of acid-induced injury. His studies provided the first evidence that mucosal impermeability to H^+ back-diffusion is an essential mechanism of gastric mucosal protection and that a derangement in mucosal permeability could produce mucosal damage, but only if H^+ back diffusion overwhelmed the capacity of supporting mechanisms of protection. using the fundamental concepts established by Davenport, other investigators have attempted to define the precise physiologic mechanisms that maintain the gastric mucosa's impermeability and that otherwise, protect the gastric mucosa from its own secretions.

In recent years, our knowledge of the physiology and anatomy underlying the gastric mucosa barrier has expanded to include such elements as the combination of surface mucus and epithelial

HCO^-_3 secretion, the apical membrane, active ion transport, the production and transport of HCO^-_3 via mucosal blood flow, and cytoprotection by endogenous prostaglandins. Meanwhile, the only physical structure between gastric acid in the lumen and the surface epithelia cells of the mucosa is the gelatinous mucus that tenaciously adheres to the mucosa surface. It is only natural then that mucus has long attracted attention as a potential mechanism of gastric mucosal protection from acid-induced injury.

The protective role of mucus is best explained by the hypothesis of Heatley (1959). He suggested that if mucus secreting cells secrete some bicarbonate and the cells are covered by a layer of mucus, there will be a gradient of pH changes through the mucus layer, low pH levels existing on the luminal side, alkaline pH levels on the cell surface plane. Though the acid may penetrate through the mucus layer, there will always be a layer, albeit infinitesimally small, of mucus penetrated by alkaline on the cell surface. This theory may be relevant as it has been shown that gastric ulcers occur only in the antral mucosa which has an alkaline secretion. It does not explain the absence of ulcers in the body of mucosa, in which case the mucus layer would be exposed to acid secretion by the parietal cells on the cell wall surface of the mucus layer and the acid contents of the gastric lumen on the other side. Although hydrogen ions diffuse through mucus as rapidly as through water, it has been suggested that the lattice - like architecture of mucus gel retards mixing of acid-pepsin with slightly alkaline fluid secreted by the surface epithelial cells,

and leaves them in contact with a thin layer of neutral or slightly alkaline fluid regardless of the concentration of acid in the lumen (Carter, 1980).

However, whether mucosal damage actually develops following exposure to agents that compromise the mucus-bicarbonate barrier may depend on the integrity of other mechanisms of gastric mucosal protection.

1.4:11 Cytoprotection

Several prostaglandins, given at non-antisecretory doses protect the rat gastric mucosa from necrosis produced by a variety of agents applied to the lumen, such as absolute ethanol, 0.6M Hcl, 0.2M NaOH (Robert et al, 1979), acidified aspirin (Guth et al, 1979) and taucholate (Chaudhury and Robert, 1980). This protection was further demonstrated in humans [prevention of gastric bleeding by aspirin (Cohen, 1978) and indomethacin (Johansen et al, 1980), of gastric erosion by aspirin (Gilbert, 1984), and of gastroduodenal damage produced by ethanol]. This effect of prostaglandins was called "cytoprotection" (Robert, 1977), which is defined as the property of certain prostaglandins to protect gastric mucosal tissue located under the surface epithelium from becoming hemorrhagic and necrotic after exposure to noxious agents. In most studies on "cytoprotection" this term is used to describe the macroscopic phenomenon of a substance preventing the appearance of macroscopically visible red or dark streaks. Silen and Ito (1982) emphasized in a letter on Gastroenterology that cytoprotection means protection from cell

damage which can be demonstrated only by microscopic examination.

The precise mechanism(s) whereby prostaglandins cause or mediate protection is still unknown. Many studies deal with mechanisms above or below the epithelium - mucus and bicarbonate secretion (Miller et al, 1983). Several studies have noted an increase in gastric juice viscosity or the intragastric concentration of carbohydrate constituents of mucus glycoprotein following gastric exposure to prostaglandins, suggesting that prostaglandins increase mucus secretion (Ruppin et al, 1981 and Lamont et al, 1983). However, these are indirect measures of mucus secretion that could just as easily reflect increased solubilization and erosion of mucus without a concomitant increase in mucus secretion. More definitive evidence that prostaglandins can affect the mucus component has evolved from a system developed by Bickel and Kauffman (1981) to directly measure the thickness of the insoluble mucus gel covering the gastric mucosa. These investigators recorded a 140% increase in gel thickness when rat gastric mucosa was exposed to a prostaglandin. A similar increase in gastric mucus gel depth was reported by Takeuchi and co-workers (1983) with frog gastric mucosa.

Other mechanisms of cytoprotection which has been proposed include; increase in mucosal blood flow, stimulation of mucosal to serosal transport of sodium (Chandry and Jacobson, 1978) or chloride (Schiessel et al, 1980) and increase in phospholipid mucosal coating (Lichtenberger et al, 1983).

1.4:12 Pathogenesis of Gastric Mucosal Damage

A discussion of the gastric mucosal protection provided by the anatomic and physiologic gastric mucosa barrier - an alliance of such elements as the mucus - bicarbonate barrier, the surface epithelial cells, and active ions transport supported by mucosal blood flow, Hco^{-}_{3} formation/transport, and cytoprotection - is simultaneously a story of the pathogenesis of gastric mucosal injury. If compromised, each of the factors contributing to the maintenance of intramural pH at a level compatible with cellular viability represents a potential pathway of gastric mucosal damage manifested by gastric erosions, gastritis, or gastric ulcers. Even though the mechanism may be different, depending upon the damaging agent or event, the proximate cause of damage would seem to be the same - accumulation of influxing H^{+} in the mucosa. A small amount of luminal acid would therefore seem to be necessary for gastric mucosal injury. An increase in mucosal permeability due to, for example, the compromise of the mucus - bicarbonate barrier could give rise to intramural acidification (Guth, 1984).

CHAPTER TWO

LITERATURE REVIEW

There is a considerable difference of opinion between clinicians and physiologists on the role of gastric acid and mucus in the pathogenesis of peptic ulceration. As a result, despite much research efforts the aetiology of peptic ulceration still remains enigmatic. Although, gastric acid seems essential for the formation of peptic ulceration (Flemston and Turnberg, 1984), very few clues have emerged from studies of acid secretion rates in patients with peptic ulcer. The problem lies in the observation that many patients with duodenal ulcer and most patients with gastric ulcer secrete acid at rates indistinguishable from normal controls. In these circumstances, it seems likely that, although, acid plays a permissive role, it is not a primary cause and attention has thus focused on the factors which protect the mucosa from acid and peptic secretions. Therefore, it has become tradition to discuss the pathogenesis of peptic ulcer as an alteration in the balance between opposing forces of attack upon the mucosa and mucosal defence mechanism. Advances in understanding the roles of gastric acid and mucus in the aetiology of peptic ulceration may raise the hope that an effective non-surgical therapy for ulcer disease will be developed in the near future,

This review discusses the mechanisms of mucosal defence against various insults caused by various aggressive forces under the broad headings of pre-epithelial protection by mucus, mediators of mucosal injury like hydrochloric acid, that produces

unphysiological mucosal damage and their possible implications in the pathogenesis of gastric mucosa damage. In a third section, which of necessity, is speculative, the review will also attempt to discuss the possible role of food habits in the pathogenesis of peptic ulcer disease.

2.1:0 Gastric Acid Secretion

Schwarz's dictum, "without gastric acid, no peptic ulcer" remains a generally reliable rule with few exceptions (Isenberg et al, 1971; Duberstein and Efrusy, 1977). Operations that diminish acid secretion usually cure duodenal (Golighter et al, 1968) and gastric ulcers (Duthie and Branson, 1979), as do procedures that divert the acid away from the ulcer (Monyniham, 1982). Administration of H2-receptor blockers viz cimetidine, which reduces gastric acid secretion usually produces healing of duodenal ulcers (Winship, 1978) and gastric ulcers (Freston, 1978). Acid secretion need not be abnormally high to provoke peptic ulceration since gastric ulcers are associated with normal or low output (Baron, 1963), and between one half and five sixth of duodenal ulcer patients secrete acid within the normal range when measured by the augumented histamine and maximal histalog stimulation test (Baron, 1963; Wormsely and Grossman, 1965). There is evidence that acid hypersecretion develops in some duodenal ulcer patients only after ulceration has occurred (Sircus, 1968; Hobsley et al, 1975; Fiddian-Green et al, 1976). It has not been shown that acid secretion falls during spontaneous remission of peptic ulcer (Grossman, 1981), although, acid output can fall spontaneously in ulcer patients (Desai et

al, 1971).

A link has also been demonstrated between acid output and ulcer location. High gastric ulcers are associated with normal or low acid output and duodenal ulcers are associated with high acid outputs (Baron, 1963; Meeroff, 1973; Relinski, 1975; Fiddian-Green et al, 1976; Bittner et al, 1979). One possibility is that acid output is related to the parietal cell mass (Cards and Marks, 1960), and the parietal cell is greater in association with distal ulcers (Cox, 1952). So it is possible that cellular background influences both acid output and ulcer site. The part played by acid in determining localized mucosal breakdown remains obscure. Acid exerts a field effect within the gastroduodenal lumen. Since fundic mucosa appear to be resistant to acid attack (Oi et al, 1959; Capper, 1967), ulceration should occur always in the first susceptible mucosa, which is in the antrum, and which has the greatest contact with the highest concentration of acid. Ulceration is expected to occur fortuitously in the exposed area, but less than 5% of the gastric mucosal is susceptible to chronic ulcer (Oi et al, 1959). Acid attack could determine the site of a solitary peptic ulcer if its effect were concentrated in one area (Kirk, 1982). From experiments in dogs it was suggested that a jet of acid emerging through the pyrolus impinges on the bowel well beyond, causing mucosal breakdown (Mann and Bollman, 1982). This would require the jet to strike always in the same place. Studies on operations (Kirk, 1968), and through the endoscope (Kirk, 1975), suggested that no part of the duodenal wall is uniquely exposed to or protected from contact with a jet emerging through the pyrolus.

Thus both experimentally and clinically, the ulcer associated with hypersecretion of acid is duodenal in location. According to Grossman (1960), this hypersecretion of duodenal ulcer patients manifest itself in all types of gastric secretory studies, whether such studies are on the interdigestive secretion during the day of night, or on the secretion in response to a meal or to drugs such as histamine, insulin or pentagastrin. The difference between normal persons and peptic ulcer disease patients is greatest in studies on the basal interdigestive secretion in response to caffein.

2.1:1 Basal Acid Output

In duodenal ulcer, the typical finding upon gastric analysis are; a fasting juice of greater volume than normal and of high acidity; and a curve of gastric acidity after a test meal which rises above the normal maximum stimulation (Best and Taylor, 1973). The role of basal gastric secretion in the pathogenesis of peptic ulceration has not been satisfactorily defined. Most, but not all, authors agree that on the average, basal secretion is greater in patients with ulcer than asymptomatic patients, but equally, it is agreed that there is a considerable overlap between different groups in terms of individual subjects (Faber and Hobsley, 1976; Baron, 1978). In order to answer the question of whether an increase in basal acid secretion is an etiological factor in development of peptic ulcer, Faber and Hobsely (1976), examined basal acid secretion in control subjects and patients with peptic ulcer disease in terms of conventional indices of

secretion; volume, titratable acidity and acid output. In all the groups, secretion was less in the second than the first hour of a one hour basal collection. They concluded that basal secretion was found on the average to be greater in peptic ulcer patients than control subjects.

An increase in basal acid output suggest the presence of an increased basal drive, and is compatible with the concept of increased vagal tone as proposed by Dragstedt (1956). More investigators indeed agreed that duodenal ulcer patients have on the average, an increased basal acid output (Baron, 1963; Faber and Hobsley, 1977; Feldman et al, 1980). Most researchers agree that total amount of basal acid secreted rather than the concentration distinguish duodenal ulcer patients from normal subjects. Based on this, Levi et al; (1960) carried out a twelve hour overnight aspiration under two circumstances in healthy subjects and peptic ulcer patients. The subjects in one group fasted twenty-four hours before aspiration, while in the other group, aspiration began after an ordinary evening meal. Under both circumstances, basal secretion was found to be comparably higher in peptic ulcer patients than in normal subjects. A year later, Levin and his associates showed that one hour morning collection of basal secretion was equivalent to a 12-hour overnight collection. A single 15-minute period of anacidity during basal morning hour was very unusual among duodenal ulcer group. Interestingly, results from one hour basal collection were more reproducible than those from standard histamine test when repeated in the same individual (Levin et al, 1961). Studies by Hunt and Kay (1964) and by Hunt (1967), indicated that

the ratio of basal secretion to stimulated secretion is the same in ulcer patients as in normal control subjects, and hence increased basal secretion rises.

