A Study of the Effects of Sucralfate in the *Bile Duct Ligated Pig* Peptic Ulcer Model with Particular Reference to the Effects on the Physico-chemical Properties of Gastric Mucus and Including Comparisons With Famotidine and Misoprostol.

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Dedication

To Melinda, Andrew, and Caroline:

The compilation of this manuscript has resulted in a sacrifice of valuable time I might have spent with you, time which is lost for ever. I hope in the long run it will be worthwhile, but thank you so very much for your patience, understanding and support.

To Ridley and Marion:

I owe so much to you for giving me the opportunities to be in a position to submit this manuscript. Thank you.

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CONTENTS

FOREWORD

Sucralfate is a drug that effectively heals duodenal, gastric and oesophageal ulcers. It is not absorbed systemically and it has been shown to act locally by coating the ulcer base. However when it was also shown to prevent stress ulcers and ethanol-induced gastric mucosal lesions, it seemed likely that it acted in some way to improve the effectiveness of the gastric mucosal barrier. Some investigators suggested that it did so by stimulating local prostaglandin release. The Slomiany group, on the basis of in vitro work on the effects of Sucralfate on pig gastric mucus, claimed that Sucralfate acted by altering the physico-chemical properties of mucus to increase the viscosity and retard the back diffusion of H+ ions.

The work described in this dissertation set out to verify, *in vivo*, these claimed effects on mucus, using an experimental porcine model of peptic ulceration, the bile duct ligated pig. In addition, the effects of Sucralfate were compared with those of Famotidine and Misoprostol, and changes in mucous prostaglandins, gastric juice pepsin and gastric flora were sought.

By way of introduction, the known and postulated actions of Sucralfate, current understanding of gastric mucus physiology and pathogenesis of peptic ulceration, have been reviewed, as have experimental animal models of peptic ulceration, in order to justify using the bile duct ligated pig model.

I. INTRODUCTION

A. GASTRIC MUCOSAL DEFENCES

Current understanding indicates that the most significant physiological roles of the stomach are to accept at intervals relatively large quantities of food, to mix components of a meal, to deliver manageable quantities of this prepared food to the duodenum, to reduce the bacterial content of ingested material, to detect and eject ingested materials which might be harmful to the body, to initiate digestion of protein and possibly carbohydrate, to facilitate absorption of certain specific minerals and other nutrients and to participate in an intricate interplay of endocrine influences.[1]

The digestive elements of gastric secretions, HCl and pepsin, have the potential to digest the gastric epithelium, but under normal circumstances this is prevented by a variety of mucosal defence mechanisms acting in concert. The components of the mucosal defences are thought to be the mucus layer lining the surface epithelium, the bicarbonate ions secreted by the surface epithelial cells, interstitial bicarbonate, the mucosal blood flow, the restitutional capacity of surface epithelial cells and the hydrophobic property of the surface epithelial cells.[2]

1. MUCUS

The mucus secretion lining the mucosal surface is a viscous gel with properties of both liquid and solid, which may vary in physiological and pathological conditions. Mucus from different organs and sites has specialized features to fulfil the special function that is required. Normal mucus forms a layer about 0.1-0.5 mm thick, lining the internal tracts of the body. It contains inorganic ions typical of extracellular fluids, specialized glycoproteins called mucins, several secreted proteins (e.g. immunoglobulin A, lysozyme, lactoferrin), and transuded plasma proteins (e.g. albumin) and in some mucins, galactose-rich pure polysaccharides. Exfoliated epithelial cell membranes

contribute glycosphingolipids, phospholipids and various other glycoproteins. The organic constituents account for 5-10% of mucus gel weight.[3]

Gastroduodenal mucus exists in two distinct physical forms known as adherent mucus, which is a stable gel insoluble in water, and soluble mucus, which mixes with the luminal juice

Adherent mucus adheres to the mucosal surface in a continuous cover varying in thickness from 50 to 450um in humans[4]. It provides the stable unstirred layer considered necessary to support surface neutralization and provide the mixing barrier for luminal acid and mucosal bicarbonate. It also prevents access of luminal pepsin to the underlying epithelial cells. Thickness of the adherent mucus layer can be increased by stimulation with prostaglandin E_2 and carbachol.