In a study to confirm the correlation between basal gastric secretion with peptic ulcer activity, Bolo et al (1961) compared basal output of gastric acid in 19 patients with peptic ulcer in relapse and remission, one test being performed in each category. Hyperchlorhydria by their arbitrary definition was present in 79 percent of those cases with peptic ulcer activity and 41 percent in remission. Further evidence suggesting higher output with ulcer activity was that hyperchlorhydria was found in 85.9% of 144 patients with active ulcer and 36.1% of 47 other patients with quiescent ulcer. This lead Littman (1962) to conclude that basal output of hydrochloric acid is higher and more variable in patients with peptic ulcer disease than control subjects. The statement that gastric hypersecretion is probably not the cause of ulcer is strictly confined to maximal gastric secretion. The important influence may still be an excess of acid entering the duodenum, but under submaximal conditions (Hobsley et al, 1975). It may be that not the maximal capacity of the stomach to secrete acid, but the actual amount secreted day in and day out, fasting and sleeping, is the factor that matters. Despite this, the impression remains that basal secretion is an etiological factor in the development of peptic ulcer if only it could be accurately determined (Faber and Hobsley, 1976).

Baron (1978) observed that the size of the stomach and the quantity of its secretion may be related to constitutional

factors such as weight, height, sex and age. The separate relationship are still not all clearly understood and the problem is complicated by both historically and statistically by the interdependence of these biological factors. All studies of gastric secretion, basal or stimulated, control subjects and patients with peptic ulcer, have shown higher values for men. Thus sex difference is not found in animals of the same weight (Shay et al, 1964) nor in human child (Ghai et al, 1965) or infant (Rodbro, 1967). The significant positive correlation found between maximal acid output and body weight in control subjects according to Marks (1961) helps to explain a portion of the difference between the mean maximal output in female and male control subjects. Sex difference have shown to be insignificant in normal subjects (Baron, 1969; Anderson et al, 1970) and patients with duodenal ulcer (Baron, 1969; Anderson et al, 1970; Borgstrons et al, 1973), but not in patients with gastric ulcer (Borgstrons et al, 1973).

A decline was found with age in food stimulated secretion (Baron, 1978). Levin et al, (1971) confirmed these decrease for acidity, volume and acid output. They showed in addition, that basal acid output also declined with age. Comparable decrease with age have been found in normal subjects after submaximal dose of histalog (Baron, 1964). Acid secretion after maximal dose of stimuli have been reported as decreasing significantly with age in normal men and women (Baron, 1963) and men but not women with duodenal ulcer (Wormsely and Glossman, 1965) and gastric ulcer (Marcussen et al, 1970; Borgstrons et al, 1973). Vakil and Mulekar (1965) studied 208 hospitalized Indian patients with

maximal histamine test. They found that acid outputs were significantly higher in men than women of comparable age. Acid output rose with an increase age in both men and women until 50 years of age after which there was a decrease of output in both sexes. Koster (1966) in a report from Copenhagen indicated a statistically significant correlation between age and peak acid output in men and women with peptic ulcer disease, the output decreasing by 5 mmol/hr for each 10 year increase in age. In women, the acid output fall approximately 2 mmol/hr for each 10 year increase in age, but the statistical significance of this trend was less later.

Gastric acid production and secretion curves seems to be very similar for normal Nigerians (Tai-da-Roucha-Afodu and Adesola, 1972), indians (Goyal et al, 1966), Singapore (Fung, 1968) but lower than those reported from Britain (Bruce et al, 1959) and America (Sun et al, 1967). The basal acid output quoted in standard textbooks (Irvine, 1972) which are indicative of duodenal ulceration do not appear to be applicable to Nigerians in the light of the study conducted by Solanke and Lewis (1976). In the study, there were significant differences in the values of basal acid output between the control group and the uncomplicated duodenal ulcer group.

2.2:0 Gastric Mucus Barrier

The only structure between gastric acid in the lumen and the surface epithelial cells of the mucosa is the gelatinous mucus that tenaciously adheres to the mucosal surface. It is only natural then that mucus has long attracted attention as a

potential mechanism of gastric mucosal protection from acid-induced injury. Although, several years have passed since Davenport (1964a, 1964b and 1965) published his classical studies describing the unique properties of the gastric mucosal barrier and how it is compromised by damaging agents, the physico-chemical nature of this protecting lining is still undefined. The physiological role(s) of gastrointestinal mucus remains disputed, despite the fact that these substances have long been known to be secreted in copious amount throughout the digestive tract (Pfeiffer, 1981).

This alleged attribution of mucus in the protection of gastro-duodenal surface epithelium against acid-proteolytic action of gastric juice has gathered wide support in recent years as a result of histochemical and in vivo secretory data documenting reductions of intracellular mucus or changes in carbonhydrate or protein content of secreted mucus after administration of irritants or ulcerogenic agents (Hakkinen and Hartiala, 1966; Kent and Allen, 1968; Johansson and Lindquist, 1971; Williams et al, 1971; Arnthorsson et al, 1971).

Hollader (1954) stated that the mucosal barrier of the stomach involves two components; an external mucus layer and the epithelial cell layer. Heatley (1959) hypothesized that mucus protect the stomach forming an unstirred diffusion barrier in which hydrogen ions from the lumen is neutralized by HCO^-_3 secreted by epithelial cells. This model was later extended by Allen and Garner (1980) by suggesting that on the basis of physico-chemical consideration, the mucus gel would maintain a pH gradient by preventing secreted HCO^-_3 from being rapidly

neutralized by luminal pH. This model was supported by the study of Williams and Turnberg (1981) who measured the pH in rabbit gastric mucus gel using micro electrodes. They found a stable pH gradient from 2.31 on the luminal side to 7.26 on the epithelial side of the gel layer. Furthermore, intestinal mucus glycoprotein have recently been shown to retard diffusion of hydrogen ion (Williams and Turnberg, 1980; Pfeiffer, 1981).

A clear and accepted role for gastric mucus is that of a lubricant protecting the underlying mucosal cells from mechanical abrasion (Hollander, 1954; Florey, 1955). Another role is that of providing a mixing barrier for the containment of HCO_3^- secretion and restriction of H^+ access to the mucosal surface. In either of these cases, the effectiveness of the surface mucus gel will depend on its depth. The layer of mucus gel is eroded because of the action of pepsin (Allen, 1978; Scawen and Allen, 1977) as well as by mechanical factor such as food (Allen and Garner, 1980).

Therefore, secretion of the glycoprotein will not only need to be at a sufficient concentration to exceed the threshold for gel-formation but also at a rate that will maintain an effective depth of gel on the surface despite erosion. Several studies, using human gastric aspirates or experimental animals with gastric fistulae, have shown increases in luminal glycoprotein with different stimuli (Horowitz, 1978; Glass and Slomiany, 1977; Vagne and Perret, 1976), for example, secretin (Kowalewski et al, 1976) and prostaglandins (Bolton et al, 1978; Domsche et al, 1978). However, increases in the amount of luminal mucus glycoprotein cannot necessarily be interpreted as an increase in

secretion of mucus gel, as such, rise could be due solely to increased erosion by pepsin or other factors (Allen, 1979). In the absence of a concomitant stimulation of secretion this would, in fact, result in a thinner layer of gel on the mucosal surface.

2.2:1 The Mucin Component

Gastric mucus, the slimy secretion of the stomach linings is a complex mixture of enzymes, cellular products, serum proteins, electrolytes and mucin, a high molecular weight glycoprotein with a viscous and gel forming properties (Glass, 1967). The viscous and gel-forming properties of mucus secretions are derived from mucin glycoprotein constituents, which in adherent gastroduodenal mucus gel constitute about 5% (50mg mucin/ml) by weight of the secretion (Bell *et al*, 1985; Neutra and Forstner, 1987; Allen, 1989; Allen *et al*, 1993). When purified, gastroduodenal mucin are reconstituted at their *in vivo* concentrations, they form a gel with the same rheological properties as the native secretion (Bell *et al*, 1984; Bell *et al*, 1986). Thus mucin alone can account for the viscous and gel-forming properties of the secretion.

Pfeiffer (1981) hypothesized several mechanisms of protection of gastric surface by mucin. These include; possible buffering of the acidic gastric juice; physical binding of digestive enzymes attributable to the three dimensional structure of mucous glycoproteins at physiological conditions; possible barrier to the diffusion of hydrogen ions towards the epithelial cell surface; and antiproteolytic action of mucus directed against gastric protease. The concept of protection due to

macromolecular structure of mucus glycoproteins (Schrager, 1964; Waldon-Edward, 1970) rest upon the hypothesis that the three-dimensional matrix formed by mucus on the mucosal surface, a condition constantly being degraded by secreted acid and renewed by secretion of mucus, inhibits the influx of high-molecular weight proteolytic enzymes. However, it has been suggested that such a protective layer would allow ready permeation of small ions such as hydrogen ions (Pfeiffer, 1981). Proteolytic enzymes *(e.g. pepsin) and mucolytic agents that reduce peptide disulfide* bridges (e.g. N-acetylystein) dissolve mucus gels. This mucolytic action has been attributed to cleavage of the protein core of the component mucin molecules to yield smaller glycoprotein units with lower viscosity and no gel-forming properties (List et al, 1978; Bell et al, 1986).

Mucins are very complex molecules and the difficulty in their isolation has been the rigorous exclusion of proteolysis. The intact polymeric structure of the mucin is essential for gel formation and hence the mucolytic action pf proteinase (e.g. pepsin), these digest the nonglycosylated peptide regions of the mucin, containing the interchain disulfide bridges, to form degraded glycopeptide fragments of still relatively high molecular mass (Allen et al, 1993). In solution, polymeric mucin molecules are highly expanded and hydrated, occupying a large volume of solution. Gel formation occurs when the concentration of polymeric mucin molecules in solution is such that their expanded conformations overlap and noncovalent intermucin interactions occurs. The nature of these gel-forming noncovalent interactions between the polymeric mucin molecules has yet to be

defined, and models proposed range from relatively nonspecific interdigitation of carbohydrate chains (Bell et al, 1984) to specific interactions (Allen et al, 1993). Following on from this model of gel structure of mucus, where the glycoprotein molecules are permeating the whole of the matrix, it can now be appreciated that mucus gel provides an excellent unstirred layer retarding any mixing of ions within its interstices with those in the bulk phase of the gastric lumen.

For over 20 years, the contribution of mucin to the contribution of gastric mucosal-barrier, has been dismissed as being minimal, as offering little or no protection (Davenport, 1967; Fromm, 1981). The view was based on the early report of Heatley (1959) that mucin, when compared with water, did not reduce the speed at which hydrogen ion and pepsin would diffuse to the mucosal surface. In recent years, the results of experimental methods more sophisticated and quantitative than those employed by Heatley, provide somewhat different perspective. According to the results of Williams and Turnberg (1980), gastric mucus may represent at least a slight barrier to H^+ back diffusion co-efficient through a known thickness of pig gastric mucosa was one fourth that through an equivalent thickness of unstirred water solution.

Defects in mucin have been described in patients with peptic ulcer. Less, N.acetynemaminic acid escape into the lumen of subjects with gastric ulcer (Domsher et al, 1977) and radio labelled percussor sugar incorporated into glycoproteins were reduced in gastric mucosal biopsies of patients with gastric ulcer (Park and Symons, 1977). The mucus taken from gastrectomy

specimens of patients with ulcers were recently found to be more graded than normal (Yonman et al, 1982). A similar but less marked defects was found in duodenal ulcer patients. The results suggested that mucus produced by ulcer patients was either more easily degradable or that it was subject to a greater than normal enzymatic attack (Flemstron and Turnberg, 1984). Goodman (1975), suggested that the alienated disease of the gastrointestinal tract, such as peptic ulcer disease might be due to some defects in the production of mucin which impairs its normal protecting power. Excess Lysozyme, which is found in the stomach of ulcer patients has been found to destroy mucus (Meyer et al, 1964).

The notion that mucus glycoprotein are antipectic, especially those containing high sulfate content has been proposed (Waldon-Edward, 1970); secreted mucus from peptic ulcer patients has been observed to contain low sulfate content, and sulfate glycoproteins have been shown to protect proteins against peptic digestion (Levy and Sheinfield, 1954). Increase in the amount of luminal mucus glycoprotein cannot necessary be interpreted as an increase of mucus gel, as such, a rise could be due solely to increased erosion by pepsin or other factors (Allen, 1979). Compounds that either inhibit or stimulate mucin production might be expected to have ulcerogenic or anti-ulcer properties respectively. For example, while a wide variety of agents have been reported to change luminal mucus glycoprotein levels (Vagne and Perret, 1976; Glass and Slomiany, 1977) it is not possible to define whether this represent changes in the rate of secretion or erosion of the mucus gel. Glass (1976) suggested that it is possible that the deficiency of mucus secretion

renders the gastric mucosa susceptible to acid-pepsin digestion, in turn contributing to the mucosa damage.

The mucosubstance of the human stomach can no longer be though of merely as mechanical lubricants and troublesome slime, but should be considered to be what they are; materials which have a wide and deep biological significance that extends far beyond the confines of gastrointestinal tract.

2.3:0 Food and Social Habits

Peptic ulcer, although, accompanied with gastric secretory abnormality, has shown to be markedly associated with smoking (Piper et al, 1982), cola, coffee and alcoholic beverages (McArthur et al, 1982). The protein, fats and carbohydrate and caloric values of meals that has been used to study gastric secretions varies greatly and there is no apparent relationship with gastric acid output. A low protein diet is said to increase susceptibility to peptic ulcer (Cleave, 1962), as may a high intake of carbohydrate. Fat has been indicted (Spura, 1956), and so has ingested alcohol. If dietary treatments reflect ideas on aetiology, they vary so widely, that no pattern emerges.

Cleave (1974) suggested that the introduction of refined carbohydrates (white flour, polished rice and sugar) or of starchy foods results in a loss of buffer and that this may be an important factor in the production of acid. The possible effect of natural buffer in unrefined food on gastric secretion has been investigated (Tovy, 1974) and the picture is confusing. After an initial fall in acidity, they may act as antral stimulants. Chowdry (1974), suggested that the amount of

mastication required in consuming the staple carbohydrate food may be important because alkaline and mucus content of the saliva protects the gastric mucosa. The possibility that differences in diet might be of aetiological importance, contributing to the differences in incidence of peptic ulcer disease is supported by the report of Malhotra (1978). He described a group of 50 patients in Bombay with radiologically proven duodenal ulcers who were hospitalized until their ulcers were symptomatically and radiologically healed. After this, one half continued on their normal rice diet and one half changed to unrefined wheat and milk. At the end of five years, 21 patients were left in each group. Among those on the unrefined wheat and milk, only three (13%) had relapsed in comparison with 17 (81%) of those on their normal rice diet. These findings would support either the concept that there may be ulcerogenic or protective factors in the diet.

Bland diets have been prescribed for generations in patients with peptic ulcer disease (Sippy, 1915). The beneficial effect of these diets was first questioned in the 1930s by both experimental and clinical observations (Mann and Bollman, 1932; Palmer, 1933). At least, clinical trials testing bland diets versus regular diets have failed to reveal any differences on ulcer symptoms or healing (Baron, 1972). Infact, hourly milk and cream result in a higher gastric acidity than three regular meals per day (Kirsner and Palmer, 1940). Ippoliti et al (1976), demonstrated that milk stimulated gastric acid secretion to 23% of the maximal acid response to pentagastrin. The results of McArthur et al, (1982) demonstrated that a 12-oz glass of milk

is as potent a stimulant of gastric acid secretion as the maximal response to pentagastrin.

Alcohol does not appear to increase gastric acid secretion in humans (Cohens and Booth, 1971). However, the effect of intragastric alcohol on gastric acid secretion has not been evaluated using either the intragastric titration or serial dilution indicator methods. Intravenous alcohol produces a slight increase in acid secretion within 30 minutes of administration (Hirschowitz et al, 1956). Thus, the effect of alcohol may be indirect and occur after its absorption. Current evidence suggests that mild to moderate alcohol consumption may have little or no effect on liability of ulcer, while the effects of heavy consumption are unclear.

Death rates with peptic ulcer are higher in smokers than in non-smokers (Doll and Hill, 1964), and ulcer itself is found more often in smokers than in non-smokers. Examination of the smoking habits of Massachusetts physicians (Mason, 1970) showed that those with gastric or duodenal ulcers had smoked more than those who did not have ulcers, and those with duodenal ulcer had started smoking at an earlier age. However, when smokers only were considered the patients and controls were similar in the number of years for which they had smoked, the amount smoked per day and in the proportion who had ultimately stopped, suggested that liability to ulcer and to smoke were separately derived from some other cause. Further evidence to support the suggestion that smoking may be a causal factor of peptic ulceration comes from the data of Paffenbarger and his colleagues (1974), who compared the smoking habits found in college students with their

later histories of ulcer. Smoking was more prevalent in those who developed ulcers later, though the association was weaker than those detected in the same students with coffee or with soft drinks consumption.

CHAPTER THREE

MATERIALS AND METHODS

Clearance to carry out the research was obtained from the Scientific and Ethical Committee on Human Research, Institute of Health, Ahmadu Bello University, Zaria.

Thirty healthy subjects and thirty patients with endoscopically confirmed peptic ulcer from the Gastrointestinal Disease Clinic, Ahmadu Bello University Teaching Hospital, Zaria, were studied using the gastric intubation technique as described by Hassan and Hobsley (1970). All the subjects used for the study were adults with ages ranging between 18 to 50 and their consent for the investigation was obtained after explaining thoroughly the methodology to them. All healthy subjects served as the control group.

3.1:0 Materials

- Adult humans
- Nasogastric tubes (8FR - 120cm)
- Specimen bottles
- 20ml syringes
- Cotton wool
- K-Y jelly
- Hand gloves
- Corning's colorimeter (253)
- Philip's pH meter with electrode (PW 9418/30)
- Water bath with shaker
- 250ml beakers

- 50-500ml cornical flasks
- 10-250ml measuring cylinder
- 50-100ml volumetric flasks
- Test tubes
- Centrifuge
- Pipette (1-10ml)
- Burette (30ml)
- Burette holder
- Emesis basin

3.1:1 Reagents

- Phenolpthalein indicator
- 0.1 - molar sodium hydroxide
- 0.1 molar hydrochloric acid
- 2% Sodium carbonate
- 10% trichloroacetic acid
- 1% potassium tartrate
- Copper sulphate
- Folin - ciocalteau phenol reagent
- Egg Albumin
- Distilled Water.

3.1:2 Technique of Collecting Gastric Juice

The procedure was thoroughly explained to the patients. All subjects were instructed to take no food or drink after bedtime the evening before, and all drugs were stopped 24 hours or more before the test.

At 8:30 am. after an overnight fast, basal gastric juice was obtained for a period of 1 hour using a nasogastric tube (8FR - 120cm) from the subjects. The tube was passed into the stomach through the nose to approximately 55cm. The tube's tip should lie in the gastric antrum when properly placed. This was confirmed by a simple method described by Hassan and Hobsley (1970). This method consist of the rapid injection of 50ml of water through the gastric tube into the stomach. If at least 45ml can be recovered by immediate aspiration, the tip is in proper position. After this, the tube was then taped to the skin at the corner of the mouth to keep it in place.

The subjects were kept lying in the left lateral position and asked to expectorate all saliva into an emesis basin throughout the test. A 20ml syringe was then attached to the tube, and the residual gastric contents was then gently aspirated continually for one hour. The tube was occasionally cleared by injecting a small quantity of air. After aspiration, the volume, pH and titrable acid were measured in each sample. The remaining gastric juice were immediately frozen for gastric mucin analysis later.

3.1:3 Technique for Gastric Acid (Hcl) Measurement

The volume of each gastric juice was measured carefully with a measuring cylinder and recorded. The pH of the specimen was measured using Philip's pH meter with glass electrode. Titration was performed on individual sample with 0.1M NaOH from standard burette after two drops of phenolphthalein indicator have been added to 10ml aliquot which was removed from the specimen. Titration was then performed carefully to the end point, which was the development of a slight pink colour from the previous colourless specimen. The volume of 0.1M NaOH required to produce this change was recorded.

Basal acid output was then calculated as follows (Boyce and palmer, 1975):

ml of 0.1M NaOH x 10 = mmol/L Hcl in specimen

mmol/L Hcl x Volume/hr specimen in litres = mmol Hcl/hr specimen.

Basal acid output were expressed as millimole of Hcl output per hour (mmol/hr).

3.1:4 Determination of Gastric Mucin Fractions

A detailed analysis of gastric mucin and its fractions using biochemical techniques of Glass (1967) and Guslandi et al (1978) was carried out on each specimen after aspirating the gastric juice by the method earlier described.

The rationale behind the use of biochemical technique to quantitate gastric mucin into its fractions is based on the fact that gastric mucin is formed when mucus glycoproteins interact

in the presence of a solvent system consisting of aqueous salt. In comparison with other gels, gastric mucin has a complex structure because it contains organic molecules other than mucus glycoproteins. These include proteins, lipids and nucleic acid which are secreted from the cells in the mucosa. A thin layer of gastric mucus normally covers the gastric mucosa. As this protective layer breaks down, the components of the gel are released into the lumen of the stomach as part of the gastric secretion. In this investigation, therefore, mucin output is represented by the combined weight of protein and glycoprotein that accumulate in the gastric juice during basal secretion.

Experimental Procedure

Eight mililitre of the specimen of gastric juice was homogenized and any visible mucus from the gastric juice was removed by centrifugation. To 3ml of the supernatant, ½ volume of 10% trichloroacetic acid (TCA) was added. This was to precipitate the soluble gastric mucin and some other proteins derived from serum. The deposit was discarded.

Glass: Fraction of Gastric Mucin

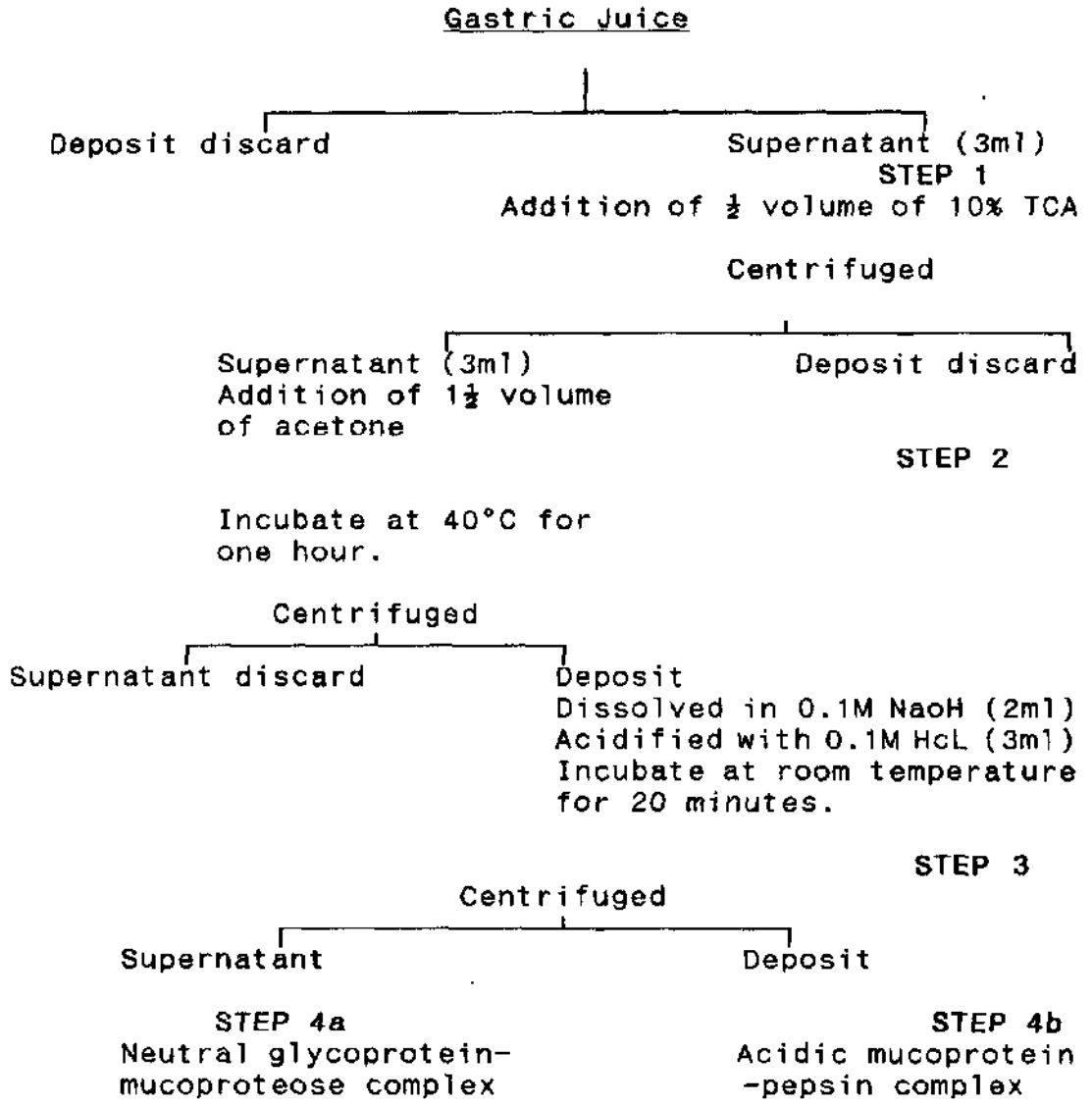


Fig. 4: Fractionation of dissolved mucin into acidic mucoprotein-pepsin complex and neutral glycoprotein-mucoproteose complex.

The solution was then centrifuged for about 10 minutes after addition of 10% TCA. To 3ml of the supernatant, 1½ volume (i.e. 4.5ml) of acetone was added. The solution was incubated, using water bath, at 40°C for one hour. The deposit was discarded. After an hour, the solution was removed from the water bath and centrifuged. The supernatant was discarded and the deposit was dissolved in 2ml of 0.1M NaOH and was acidified with 3ml of 0.1M Hcl. The solution was then incubated at room temperature for 20 minutes after which it was centrifuged. The supernatant represents the "Neutral glycoprotein - mucoproteose complex" fraction while the deposit represent the "acidic mucoprotein - pepsin complex" fraction. The details of this fractionation method are shown in Figure 4.