Soluble mucus mixes with the luminal juice and, being quite viscous, it is an excellent lubricant, minimizing physical damage to the adherent mucus gel and epithelial cells. It can be removed from the mucosal surface by gentle washing. It contains a greater proportion of lower molecular weight glycoproteins and lower concentration of polymeric mucin. It is formed by the mucolytic action of pepsin on the luminal surface of the adherent mucus, as well as secretion of polymeric mucin. Therefore changes in soluble mucus output do not necessarily reflect changes in efficacy of the protective adherent mucus gel.[5]

A) MUCIN STRUCTURE

The most widely accepted model of the structure of gastric mucin, the principal gel-forming constituent of mucus, is that proposed by Allen et al[6]. This model proposes that one subunit of 70 000 daltons is linked by disulphide bonds to 4

other rigid "bottle-brush" subunits each consisting of a core polypeptide with many branched oligosaccharide chains attached. These large "windmill" units may be further polymerized into much larger molecules with markedly visco-elastic properties in concentrated solution.

Mucins, comprise 10-15% protein, constituting the polypeptide core, to parts of which are attached hundreds of oligosaccharide chains (the "bottle-brush" regions). The cysteine-rich, non-glycosylated regions of the polypeptide cores are believed to be the sites of disulphide bridges which link subunits. In the regions of the polypeptide core that bear the oligosaccharide chains, serine, threonine, and proline are the predominant amino acids .

The oligosaccharide chains have a typical complement of sugars, including galactose, fucose, N-acetyl galactosamine, N-acetyl glucosamine, and negatively charged sialic acids. In the case of gastric mucins these chains comprise up to 11-20 residues and may be branched. Negatively charged sulphate is also a feature of mucins - either together with sialic acids or as ester sulphate. Some of the oligosaccharides are identical to those found in glycolipids and glycoproteins of red cell membranes which confer upon them the antigenic character of the ABO and Lewis systems. Carbohydrate comprises 70-85% of total mass of mucin and the carbohydrates are attached to serine and threonine hydroxyl side chains. Because the packing is so close and the tertiary structure so tight, the carbohydrate-bearing regions of the protein cores are rather resistant to proteases.

Disulphide bonds cross-link the non-carbohydrate-bearing (naked) regions of the polypeptide chains, either to each other or to linker subunits. The oligosaccharide side chains are mostly negatively charged and tend to repel each other, forming expanded and rigid "bottle-brush" structures. As the concentration of highly hydrated

mucins is increased, interdigitating carbohydrate side chains interact by means of hydrogen bonds or salt bridges between charged groups, and at concentrations of 20-50 mg/ml visco-elastic gels are formed. The overlapping domains of extended macromolecules cause the entire solvent to become "floppily-solid" so that other macromolecules cannot penetrate the interstices of the gel. Small molecules diffuse readily through the liquid phase of the gel, although not as freely as in free solution, because of ion exchange phenomena and the absence of a stirring mechanism. Purified mucins have nearly the same properties as crude mucus when brought to appropriate concentrations, and they are believed to be mostly responsible for the physico-chemical behaviour.[7]

Proteolysis of non-glycosylated polypeptide regions and/or cleavage of disulphide bonds significantly decreases the viscosity of mucus. High salt concentrations cause shrinkage of the mucin domains and diminish their entanglement. Certain proteins enmeshed in the mucins can enhance gelation, and the lipid components probably also have a promoting role in this regard.

Carlstedt and Sheehan[8] have proposed an alternative model of mucin structure, in which mucins are built up of a linear array of glycosylated domains interconnected by naked protein regions. They believe that mucin subunits are joined end-to-end by disulphide bonds to produce linear flexible chains with properties in solution approaching those of a random coil.[8,9]

The Slomiany group have proposed a modification of the Allen model[10], based on their observations that gastric mucins also contain lipid in the form of neutral lipids, glycolipids and phospholipids. They have noted differences in the glycolipid composition of intracellular and surface mucus, surface mucus having a higher proportion of glycosphingolipids which are derived from exfoliated epithelial cell

membranes, whereas intracellular mucus lipid is almost exclusively glucoglycerolipid.[11] It is claimed by these investigators that the glycoprotein polymer forms a dynamic continuum with lipids and secreted and transuded proteins and that, while mucin is the only component of mucus capable of gel formation, the proteins and lipids exert significant effects on the physico-chemical characteristics of gastric mucus .

2.BICARBONATE

Gastric antral and fundal mucosa secretes bicarbonate into the lumen under the influence of various stimuli and inhibitory signals. Duodenal mucosa has a similar ability to secrete bicarbonate, but the rate of secretion is higher than in the stomach, and the processes of transport of bicarbonate and control of secretion differ.[12,13] Bicarbonate secretion has been shown to be considerably greater in the proximal duodenum than the distal duodenum, and it seems likely that a substantial proportion of acid delivered to the proximal duodenum is neutralized by surface epithelial bicarbonate secretion prior to contact with pancreatic or biliary secretions.[14]

Studies using pH-sensitive microelectrodes have shown that pH at the epithelial surface is nearly neutral, while that of the luminal solution is 2-3. The mucus forms a continuous layer of water-insoluble, visco-elastic gel adherent to the surface epithelium. It is not well understood how cells lining the gastric crypts resist acid-peptic digestion, as only the surface epithelium is covered by mucus.