The two fractions were separated into two different test tubes. The tyrosine content of both fractions was then estimated using Folin-ciocalteau's reagent. This is in view of the presence of a distinct protein moiety in each of these materials and proteins react with folin-ciocalteau reagent to give a colour complex. The folin-lowry method of protein assay is outlined below;

Five millilitre of alkaline solution (2% $NaCO_3$ in 0.1M NaOH and 0.5% $CuSO_4$ in 3% $Na^{+}K^{+}$ tartrate) was added to 1ml of each mucin fractions. The solution was then mixed thoroughly and allowed to stand at room temperature for about 10 minutes or longer.

Half (0.5) millilitre of diluted Folin-ciacalteau reagent was then added to each solution rapidly with immediate mixing. After 30 minutes, the absorbance was read against appropriate

blank at 550 nm wave length, using a Corning's colorimeter (253).

The protein concentration of each fraction was then estimated after preparing a standard curve (appendix 7).

The ratio of neutral glycoprotein-mucoproteose complex to acidic mucoprotein-pepsin complex was expressed as a "mucoprotective index". Calculated from the formula below (Guslandi et al, 1978);

$$\frac{B}{A+B} \times 100 = \text{mucoprotective index}$$

Where A = acidic mucoprotein-pepsin complex (µg/ml)

B = neutral glycoprotein-mucoproteose complex (µg/ml).

3.2:0 Data Collection for dietary intake

Patients with peptic ulcer disease were asked by means of a structured questionnaire, their pattern and type of food intake and possible attributable risk factors such as smoking, drinking of alcohol (beer), coffee, beverages (soft drinks and various types of food drinks) and milk during two time periods; (i) the life time period until onset of initial symptoms (2) six months before onset of initial symptoms.

3.3:0 Statistical Analysis

The arithmetic mean and the standard error of mean (SEM) of various parameters such as age, pH, volume of gastric juice, basal acid output, acidic mucoprotein-pepsin complex, neutral glycoprotein-mucoproteose complex and muco-protective index were

determined and results were expressed as mean (±SEM). The mean values of the various parameters in the control subjects and patients with peptic ulcer disease were compared statistically using the Z-test. The difference between males and females in each group was determined using the student t-test. The Pearson product-moment correlation coefficient (r) was used to determine the correlation between parameters in each group. Results were considered significantly different if the P value was <0.05 (i.e. statistically significant at 95% confidence limit).

The dietary intake and ingestion of attributable risk factors was determined using the method described by Whittemore (1983). This statistics, also called the aetiology fraction, refers to the percentage of the total prevalence of disease amongst a group, which can be attributed to exposure to certain factors and therefore it indicates the proportion of disease that would not have occurred had such exposure be absent. The dietary intake and ingestion of the attributable risk factors was calculated as a percentage and derived from the numbers in the patients exposed to these factors.

CHAPTER FOUR

RESULTS

The control group of thirty patients were subjects not suffering from dyspepsia or peptic ulcer disease. There were thirty patients with uncomplicated peptic ulcer disease referred from the Gastrointestinal Clinic, Ahmadu Bello University Teaching Hospital, Zaria. There were ten female and twenty male subjects in the control group. Seventeen of the thirty patients with peptic ulcer were males while the remaining thirteen were females.

The mean ± (SEM) ages in the control group and peptic ulcer group were 23.3 ± 0.68 years and 26.7 ± 1.35 years respectively (Table 1). The mean ± (SEM) age of males in healthy control was 23.5 ± 3.61 years and 27.5 ± 1.52 years in patients with peptic ulcer disease (Table 2). The mean ± (SEM) age of females in both healthy controls and those with peptic ulcer disease were 20.0 ± 0.33 years and 24.5 ± 2.32 years respectively (Table 3). There was no significant difference between the ages of males and females in both healthy controls and those with peptic ulcer disease ($P>0.05$). Meanwhile, there was no significant correlation between age and basal acid output ($r = -0.215$, $P>0.05$), acidic mucoprotein-pepsin complex ($r = -0.23$; $P>0.05$) and neutral glycoprotein-mucoproteose complex ($r = 0.114$; $P>0.05$) in patients with peptic ulcer disease.

4.1 Gastric Juice

The mean (±SEM) values obtained for total gastric juice secreted during basal period in healthy controls and patients with peptic ulcer are given in Table 1. The values were 25.5 ± 1.49 ml/hr for healthy controls and 42.0 ± 2.22 ml/hr for patients with peptic ulcer disease. There was a significant difference between the two groups ($P<0.05$).

Tables 2 and 3 shows the mean (±SEM) values obtained for males and females separately in the two groups. The mean (±SEM) gastric juice secreted by male healthy controls was 24.25 ± 2.07 ml/hr and 39.76 ± 2.94 ml/hr in male patients with peptic ulcer. The difference between the two means was significant ($P<0.05$). The gastric juice secreted by females with peptic ulcer was significantly raised ($P<0.05$) when compared with healthy controls. The mean (±SEM) values were 28.0 ± 2.23 ml/hr for female healthy controls and 45.31 ± 1.67 ml/hr in the peptic ulcer disease group. There was no significant difference in gastric juice secreted by females and males of both control groups and those with peptic ulcer group ($P>0.05$).

4.2 Basal Acid Output

The mean (±SEM) basal acid output in healthy controls and patients suffering from peptic ulcer were 1.32 ± 0.39 mmol/hr and 3.04 ± 0.33 mmol/hr respectively (Table 1). The mean basal output of ulcer patients was significantly higher than those of healthy controls ($P<0.001$). Tables 2 and 3 shows the mean basal acid output of males and females respectively. The mean (±SEM) in male controls and those with peptic ulcer were 1.36 mmol/hr

and 3.42 mmol/hr respectively and the difference was highly significant (P<0.001). The mean (±SEM) values obtained for female healthy controls and those suffering from peptic ulcer were 1.21 ± 0.26 mEq/hr and 2.54 ± 0.25 mmol/hr respectively and the difference between the means was highly significant (P<0.001). Meanwhile, when the basal acid output obtained for males was compared with that of females in each group, the mean output did not differ significantly in both cases (P>0.05).

There was no significant correlation between age and basal acid output (r = 0.216, P>0.05). Similarly, there was no significant correlation between basal acid output and acidic mucoprotein-pepsin complex (r = -0.382, P>0.05) and neutral glycoprotein-mucoproteose complex (r = -0.411, P>0.05).

Frequency distribution of basal acid output levels in healthy controls and patients with peptic ulcer disease is shown in Figure 5. It shows that 3.3%, 26.7%, 20% and 50% of peptic ulcer patients secretes basal acid output of less that 1 mmol/hr, 1.1 and 2.0 mmol/hr, 2.1 and 3.0 mmol/hr, 3.1 mmol/hr and above respectively. The percentage of normal healthy controls secreting basal acid output of less than 1.0 mmol/hr was 30%, between 1.1 and 2.0 mmol/hr (63.3%), 2.1 and 3.0 mmol/hr (6.7%). None of the normal healthy controls secretes acid above 3.0 mmol/hr.

4.3 pH (Hydrogen ion Concentration

The mean (±SEM) values obtained for pH levels in healthy controls and peptic ulcer patients along with values obtained for males and females separately are given in Tables 1, 2 and 3 respectively. The mean (±SEM) values were 2.22 ± 0.05 (healthy controls), 1.8 ± 0.07 (peptic ulcer patients), 2.23 ± 0.07 (male controls), 1.7 ± 0.007 (males with peptic ulcer), 2.23 ± 0.09 (females controls) and 1.98 ± 0.01 (females with peptic ulcer).

There was a significant difference between the pH of healthy controls and peptic ulcer patients ($P<0.05$). The difference was also significant in males ($P<0.05$) but not in females ($P>0.05$). There was no significant difference between mean values obtained for males and females suffering from peptic ulcer nor in the control group when they were compared ($P>0.05$).

The correlation between pH and age was not significant ($P>0.05$) nor was there a significant correlation between pH and acidic mucoprotein-pepsin complex (0.461, $P>0.05$) and neutral glycoprotein-mucoproteose complex ($r = 0.216$, $P>0.05$) in patients with peptic ulcer disease. The frequency distribution of pH levels in healthy controls and those with peptic ulcer disease is shown in Figure 6. It shows that 13.3%, 56.6%, 23.3% and 6.7% of peptic ulcer patients had pH levels of between 1.0 and 1.4; 1.5 and 1.9, 2.0 and 2.4, 2.5 and above respectively, while in normal healthy controls, no subject had pH level below 1.4. Meanwhile, 16.7% had pH level that fell between 1.5 and 1.9, 56.7% (2.0 - 2.4) and 26.3% (2.5 and above).

Table 1: Basal acid output, gastric juice secretion, pH and ages of control subjects and patients with peptic ulcer disease.

Parameters	A Control (30)	B Peptic Ulcer (30)	P A Vs B
Gastric Juice (ml/hr) Mean ± S.E.M.	25.5 ± 1.49	42.03 ± 2.22	<0.05*
Basal acid output (mmol/hr) Mean ± S.E.M.	1.32 ± 0.39	3.04 ± 0.33	<0.001**
pH Mean ± S.E.M.)	2.22 ± 0.05	1.8 ± 0.07	<0.025**
Age (Year) Mean ± S.E.M.	22.3 ± 0.68	26.23 ± 1.35	>0.05

* Significant

** Highly Significant.

Table 2: Basal acid output, gastric juice secretion, pH and ages of healthy controls and patients with peptic ulcer disease (MALES).

Parameters	A Control (20)	B Peptic Ulcer (17)	P A Vs B
Gastric Juice (ml/hr) Mean ± S.E.M.	24.25 ± 2.07	39.76 ± 2.94	<0.001**
Basal acid output (mmol/hr) Mean ± S.E.M.	1.36 ± 0.49	3.43 ± 0.54	<0.001**
pH Mean ± S.E.M.	2.23 ± 0.07	1.7 ± 0.07	<0.001**
Age (Year) Mean ± S.E.M.	23.5 ± 3.61	27.59 ± 1.52	>0.05

* Highly Significant

Table 3: Basal acid output, gastric juice secretion, pH and ages of healthy control subjects and patients with peptic ulcer (FEMALES).

Parameters	A Control (10)	B Peptic Ulcer (13)	P A Vs B
Gastric Juice (ml/hr) Mean ± S.E.M.	28 ± 2.23	45.31 ± 1.67	<0.001**
Basal acid output (mmol/hr) Mean ± S.E.M.	1.21 ± 0.26	2.54 ± 0.25	<0.001**
pH Mean ± S.E.M.)	2.3 ± 0.09	1.98 ± 0.12	<0.05
Age (Year) Mean ± S.E.M.	20 ± 0.33	24.46 ± 2.37	>0.05

* Highly Significant.

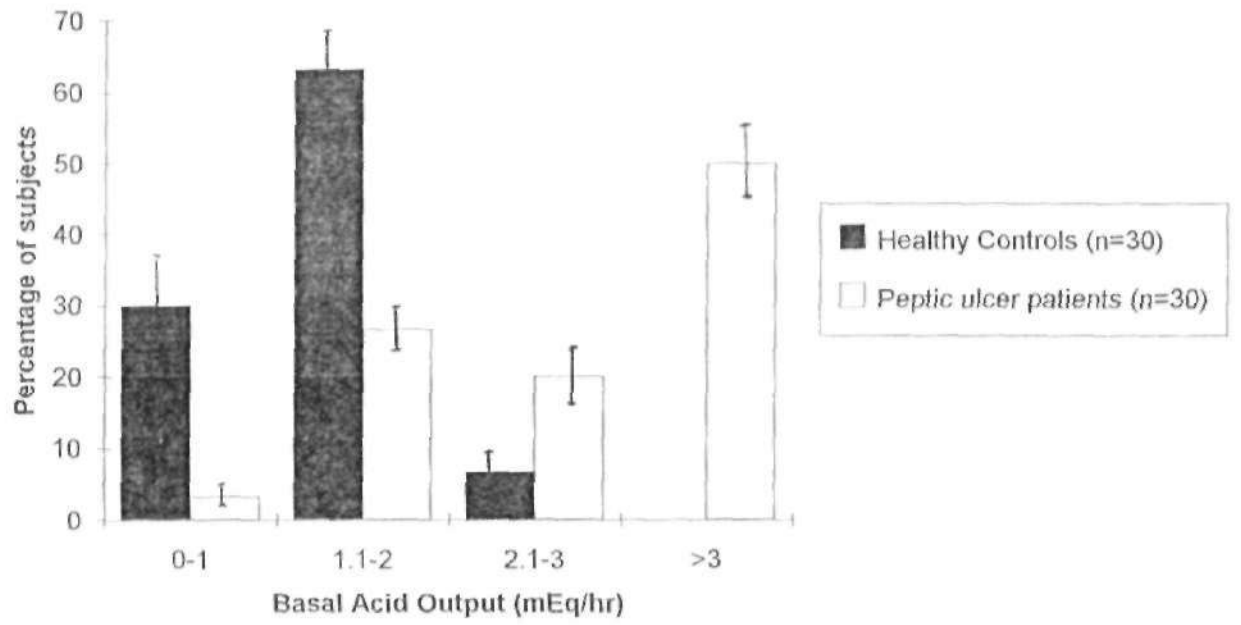


Fig 5: Frequency distribution of basal acid output levels in healthy controls and in patients with peptic ulcer disease.

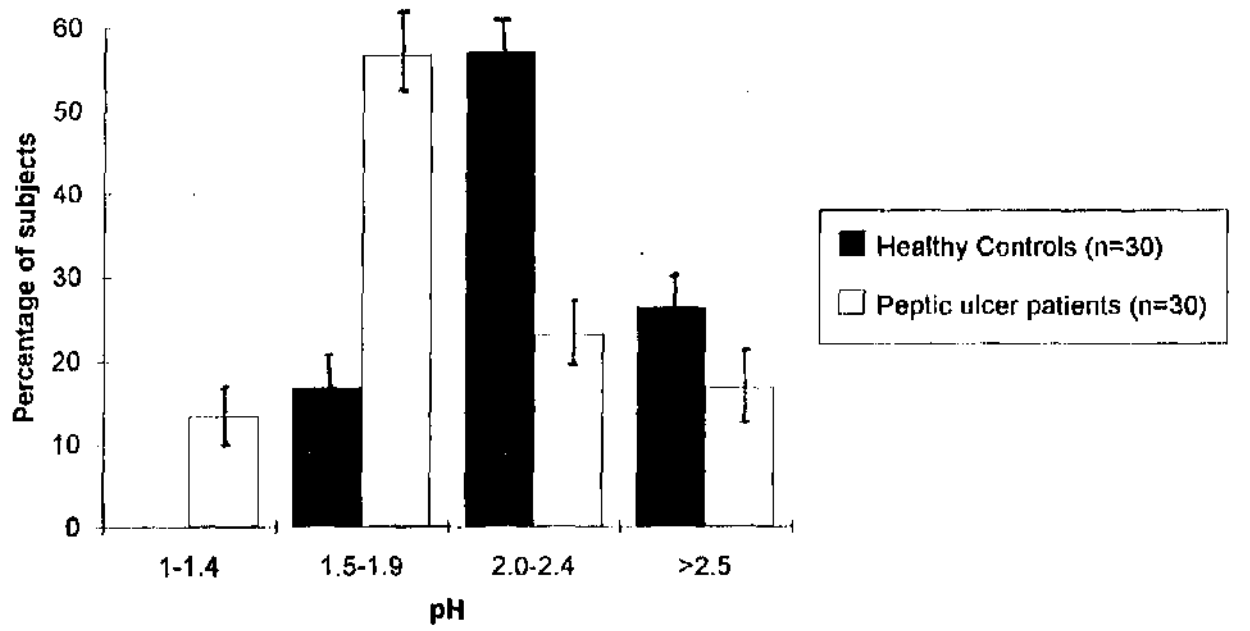


Fig 6: Frequency distribution of pH in healthy controls and patients with peptic ulcer disease.

4.4 Acidic mucoprotein-pepsin complex

The mean (±SEM) level of acidic mucoprotein-pepsin complex in healthy controls and peptic ulcer disease patients were 165.3 ± 4.71 µg/ml and 253.4 ± 6.31 µg/ml respectively. The difference between the mean of healthy controls and those with peptic ulcer disease was highly significant (P<0.001, table 4). The mean (±SEM) values obtained for both male controls and those with peptic ulcer disease were 168.7 ± 2.23 µg/ml and 254.23 ± 5.31 µg/ml respectively. The difference between the two means was highly significant (P<0.025, Table 5). In females, the mean (±SEM) values obtained for acidic mucoprotein-pepsin complex was 158.4 ± 1.85 µg/ml for healthy female controls while the mean values obtained for females suffering from peptic ulcer disease was 261.61 ± 5.61 µg/ml. The difference between the mean of female controls and those of peptic ulcer was highly significant (P<0.025; Table 6). There was no significant difference (P>0.05) in acidic mucoprotein-pepsin complex secreted by females from those of males with peptic ulcer disease and healthy controls respectively.

The frequency distribution of acidic mucoprotein-pepsin complex in healthy controls and patients with peptic ulcer disease is shown in Figure 7. The two populations differed markedly in the percentage of subjects that had acidic mucoprotein-pepsin complex concentration that fell between 151 and 200 µg/ml and those that had levels that fell between 250 µg/ml and 300 µg/ml. The percentage of subjects with levels

Table 4: Gastric mucin fractions and ages of healthy controls and patients with peptic ulcer disease.

	A Control [30]	B Peptic Ulcer [30]	P A Vs B
Acidic mucoprotein-pepsin complex (µg/ml Mean ±S.E.M.	165.3 ± 4.71	253.4 ± 6.31	<0.001**
Neutral glycoprotein-mucoproteose complex (µg/ml) Mean ±S.E.M.	211.3 ± 2.88	110.5 ± 2.66	<0.001**
Mucoprotective Index (%) Mean ±S.E.M.	56.1 ± 0.69	30.4 ± 0.72	<0.025**
Age (Year) Mean ±S.E.M.	23.3 ± 0.68	26.23 ± 1.35	>0.05

** Highly Significant

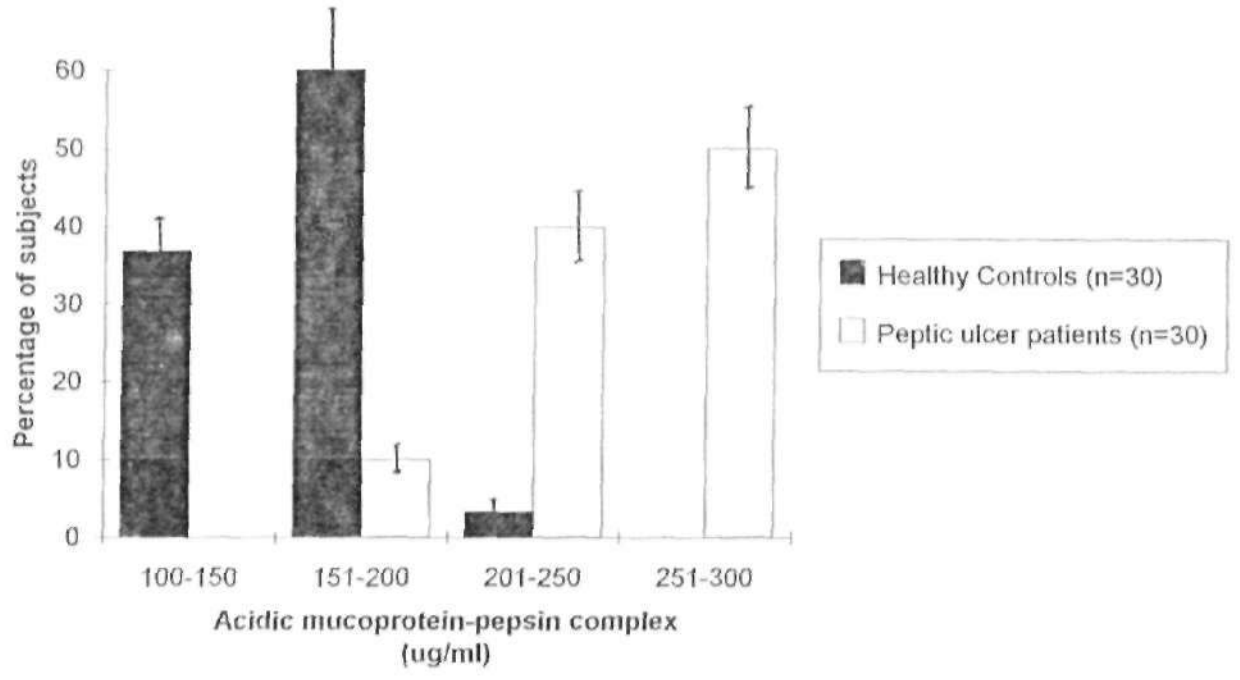


Figure 7: Frequency distribution of acidic mucoprotein-pepsin complex levels in control subjects and peptic ulcer patients.

Table 5: Gastric mucin fractions and ages of healthy controls and patients with peptic ulcer (MALES).

	A Control [20]	B Peptic Ulcer [17]	P A Vs B
Acidic mucoprotein-pepsin complex (µg/ml Mean ±S.E.M.	168.7 ± 2.23	254.2 ± 5.31	<0.025**
Neutral glycoprotein-mucoproteose complex (µg/ml) Mean ±S.E.M.	213.95 ± 1.98	108.17±1.32	<0.001**
Mucoprotective Index (%) Mean ±S.E.M.	58.8 ± 0.52	30.4 ± 0.61	<0.001**
Age (Year) Mean ±S.E.M.	23.5 ± 3.61	27.59 ± 1.52	>0.05

** Highly Significant.

Table 6: Gastric mucin fractions and ages of healthy controls and peptic ulcer patients (FEMALES).

	A Control [10]	B Peptic Ulcer [13]	P A Vs B
Acidic mucoprotein-pepsin complex (µg/ml Mean ±S.E.M.	158.4 ± 1.85	261.61±5.61	<0.025**
Neutral glycoprotein-mucoproteose complex (µg/ml) Mean ±S.E.M.	206 ± 1.78	113.5 ± 3.30	<0.001**
Mucoprotective Index (%) Mean ±S.E.M.	56.6 ± 0.41	30.3 ± 0.51	<0.001**
Age (Year) Mean ±S.E.M.	20 ± 0.33	24.46 ± 2.37	>0.05

** Highly Significant.

that fell between 151 µg/ml and 200 µg/ml were 60% (normal controls) and 10% of patients with peptic ulcer disease. On the other hand, 50% of patients with peptic ulcer had levels that fell between 251 µg/ml and 300 µg/ml while there was none from the normal healthy controls. Meanwhile, the frequency distribution of this fraction at levels that fell between 100 and 150 µg/ml were 36.7% (healthy controls) and none from the peptic ulcer disease group. 40% of patients with peptic ulcer disease had levels that fell between 201 µg/ml and 250 µg/ml while 3.3% of healthy controls had values that are within this range.

The correlation between acidic mucoprotein-pepsin complex and neutral glycoprotein-mucoproteose complex was not significant ($r = 0.175$, $P>0.05$). The relationship between this fraction and basal acid output was not significant ($r = 0.382$, $P>0.05$). The relationship was also not significant when compared to age ($r = 0.023$) and pH ($r = 0.461$) in patients with peptic ulcer disease.

4.5 Neutral glycoprotein-mucoproteose complex

The mean (±S.E.M.) values obtained for neutral glycoprotein-mucoproteose complex levels in healthy controls and in patients with peptic ulcer along with mean (±S.E.M.) values obtained for either sexes are given in Tables 4, 5 and 6 respectively. The mean values were 211.3 ± 2.86 µg/ml for healthy controls and 110.5 ± 2.66 µg/ml for patients with peptic ulcer disease. The difference between the control group and those with peptic ulcer was highly significant ($P<0.001$, Table 4). In males, the mean

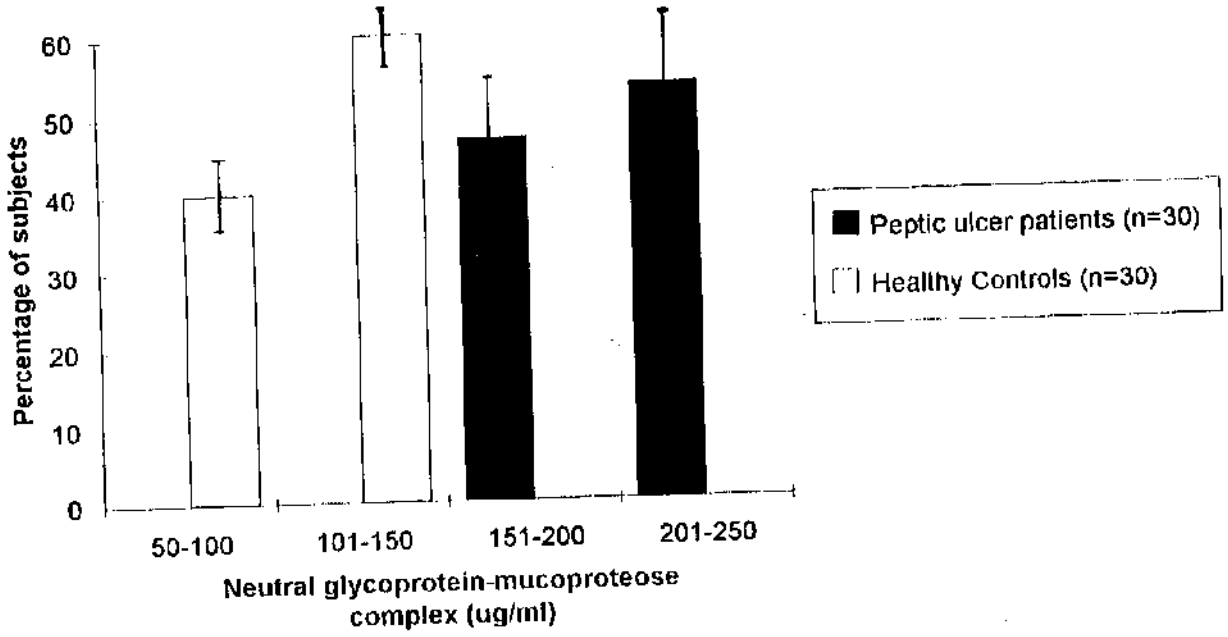


Figure 8: Frequency distribution of neutral glycoprotein-mucoproteose complex levels in control subjects and peptic ulcer patients.