Three mechanisms act in concert to control bicarbonate secretion:

Neural stimulation, together with acid, by the vagi. It can be inhibited by anti-cholinergic drugs, and it is thought to be mediated by intracellular c-GMP.

Local mucosal linkage between the processes of H^+ secretion and bicarbonate secretion, by parietal cells and surface mucosal cells respectively

Stimulation of bicarbonate secretion by the presence of acid in the lumen. This is probably mediated by prostaglandins, humoral factors, and neural mechanisms. Prostaglandin E_2 stimulates bicarbonate secretion, more potently when administered intraluminally than parenterally. Prostaglandin F_{2a} also stimulates bicarbonate secretion but less potently than PGE_2. Peptides such as endorphins, VIP, and pancreatic polypeptide are also known to stimulate bicarbonate secretion, possibly as neurotransmitters.[15]

B. PATHOPHYSIOLOGY OF PEPTIC ULCER

The term "peptic ulcer" embraces a number of different categories of ulceration of the mucous membrane of the gastrointestinal tract and it is very important, in trying to understand their pathophysiology, to be aware of the different mechanisms involved. A useful classification is that proposed by Brooks in a recent review[16]. He recognized two broad groups, viz. acute and chronic. The acute ulcers are all gastric and include the ulcers occurring in patients with severe burns and CNS lesions, ulcers occurring in patients subjected to severe non-specific stress such as shock and sepsis, ulcers occurring after ingestion of aspirin and other non-steroidal anti inflammatory drugs (NSAIDs) and excessive ethanol, or exposure to bile reflux, as well as ulcers occurring at the extremes of age. The chronic ulcers are subdivided into gastric, duodenal, oesophageal, hormonally induced, recurrent after surgery, and those adjacent to a Meckel's diverticulum. The chronic gastric ulcers are further sub-divided, as suggested by H. Daintree Johnson[17], into lesser curve ulcers occurring at the angulus incisura or, more precisely, at the junction of body and antral mucosae[18] (Type I); gastric ulcers secondary to duodenal ulceration or scarring (Type II); and prepyloric/pyloric channel ulcers (Type III).

Ulceration in the Zollinger-Ellison syndrome is clearly related to excessive gastric acid secretion secondary to uncontrolled gastrin secretion by a gastrinoma. Duodenal ulcer disease is associated with high acid secretion in many, but by no means all patients and patients with types II and III gastric ulcers have similar acid profiles to those with duodenal ulcers. Patients with type I gastric ulcers, however, tend to have normal or, more often, lower than normal levels of gastric acid secretion. So acid, although clearly important, is not the only factor contributing to ulcer formation. Indeed, it is essential to remember that, as Venables has written, "acid has never on its

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own been shown to induce an ulcer"[19]. Like acid, peptic activity is an indispensible component of the pathogenesis of peptic ulcers, even in Zollinger-Ellison syndrome, for acid without pepsin has little digestive power, and pepsin is inactive without acid.

Pepsin, in an acid environment, is a powerful protease that can digest mucus and cell membranes, and concentrations of pepsin in gastric juice follow a similar trend to acid in the different categories of ulcer. However, analysis of the pepsin profiles in normal subjects and patients with peptic ulcers has revealed a higher proportion of total peptic activity accountable to pepsin 1 in the gastric juice of ulcer patients - 16.5% in duodenal ulcer (DU) patients and 23% in gastric ulcer (GU) patients compared with 3.6% in controls[20,21,22]. This may well be significant because, firstly, pepsin 1 has been shown to digest mucus more avidly than the other pepsins, and secondly pepsin 1 retains its peptic activity at higher pH levels (4-5) at which other pepsins are inactive.[23]

The mucous membrane of the stomach and duodenum is uniquely adapted to resist the corrosive power of acid and pepsin. The adherent mucus gel, whilst being digested by pepsin on its luminal surface, is impermeable to pepsin and so protects the epithelium from digestion. The adherent mucus also retards diffusion of H^+, so allowing effective neutralization at the epithelial surface by bicarbonate secreted by the epithelial cells. Mucus probably also assists the healing process once epithelial erosion has occurred, by acting as a template for fibrinogen-fibrin conversion and preventing access of pepsin to the clot[25,26]. The epithelial cell membranes have an inherent resistance to damage by acid and the mucous membrane has the ability to rapidly regenerate its epithelium when this is breached. The integrity of this mucosal barrier is dependent on adequate blood supply, normal homeostasis and cell metabolism and, possibly local prostaglandins[27], and disturbance of this barrier may also contribute to ulcer formation.

Failure of the mucosal barrier is certainly the primary fault in the genesis of acute gastric stress ulcers, acid and pepsin being only secondary co-factors. The primary mechanism involves impaired mucosal blood flow, increasing tissue acidosis, depletion of ATP and epithelial cell death, with impaired secretion of mucus and bicarbonate and consequently increased susceptibility to acid and pepsin, as well as bile reflux which may increase as a result of gastrointestinal atonia. Impaired mucosal blood flow also results in impaired mucosal restitution.[28]

Peptic ulceration can thus be viewed, perhaps simplistically, as developing as a result of disequilibrium between the aggressive factors (acid and pepsin) and the defence of the mucosa. However this does not explain why, at a particular time, the mucosal defence is weakened to the point that it is overwhelmed by the "aggressors", and neither does it explain the typical locations of chronic ulcers

Whereas chronic gastric ulcers were recorded in ancient history[29], duodenal ulceration, although described in the 19th century, only emerged as a common disease at the beginning of the 20th century and Wormsley argues, on the strength of this observation, for the influence of an "environmental ulcerogen (or deficiencies of anti-ulcerogenic factors)"[30], which allows the initial mucosal damage. He suggests that, possibly, a deficiency of epidermal growth factor in saliva or other "growth factors" may delay healing of epithelial damage, allowing ulcers to form and become chronic. Another suggestion is that gastric and duodenal ulcers are caused by an infectious or chemical ulcerogen, herpes simplex virus being proposed as a possible culprit.

Helicobacter pylori has been proposed as a possible infective cause for peptic ulceration[31], although this is controversial[32] and it is currently felt to be an important co-factor in the pathogenesis of duodenal ulcer disease. This Gram-negative, motile, spiral, urease-producing bacterium was first successfully cultured in 1984 and was

initially thought to be a member of the Campylobacter family. It is uniquely adapted to live in the stomach, immediately above the surface epithelial cells and deep to the adherent mucus gel, shielded from the gastric acid to which it is vulnerable after more than 30 minutes at pH < 2. Its urease converts urea to ammonia, which also neutralizes acid and presumably protects it. It is also found in the duodenal bulb, but only in relation to antral-type epithelial cells. In vitro, it is sensitive to numerous antibiotics, but in vivo it is resistant to most of them, with the exception of metronidazole and tinidazole. This is presumably because of its location deep to the mucus gel, out of reach of the antibiotics. Colloidal bismuth subcitrate also exerts anti-bacterial activity against the organism. As pathogen, there is good evidence that it causes type B gastritis[33,34], but evidence for its role in the aetiology of peptic ulcer disease remains circumstantial. Certainly, the current data does not indicate any association with gastric ulceration, although the organism is frequently detected in the antra of patients with duodenal ulcers, and it may play an indirect role in the development of duodenal ulcers. It has been claimed that this organism degrades mucus and in this way initiates mucosal damage.[35] This seems highly improbable, given its unique adaptation and apparent dependence on the mucus to shield it from the gastric acid.

In similar vein, Szabo has drawn attention to the similarities in the epidemiology of duodenal ulcer disease and Parkinson's disease, and feels that "exposure to environmental agents or food products may trigger biochemical defects that can result in duodenal ulcer". His experimental work with cysteamine, showing deficiencies of dopamine both locally in the stomach and duodenum and centrally in parts of the brain, and disorders of duodenal motility, remind us of the importance of the brain and motility in the pathogenesis of "peptic ulcer".[36]

C. MODELS OF PEPTIC ULCERATION

1. IN VIVO

To date no experimental model of peptic ulceration has been entirely satisfactory as none exactly mimics the human disease and they all involve creating abnormal physiological circumstances or exposure to highly toxic chemicals. Besides this, the human disease is not a uniform single entity, but rather a spectrum of different types of peptic ulceration.

One of the earliest observations of peptic ulcer was the association with acid, as indicated by the dictum "no acid , no ulcer" enunciated by Schwarz in 1925, so it is not surprising that the earliest animal models involved abnormal exposure of mucosa to acid, either by diverting alkaline secretions away from the site of acid exposure, or by increasing acid[37,38]. Other models more closely mimic the acute erosive gastritis/stress ulcer syndrome, and several models are themselves poorly understood. Some are ingenious experiments, while others were serendipitously discovered in the process of other lines of research and subsequently exploited. Yet others involve bizarre, unphysiological injuries to the gastric mucosa with e.g. boiling water or 100% ethanol, which can have little relevance to clinical practice[39]. The following is an attempt to classify various ulcer models, but is by no means a complete list of all the methods used.