(±S.E.M.) were 213.95 ± 1.98) µg/ml (controls) and 108.17 ± 1.32 µg/ml (peptic ulcer group). The difference between the two groups was statistically significant (P<0.001, table 5). The mean (±S.E.M.) values obtained for females were 206 ± 1.78 µg/ml and 113.5 ± 3.30 µg/ml in healthy controls and patients with peptic ulcer respectively. There was a significant difference between the mean of healthy controls and those with peptic ulcer disease (P<0.001). The difference in neutral glycoprotein-mucoproteose complex levels secreted by males was significantly different from that of females with peptic ulcer disease.

A comparison of the frequency distribution of neutral glycoprotein-mucoproteose complex levels in controls and peptic ulcer patients showed that the two populations differed markedly in the percentage of subjects with levels that fell between 90 µg/ml and 150 µg/ml (peptic ulcer patients) and levels that fell between 151 µg/ml and 250 µg/ml (healthy controls). This is shown in Figure 8. By the criteria used, 40% of ulcer patients had values that fell between 50 µg/ml and 100 µg/ml and none from the control subjects. Of the 30 control subjects, none had levels that fell below 150 µg/ml. Meanwhile, 60% of the peptic ulcer patients had values that fell between 101 µg/ml and 150 µg/ml. Neutral glycoprotein-mucoproteose complex levels that fell between 151 µg/ml and 200 µg/ml was recorded in 46.7% of the healthy controls while the remaining 53.3% of the same population had levels that fell within the range of 201 µg/ml and 250 µg/ml. None of the ulcer patients had values above 150 µg/ml.

The correlation between neutral glycoprotein-mucoproteose complex and age was not significant (r = 0.001, P>0.05) nor was

there a significant correlation with basal acid output (r = -0.411, P>0.05). Also, the correlation with pH was not significant (r = 0.051) in patients with peptic ulcer disease.

4.6 Mucoprotective Index

Mucoprotective index was significantly decreased in peptic ulcer patients when compared to healthy controls (P<0.025). The mean (±S.E.M.) values were 56.1 ± 0.69% and 30.4 ± 0.72% for healthy controls and patients with peptic ulcer disease respectively (Table 4). The mean (±S.E.M.) values obtained for either sexes are given in Tables 5 and 6 respectively. The mean (±S.E.M.) values obtained for male healthy controls was 58.8 ± 0.52% while the mean (±S.E.M.) values obtained for those with peptic ulcer was 30.4 ± 0.61%. The difference between the two means was significant (P<0.001). For females, the mean (±S.E.M.) values obtained were 56.6 ± 0.41% (controls) and 30.3 ± 0.51 (peptic ulcer group). Mucoprotective index was significantly decreased in females with peptic ulcer when compared with female healthy controls (P<0.001).

Table 7: Food and Social Habits of patients with peptic ulcer and control subjects.

Food & Social Habits	Percentage	
	Peptic Ulcer	Normal Subjects
Rice	93.3 (28)	96.7 (29)
Yam	56.7 (17)	96.0 (27)
Cassava (Garri)	50.0 (15)	73.3 (22)
Corn Flour (Tuwo)	46.7 (14)	56.7 (17)
Beans	83.3 (25)	60.0 (18)
Pounded Yam	16.7 (5)	50.0 (15)
Milk	66.7 (20)	33.3 (10)
Coffee	33.3 (10)	16.7 (5)
Beverages	60.0 (18)	33.3 (10)
Alcohol	36.7 (11)	16.7 (5)
Smoking	33.3 (10)	23.3 (7)

4.7 Food and Social Habits

The estimate of peptic ulcer disease attributable to exposure to various diet, milk, coffee, alcohol consumption and smoking are shown in Table 7. As any exposure to these factors, a patient may have had was recorded in the life time period, data for this period were considered the most appropriate for attributable risk estimates. These estimates are not additive and the estimates of peptic ulcer risk attributable to exposure to any of factors, either alone or in combination, cut across the sexes.

The findings indicates that in the groups under study, 93.3% of peptic ulcer patients had rice as part of their staple diet while 56.7%, 50%, 46.6% and 16.7% had yam, cassava (Garri), corn flour (Tuwo) and pounded yam as part of their staple diet respectively.

These food items contain high proportion of carbonhydrate and beans, the only food item in the group that contains high amount of protein content was found to be among the staple diet of 83.3% of the ulcer population. The results also indicated that 33.3% of the peptic ulcer patients are smokers while 36.7% of the patients were taking alcohol. The percentage of patients taking coffee, milk and other beverages were 33.3%, 66.6% and 60% respectively. The distribution of these food items in the normal control group is given in Table 7.

CHAPTER FIVE

DISCUSSION

Gastric juice analysis is most commonly done on single samples of fasting juice after aspiration. This practice has the advantage that multiple investigations, such as gastric inspection, gastric acid output, pH and dissolved gastric mucin fractions may be accomplished with minimal disturbance to the patient. The passage of any gastric tube may stimulate unnatural bile reflux and such artifactual bile reflux may lead to inaccuracies in gastric juice analysis. In this study, the smallest effective nasogastric tube was used; this was to minimize gastric juice artifacts and it was done by delaying aspiration for about thirty minutes after placing the tube to allow a steady state to develop.

Basal Acid Output

It is probable that most peptic ulcers are caused by the hypersecretion of gastric juice of nervous or vagus origin. The older gastroenterologist recognised that peptic ulcer patients secretes more gastric juice in response to Edwald meal or the stimulus of alcohol or histamine and pentagastrin than do normal people. This increase may not be very great and it was concluded that its determination was of little diagnostic value. But it has been pointed out that peptic ulcer patients frequently secrete twice or two to three times as much as acid in the basal fasting secretion as did normal people though they secrete only 25% to 50% more acid in response to histamine or food (Grossman, 1960; Dragstedt, 1956). Accordingly, it seems more appropriate

to learn how much of the corrosive hydrochloric acid, the stomach of the ulcer patients were producing when shielded from the usual physiological stimuli and gastric secretion than to learn the response of the stomach to various stimuli, which was the usual method. Afterall, it is at night when the stomach is empty and the buffering effect of food is absent that stomach or duodenum receives its greatest injury.

In this study, there was a significant difference in the values obtained for basal acid output between control subjects and the peptic ulcer disease group. The results obtained once again confirm the findings of previous reports that acid output in normal subjects and peptic ulcer disease patients is distributed over a wide range. The mean basal acid output of 3.04mmol/hr and 1.32mmol/hr obtained in patients with peptic ulcer and healthy controls respectively, is low when compared with results from other centres such as Britain (Levin et al, 1953; Kay, 1953; Bruce et al, 1959 and Sircus, 1959), America (Grossman, 1960; Marks et al, 1961; Littman, 1962) and India (Goyal et al, 1966), which vary from 1.4mmol/hr and 2.99mmol/hr in healthy controls, 2.9mmol/hr and 6.8mmol/hr in patients with peptic ulcer disease but it is fairly comparable with results from Lagos (Tai-da-Rocha-Afudu and Adesola, 1972), Ghana (Wosonu, 1972) and Ibadan (Solanke and lewis, 1976). In all of the reports by others, the mean values for patients with peptic ulcer disease were more than twice as high as for control subjects. The data of this report give a similar ratio. The variations in results obtained from different centres can be ascribed to difference in age, length of history of the disease, race and

food habits. In addition, some reports do not distinguish between results from men and women and the validity of the diagnosis of peptic ulceration is open to doubt in patients not subjected to operation confirmation.

Mean basal acid output for male controls and those with peptic ulcer disease were 1.36mmol/hr and 3.43mmol/hr respectively while that of females were 1.21mmol/hr (controls) and 2.54mmol/hr (peptic ulcer patients). The difference between the means of each group was statistically significant but the basal acid output of females when compared with that of males in both the control subjects and those with peptic ulcer, the difference was not significant. Meanwhile, the data is in conformity with other reports (Grossman, 1960; Solanke and Lewis, 1976), which have shown higher values for men. The difference among some of the series ranging from 2.9mmol/hr for male peptic ulcer patients reported by Grossman (1960) to 6.0mmol/hr by Sircus (1959) and 6.8mmol/hr by kay (1959) are interesting. This discrepancy might be explained in part by age difference. Another possible factor is the collection technique; Grossman used mechanical suction which gives incomplete aspiration more often than does the continuous manual syringe (Johnston and McCraw, 1959) but so did Sircus. After correcting for body weight, sex difference have shown to be insignificant in normal subjects (Andersson et al, 1970 and Baron, 1969) and in patients with duodenal ulcer (Borgstrom et al, 1973). However, in this series, the men studied had higher values than women even though the difference was not statistically significant. High secretion of acid in men with or without peptic ulcer probably represent

a hormonal influence independent of the ulcer.

The acid output has been found to decrease with age. Polland (1933) first confirmed these decrease for acidity, volume and acid output, followed by Levin et al (1951) who showed in addition, that basal acid output also decrease with age. Comparable decrease with age have been found in normal subjects after submaximal dose of histalog (Kirsner and Ford, 1955; Grossman et al, 1963). Acid secretion after maximal dose of stimuli have been reported as decreasing with age in normal men and women (Baron, 1969) and men (but not women) with peptic ulcer (Andersson et al, 1070; Borgstrom et al, 1973). The results of this study differ from previous studies in that basal acid output did not decrease with increase in age. This might be due to the fact that almost all the subjects in both controls and those with peptic ulcer fell within the same age range, since Levin et al, (1951) published normal ranges of basal acid output for each sex by a decade.

Basal acid secretion respective of age and sex has been correlated with maximal secretion (Baron, 1978). Therefore, basal hypersecretion in gastric disease can broadly be explained on the basis of increased parietal cells often found in the stomach of these patients. However, there may be an increased 'drive' (vagal/or hormonal) on the parietal cell because in some studies, (Baron, 1972) the ratio of basal acid to stimulated acid output in patients with peptic ulcer was greater than normal. The view that the increased parietal cell mass in peptic ulcer patients represent hypertrophy and hyperplasia of the parietal cell may be as a result of long continued stimulation rather than

genetic factors. The augumented histamine test of Kay (1959) gives information concerning the extent of this hyperplasia and perhaps, also the sensitivity of the cells to such stimulation. The measurement of the fasting secretion in the absence of the usual physiological stimuli, provide evidence of the severity of the psychogenic stimulation of gastric secretion, which is the cause of the disease itself. For this reason, the augumented histamine test should not replace measurement of the continuous basal secretion. However, the results obtained for basal acid secretion in this study provide strong support for the Dragstedt hypothesis of aetiology of peptic ulceration (Dragstedt, 1956) because in terms of the individual subjects, only about 50% secreted more acid than the normal

pH (Negative log of hydrogen ion concentration)

The pH of the gastric juice collected from patients with peptic ulcer disease was significantly different from that of control subjects. There was a significant correlation between acid output and pH. This is not surprising because hydrochloric acid have the same titratable acidity but have different dissociation constants and therefore, different hydrogen ion activities. The rate of gastric emptying is an important determinant of duodenal pH. Acid in the stomach normally acts as a brake on emptying but this protective mechanism is absent in patients with peptic ulcer, and acid in the stomach may actually increase the emptying rate. meanwhile, one cannot say whether this abnormal response preceeds or succeeds development of the ulcer. However, there is a general agreement that basal pH are lower than normal in patients with peptic ulcer (Carter,

1980).

Food protein is a potent stimulus to gastric secretion and is the only important buffer in food (Carter, 1980). As gastric emptying is abnormally rapid in many patients with peptic ulcer, the buffer may pass rapidly into the duodenum having triggered the acid secretion response. The resulting acid secretion would ultimately enter the duodenum without protective buffering and might explain ulceration in patients with a normal secretory capacity. Therefore, an abnormal gastric pH appears to be an important factor in the genesis of ulceration.

Gastric Mucin Fraction

Methods are needed to fractionate the macromelecules in gastric secretion, so that they may be isolated in pure forms and characterize in terms of biological, chemical and physical properties. Glass (1967) proposed a scheme for separating two fractions of the glycoprotein fractions present in human gastric juice which he named "acidic mucoprotein-pepsin complex" and "neutral glycoprotein-mucoprotoese complex". Although, Schrager (1961) questioned the measurement of tyrosine content of the fraction by folin-ciocalteau colour which he believed measure pepsin and other compounds along with the mucoprotein, Glass (1967) found a significant correlation between pepsin content and mucoprotein tyrosine. This makes it clear that one cannot accept a simplified concept of Schrager that tyrosine content of mucoprotein relates mainly to the pepsin of the fractions. Moreover, the Lowry modification which incorporate an alkaline copper solution in the Folin reagent, gives a more equal colour

yield for diverse protein and this more reliably determine protein in the precipitates.

The fractions of gastric mucin were analysed in healthy controls and patients with peptic ulcer disease because dissolved mucin has been shown to exerts its protective properties on duodenal and gastric mucosa. Gastroduodenal mucin is considered to have a protective role against (a) acid, by acting as a stable mixing barrier with the epithelial HCO^-_3 secretion (Hollander, 1954; Heatley, 1959; Allen and Garner, 1982; Flemstrom and Garner, 1982), (b) luminal pepsin by forming a diffusion barrier (Allen, 1981a; Allen, 1981b) and (c) mechanical damage by acting together with adherent mucus, as a lubricant (Florey, 1955).

By analyzing the mucin fractions in the human gastric juice as was done in this investigation, the compositional changes in the secretion, has reflected the functional status of the gastric mucosa in health and gastroduodenal disease. The results obtained for gastric mucin fractions in this study are in conformity with the published data in caucasians (Glass, 1967; Guslandi et al, 1978; Guslandi et al, 1980) and black Africans (Ali et al, 1989). The results indicated that in comparison to controls, patients with peptic ulcer disease had significantly increased levels of acidic mucoprotein-pepsin complex, that patients with peptic ulcer disease had significantly decreased levels of neutral glycoprotein-mucoproteose complex and that patients with peptic ulcer had significantly decreased mucoprotective index than controls. The degree of overlap between control subjects and patients with peptic ulcer disease shown in Figures 7 and 8 suggested that the discriminatory

ability of the gastric mucin fraction levels is as good or perhaps slightly better than what has been reported for basal or stimulated gastric acid output. It will be noted that the correlation between acidic mucoprotein-pepsin complex and basal acid output is fair and roughly parallel, as shown by the correlation coefficient of -0.382. Its concentration and output were significantly increased in patients with peptic ulcer disease when compared to controls. Although, acidic mucoprotein-pepsin complex accounts for one-quarter of the total mucin produced, it is believed to exhibit very slightly buffering effect (Glass, 1967). There is, however, evidence that neutral glycoprotein-mucoproteose fraction of the mucin may act as an inhibitor of gastric acid secretion. Brunschwig (1939) and his group first demonstrated the presence of an agent in human gastric juice which would inhibit canine gastric acid secretion. The work of Code and his co-workers (1948) and of Smith and his group (1958, 1960) has confirmed and extended this concept. A report by Glass and Boyd (1952) showed an inverse relationship between gastric acidity and the neutral glycoprotein-mucoproteose complex content of the gastric mucus in man. Glass (1977) also showed an inverse relationship between gastric acidity and gastric mucus. Our present report also shows an inverse correlation between titrable acidity and neutral glycoprotein-mucoproteose complex fraction of the gastric mucin (-0.411). It is therefore possible that the inhibition of gastric acid secretion in the literature, is contained in the neutral glycoprotein-mucoproteose fraction. However, Menguy and Masters (1965) have shown in the rat that the intravenous injection of

extracts of gastric juice from peptic ulcer patients will inhibit gastric acid secretion to a greater extent than extracts of gastric juice from normal individuals. The low levels of neutral glycoprotein-mucoproteose complex in the gastric aspirate of patients with peptic ulcer may be as a result of the destruction of the glandular apparatus of the fundus and body of the stomach in patients with peptic ulcer, since in this disease, there is absence of diluting parietal secretion. This is a strong evidence for the derivation of neutral glycoprotein-mucoproteose fraction from the surface epithelium and/or pyrolus glands of the stomach and speaks against its origin from the glandular structure of the fundus and body of the stomach.

The mucin fractions levels do not appear to be related to age, sex and pH of patients with peptic ulcer since the correlation between these parameters and the mucin fractions was not significant. Meanwhile, the correlation between acidic mucoprotein-pepsin complex with pH and acid output was fair as shown by correlation coefficient of 0.461 and 0.382 respectively. Schrager (1970) showed that, lowering the pH of gastric aspirates increases the concentration of the glycoprotein in the mucin fractions. Normally, this result in th lowering of the effectiveness of the forces which maintain the integrity of mucus with increasing ionic strength. According to Schrager, the acid-pepsin component of gastric secretion produces environmental changes, that is, increased acidity and ion-concentration and marked increase in diluting fluid which are destructive to the mucus structure. The significant increase in acidic mucoprotein-pepsin complex suppresses the electrostatic forces, reduces the

hydrogen bonding, cleaves off the sialic acid and frees the individual macromulecules. This may result in an increase in fluid, that is, gastric juice which was significantly higher in patients with peptic ulcer than healthy controls. For any system capable of gel formation there will be a limit to dilution when gel formation becomes impossible. If this condition occurs, it brings about demixture, flocculation is observed instead of gel formation. So, as long as the production of mucus do not lag behind the destruction, the protective and destructive forces are in dynamic equilibrium.

Guslandi et al (1978, 1980) have shown that mucoprotective index is a fairly reliable index of assessing the quality and quantity of gastric mucus. Meanwhile, the "normal" range of the two mucin fractions is not specified in this study, so it will be difficult to determine the degree of overlap which exist between the healthy control subjects and peptic ulcer patients. However, the diagnostic significance of a particular mucin fraction level obviously improves at lower or higher levels since the concentration of each fraction in a sample of gastric juice is a reflection of the protective property of gastric mucin. For example, a gastric juice that has an acidic mucoprotein complex of 440μ/ml and a neutral glycoprotein-mucoproteose complex of 30μg/ml will have a mucoprotective index of 7.9%. Similarly, a gastric juice sample that has an acidic mucoprotein-pepsin complex of 30μg/ml and a neutral glycoprotein-mucoproteose complex 160μg/ml will have a mucoprotective index of 84.2%. Therefore, low levels of acidic mucoprotein-pepsin complex and high levels of neutral glycoprotein-mucoproteose complex may be

considered as a protective mechanism against potential ulcerogenic agents such as smoking (Sonnerberg et al, 1978) and non-steroidal anti-inflammatory drugs (Overholt, 1969; Guth et al, 1984).

From the afore-going discussion, compounds that either inhibit or stimulate mucus production may be expected to have ulcerogenic or anti-ulcer properties respectively. The relapse of peptic ulcer in cimetidine-healed patients has been ascribed to the damaging effect of this drug on the gastric mucosa, whereas carbenoxolone has been shown to be effective in preventing relapse by restoring the protective function of the cimetidine altered gastric mucus (Guslandi et al, 1978). It is well known that salicyclates damages the gastric mucosa both in man and experimental animals. In low concentration, aspirin has been found to inhibit mucus biosynthesis (Menguy and Masters, 1965; Kent and Allen, 1967). Other non steroidal anti-inflammatory agents known to damage the gastric mucosa including indomethacin also inhibits mucus secretion (Allen and garner, 1980). The ability of prostaglandins to inhibit ulceration in laboratory models was originally noted by Roberts (1978). Inhibition of ulcer formation in the presence of exogenous acid or at prostaglandins dose below anti secretory threshold suggested that their anti-ulcer activity is mediated by a mechanism unrelated to inhibition of acid secretion. Prostaglandins especially the E-type have been reported to stimulate production of soluble mucus in rat stomach and to increase levels of sialic acid, a sugar characteristics of glycoproteins, in human gastric juice (Bolton et al, 1978;

Domsher et al, 1978).

Food and Social Habits

Diet is often considered to be important in the pathogenesis of peptic ulcer disease. The possibility that difference in diet might be of aetilogical importance contributing to the difference in incidence of peptic ulcer is supported by the report of Malhotra (1978). Cleave (1962; 1974) suggested that the introduction of refined carbonhydrates (Polish rice, wheat or corn flour) or starchy food results in a loss of buffer (principally protein) and that this may be an important factor in the aetiology of peptic ulceration. This concept fits in with the results obtained in this study where 93.3%; 56.0%; 50%; 46% and 16% of the patients had rice, yam, cassava (gari), corn flour (tuwo) and pounded yam respectively as part of their staple diet. These food items have high carbonhydrate content and they require little or no mastication before they are swallowed. As Malhotra (1964) and Chouldrie (1974) suggested the amount of mastication required in consuming the staple carbonhydrate may be important because the alkaline and mucus content of the saliva protects the gastric mucosa. This suggestion is unconvincing since the buffering capacity of saliva is low. Moreover, such diets has no specific neutralizing characteristics, nor is there any reason to believe that they are less of stimulus to gastric acid output.

Many studies have shown an association between smoking and prevalent gastric ulcer (Doll et al, 1958; Gillies and Skyring, 1968), but not with duodenal ulcer (Peterson et al, 1977). The data obtained in this study showed that only 33.3% of the peptic

ulcer population were smokers as compared to healthy controls (23.3%). However, these findings do not necessary imply that smoking is an important causal factor in peptic ulceration. Moreover, an increased frequency of smokers among those with ulcers compared with control individuals could be due in part to a common social class pattern for peptic ulceration and smoking if control subjects were similarly matched for social class. Smoking, however, has no effect on gastric acid secretion (Whitecross et al (1974). Taken overall, the association between smoking and ulcer is weak, and the habit can at best play a minor causal role in ulcer development.

It is commonly supposed that alcohol consumption both causes peptic ulceration and exercerbates its symptoms. The evidence in support of either concept is weak with regards to this study, since 36.7% and 16.7% of patients with peptic ulcer and healthy controls were taking alcohol respectively. From the literature there is no proof that excess alcohol ingestion results in peptic ulceration. However, alcohol does not appear to increase acid secretion in humans (Cook, 1972), as a result the effect of alcohol may be indirect and occur after its absorption.

Soft drinks, coffee, tea and milk are potent stimulant of acid secretion (McArthur et al, 1982). 60% of the ulcer population were taking soft drinks, tea and other food drinks while 33.3% were taking coffee over a period of five years before onset of ulcer symptoms. Traditionally, milk has been advocated in the management of patients with peptic ulcer disease (Bockus, 1974). It has been referred to as ideal agent on the basis of its caloric value and neutralizing capacity. However, it has

been observed that neither the hourly ingestion of milk nor intragastric milk drip continuously neutralize gastric acidity (Doll et al, 1956). The results of the Present study indicates that 66.7% of ulcer patients had milk as part of their diet before and after onset of symptoms. The principal gastric acid stimulant in food is protein. Amino acids derived from hydrolysis of the milk protein, stimulate acid secretion in man (Walsh et al, 1975). In addition, the calcium content of milk is comparable to 500mg of calcium carbonate. This dose increased acid secretion in both patients with duodenal ulcer and normal subjects (Levant et al, 1973). Because milk has only a transient neutralizing effect followed by a sustained rise in acid secretion, there is reason to question its frequent ingestion in the therapy of peptic ulcer.

Given the known failings of retrospective questionnaire analysis, these results must be given less weight because direct causal relationship could not necessarily be assumed since each of the item considered above could be acting as a marker for other substances not included in the survey.

CONCLUSION AND SUGGESTION

Combining the basal acid outputs and gastric mucin fractions data presented in this study, the following conclusion and suggestions seems appropriate.

This study provides a strong support for the hypothesis that peptic ulceration is an heterogenous disease. It also supports the hypothesis that patients with peptic ulcer have increased basal acid output and low mucosal resistance as indicated by a significance decrease in the mucoprotective index as compared to healthy control subjects. Therefore, it can be postulated that a high basal acid output and a low mucoprotective index are indicators of peptic ulceration.

The two major factors to be considered in pathogenesis are that acid secretion are excessive and that the gastroduodenal defence are less effective than normal in resisting autodigestion. Attention is usually focussed on the aggressive factors (hydrochloric acid) in most of the reports published earlier, but this study, has shown that inadequate mucosal defence may play a significant role and could conceivably contribute to ulceration in patients with peptic ulcer disease. Defective gastric mucosal may still be responsible for ulceration in patients with normal or reduced level of gastric acid secretion.

The separation of gastric macromolecule components by fractionation into acidic-mucoprotein-pepsin complex and neutral glycoprotein-mucoproteose complex group is of value and may serve as a spring-board for further advances in the purification of these materials.

Gastric juice pH was abnormally low in many patients with peptic ulcer. This may reflect an increased acid secretion, and an increased gastric emptying rate. It is therefore postulated that tenacious mucus may protect the pH receptors in the antral mucosa and thereby prevent the normal inhibition of acid secretion by highly acidic gastric juice.

The question with regards to dietary intake, is whether the data obtained in this study are adequate to postulate a causal relationship with peptic ulcer disease. However, it is suggested that while in any community, there are people with increased susceptibility to peptic ulceration, the absence or presence of aggressive factors in the diet may determine whether or not these may lead to actual ulceration.

The results obtained in this study indicates that gastric analysis may serve as a useful tool for evaluating the effectiveness of various anti-ulcerogenic drugs such as H_2-receptor blockers (Cimetidine, Ranitidine) and antacids as potent therapeutic agents. It may be possible to study whether there is rebound hyperacidity when treatment is discontinued or whether acid secretion remains below normal after prolonged treatment.

One of the major problems in defining etiologic factors relate to the difficulty of distinguishing between cause and effect, and this problem will continue to arise.

For the majority of ulcer patients, hypersecretion of gastric acid and lowered mucosal defence may be responsible for peptic ulceration. This bland statement summarizes the extent of our knowledge and undermines our ignorance of the basic factors which allow increased acid secretion to ulcerate the

mucosa of the upper gastrointestinal tract. Therefore, the future development in the management of peptic ulcer will depend in elucidation by further research, of the various causative factors responsible in individual cases and to recognize as to who would be a potential candidate for peptic ulcer disease.

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Appendix 1 Basal acid output, pH, age, gastric juice and sex of control subjects.

No.	Sex	Age (Years)	Total Volume of gastric juice (ml/hr)	Hcl Output (mmol/hr)	pH
1	M	38	10	1.2	2.4
2	M	23	10	1.1	2.6
3	M	20	14	1.0	1.8
4	F	19	37	1.5	2.5
5	M	24	12	1.0	2.6
6	M	22	20	2.6	2.3
7	F	20	25	1.0	2.5
8	F	19	20	1.0	2.0
9	M	26	25	1.0	2.1
10	M	26	25	1.0	2.0
11	M	25	15	1.1	2.5
12	M	23	23	1.2	2.4
13	M	26	30	1.6	2.3
14	M	25	28	1.6	2.5
15	M	21	50	2.6	1.8
16	M	19	28	1.1	2.2
17	M	22	25	1.9	1.6
18	M	24	25	1.0	2.8
19	M	21	30	1.1	2.2
20	F	22	38	1.1	2.5
21	F	20	20	1.9	1.6
22	F	19	25	1.1	2.2
23	M	22	25	1.0	2.4
24	M	21	30	1.8	1.7
25	F	21	25	1.5	2.2
26	F	19	27	0.9	2.1
27	M	20	30	1.3	2.2
28	M	22	30	1.1	2.2
29	F	20	25	1.3	2.1
30	F	21	38	1.2	2.3
Total		670	765	39.8	66.6
Mean(x)		22.2	25.5	1.32	2.22
S.D		3.71	8.15	2.13	0.30
S.E.M.		±0.68	±1.49	±0.39	±0.05

Appendix 2 Gastric juice, Basal acid output, pH, age and sex of peptic ulcer patients.

No.	Sex	Age (Years)	Total Volume of gastric juice (ml/hr)	Hcl Output (mmol/hr)	pH
1	M	37	28	1.1	1.6
2	M	21	45	3.5	1.9
3	M	24	45	4.0	1.8
4	M	22	50	2.2	1.5
5	M	33	50	4.6	1.8
6	F	21	48	2.6	1.7
7	F	40	30	1.8	1.9
8	M	35	50	1.2	1.9
9	F	20	50	3.4	2.4
10	F	18	44	2.0	2.2
11	M	24	42	3.2	1.3
12	M	21	40	3.1	2.2
13	F	19	39	2.1	2.0
14	M	18	26	6.5	1.6
15	M	27	25	1.2	1.4
16	F	18	45	2.4	2.2
17	F	25	45	3.2	1.4
18	M	25	46	9.8	1.8
19	M	37	25	1.5	1.4
20	M	36	25	1.9	1.6
21	F	26	50	1.2	1.5
22	F	26	51	1.0	1.8
23	M	24	35	2.4	2.0
24	F	22	40	2.5	1.5
25	F	18	50	3.4	2.5
26	M	27	46	5.4	1.5
27	M	25	30	3.2	2.3
28	M	35	68	3.9	1.7
29	F	45	47	3.4	2.8
30	F	20	50	4.0	1.8
Total		787	1261	91.3	55
Mean(x)		26.23	42.03	3.04	1.8
S.D		7.38	10.18	1.8	0.37
S.E.M.		±1.35	±2.22	±0.33	±0.07

Appendix 3 Gastric mucin fractions in normal control subjects.

No.	Sex	Age (Year)	Acid mucoprotein-pepsin complex (µg/ml)	Neutral glycoprotein-mucoproteose complex (µg/ml)	Mucoprotective index (%)
1	M	38	120	206	63.2
2	M	23	156	190	54.9
3	M	20	204	240	54.1
4	F	19	158	185	58.9
5	M	24	160	204	56.0
6	M	22	200	240	54.5
7	F	20	165	200	54.8
8	F	19	150	195	56.5
9	M	26	195	230	54.1
10	M	26	180	200	52.6
11	M	25	150	200	57.1
12	M	23	177	180	50.4
13	M	26	150	200	57.1
14	M	25	150	210	58.3
15	M	21	200	230	53.5
16	M	19	190	210	52.2
17	M	22	200	220	47.6
18	M	24	142	250	63.8
19	M	21	156	231	59.7
20	F	22	204	240	54.1
21	F	20	150	220	59.5
22	F	19	120	197	62.1
23	M	22	175	188	51.8
24	M	21	200	250	55.6
25	F	21	150	200	57.1
26	F	19	187	214	53.4
27	M	20	120	200	62.5
28	M	22	150	200	57.1
29	F	20	140	180	56.3
30	F	21	160	230	59.0
Total		670	4959	6340	1682.8
Mean (x)		23.3	165.3	211.3	56.1
S.D		3.71	25.9	15.9	3.79
S.E.M.		±0.68	±4.71	±2.88	±0.69

Appendix 4 Gastric mucin fractions of peptic ulcer patients.

No.	Sex	Age (Years)	Acidic mucoprotein-pepsin complex (µg/ml)	Neutral glycoprotein - mucoproteose complex (µg/ml)	Mucoprotective Index (%)
1	M	37	250	100	28.9
2	M	21	296	92	23.7
3	M	24	220	90	29.0
4	M	22	200	120	37.5
5	M	33	250	90	26.4
6	F	21	200	93	31.7
7	F	40	294	95	24.4
8	M	35	220	100	31.3
9	F	20	280	100	26.3
10	F	18	285	110	27.8
11	M	24	277	120	30.22
12	M	21	233	120	39.9
13	F	19	244	110	31.1
14	M	18	300	100	25.0
15	M	27	260	105	28.8
16	F	18	244	108	30.7
17	F	25	268	130	32.7
18	M	25	180	98	35.2
19	M	37	300	150	33.3
20	M	36	296	110	27.1
21	F	26	232	120	34.1
22	F	26	244	130	34.7
23	M	24	240	100	29.4
24	F	22	300	130	30.2
25	F	18	298	100	25.1
26	M	27	280	114	28.9
27	M	25	220	120	35.2
28	M	33	300	110	26.8
29	F	45	272	130	32.3
30	F	20	240	120	33.3
Total		787	7723	3315	911.15
Mean (x)		26.23	253.4	110.5	30.4
S.D.		7.83	34.7	14.64	3.98
S.E.M.		±1.35	±6.31	±2.66	±0.72

Appendix 5 Statistical Analysis.

1. Mean $(\bar{x}) = \frac{\Sigma x}{n}$

2. Standard Deviation (S.D.) =

$$\sqrt{\frac{\sum d^2}{n-1}}$$

3. Standard Error of Mean (S.E.M.) = $\frac{S.D.}{\sqrt{n}}$

4. Student t - test (t) =

$$\frac{\bar{x}-\bar{x}_2}{\sqrt{\frac{S.D^2}{n_1}+\frac{S.D.^2}{n_2}}}$$

5. Z - test (Z) =

$$\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\frac{S.D.^2}{n_1}+\frac{S.D.^2}{n_2}}}$$

6. Correlation Co-efficient (r) =

$$\frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 \sum (y-\bar{y})^2}}$$

7. Regression Equation; $y = mx + c$
where, Σx = summation of observations

n = number of observations
Σd^2 = squares of each deviation from the mean
y = dependent variables
x = independent variables
m = slope or gradient
c = intercept.

Appendix 6 Preparation of Reagents

GLASS METHOD

A. Prepare 2 litres of 0.1M NaOH as follows:
   1. Weigh 8g of NaOH and dissolve in distilled water to a final volume of 2000ml.

B. Prepare 2 litres of 0.1M Hcl as follows:
   1. Using a pipette, take 17.2ml of Conc. Hcl and dilute to a final volume of 2,000ml with distilled water. Add acid to 500ml of distilled water, then make up to 2 litres.

C. Prepare 1 litre of 10% trichloroacetic acid by weighing 100g of the solid trichloracetic acid and dissolving in distilled water to a final volume of 1000ml.

FOLIN-PHENOL METHOD

A. Prepare 200ml of 2% Na_2CO_3 in 0.1M NaOH as follows:
   i. Weigh 2g of NaOH and dissolve in distilled water to a final volume 100ml.

   ii. Weigh 4g of $NaCo_3$ and dissolve in solution above to a final volume of 200ml.

B. Prepare 100ml of 0.5% $CuSo_4$ in 1% Na^+K^+ tartrate as follows
   i. Weigh 1g of Na^+K^+ tartrate and dissolve in distilled

water to a final volume of 100ml.

ii. Weigh 0.5g of $CuSo_4$ and dissolve in solution (i) above.

THE FOLIN-LOWRY METHOD OF PROTEIN ASSAY

PRINCIPLE:

Protein reacts with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the buret test and the reduction of phosphomolybdate by tyrosine and tryptophan in the protein.

MATERIALS:

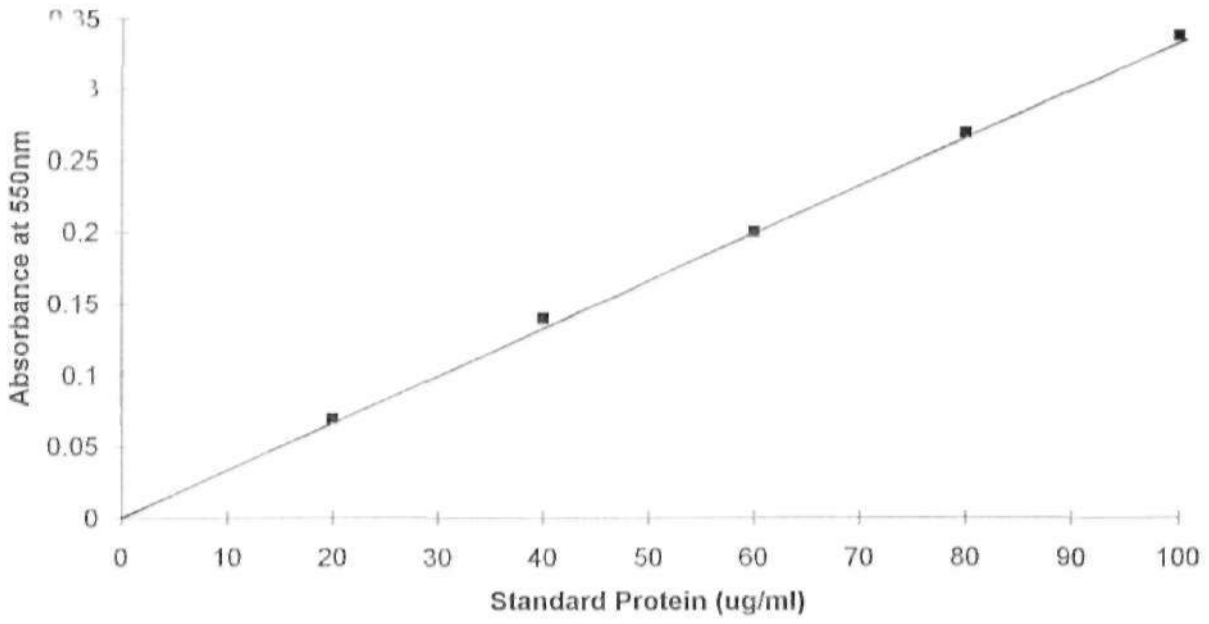
1. Alkaline sodium carbonate solution (2% $NaCo_3$ in 0.1M NaOH).

2. Copper Sulfate - Sodium Potassium tartrate solution (0.5% $CuSo_4$).

3. Alkaline solution, prepare on day of use by mixing 50ml of (1) and 1ml of (2).

4. Folin-ciocalteau reagent (Dilute the commercial reagent with an equal volume of distilled water on the day of use. This is a solution of sodium tunsgate and sodium molybdate in phosphoric acid and hydrochloric acid).

5. Standard protein (albumin solution, 20µg/ml).



Appendix 7. Standard Curve for Protein.