A) DIVERTING ALKALI

a) EXCLUSION OF DUODENAL JUICE

The best known of these is the Mann-Williamson duodenal drainage method in dogs, in which the stomach is disconnected from the duodenum, the proximal end of

duodenum is closed, the proximal jejunum is divided, and the distal end is anastomosed to the stomach (end-to-end) and proximal end anastomosed to the ileum end-to-side.

Other methods include:

a) transplanting the common bile duct and pancreatic duct to the ileum, which produces ulcers in only 50% of the dogs.

b) the Goldberg method - creating a gastric fistula by anastomosing, in antiperistaltic fashion, an isolated piece of small intestine between gastric fundus and skin, and subsequent conversion of that segment of fundus to an isolated fundal pouch (in a high percentage peptic ulceration occurred in the intestinal mucosa just beyond the gastric stoma).

b) Exclusion of bile

The Kapsinow method, which comprises drainage of the gall bladder to the renal pelvis and ligation of the common bile duct of dogs, produces ulcers in duodenum in 40% within 2 weeks.

The Bollman and Mann / Berg and Jobling method:- dogs with complete obstructive jaundice. About 60% developed perforating gastric and duodenal ulcers.

c) Exclusion of pancreatic juice

The Rous and McMaster method:- complete diversion of pancreatic juice to the exterior (in dogs) which produces ulcers in a large percentage.

In all these models the ulcers heal if the anastomoses are taken down and reconnected in the correct sequence, and they confirm the importance of defective neutralization of acid in gastric juice.

The resistance of duodenum to gastric acid has been demonstrated by the fact that only 20% of dogs whose common bile duct and pancreatic duct were transplanted to the ileum developed ulcers, and by experiments where duodenal drainage procedures were done with preservation of an inch of proximal duodenum and ulceration developed at the duodeno-jejunal anastomosis and not in the duodenal remnant.

B) INCREASING ACID

a) SHAY'S PYLORUS-LIGATED RAT

In 1945 Harry Shay and associates accidentally discovered that if rats were allowed to survive 18 hours with the pylorus ligated they consistently developed perforating ulcers and haemorrhagic glandular ulcers. It soon became the most popular experimental model of peptic ulceration because of the ease of preparation, high incidence of perforating lesions, and susceptibility to blockade by anticholinergic agents.

Pylorus ligation has been shown to stimulate gastric secretion. The ulcers can be prevented by any compound that will reduce either volume or acidity or both of gastric juice, as well as compounds that block peptic activity.

b) DRUGS

i) GASTRIC STIMULANTS

Histamine and Gastrin

Continuous maximal acid output in response to repeated IM or continuous IV administration results in duodenal ulcer formation in a high proportion of cases.

Cinchophen

Cinchophen (C16H11NO2), a drug used in the past for its analgesic and antipyretic properties to treat gout[40], increases the amount of gastric secretion without

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Cinchophen (C16H11NO2), a drug used in the past for its analgesic and antipyretic properties to treat gout[40], increases the amount of gastric secretion without

altering the level of acidity, when administered by continuous IV infusion or regular IM injections, and causes duodenal and gastric ulcers.

ii) Adrenal Steroids

Silen points out that it has been accepted as dogma that steroids play an important role in stress ulceration, yet almost all studies indicate that steroids ameliorate, rather than accentuate stress ulcers and other forms of gastric mucosal damage.[39]

iii) Non-steroidal anti-inflammatory drugs (NSAIDS)

NSAIDS produce a spectrum of gastroduodenal mucosal lesions ranging from haemorrhages and petechiae to erosions and ulcers. They probably act systemically by inhibition of prostaglandin synthesis, as well as locally to alter the mucosal barrier, either permitting increased H^+ back diffusion or impairing HCO_3^- secretion and neutralization of acid. The proposed protective role of prostaglandin in gastric physiology has been highlighted by the ulcerogenic potential of the NSAIDS.

The presence of H^+ is required to produce ulceration, but it may be that it operates by producing the undissociated form of the NSAID, which penetrates the cell membrane more easily. It is thought that the cellular damage is caused by the dissociated intracellular anion rather than H^+ itself.[41] Several NSAIDS inhibit glycoprotein biosynthesis by the epithelial cells, so although they do not alter the secreted mucus gel acutely, they may cause depletion of mucus with chronic use.[42]

Interestingly, acute parenteral injection of aspirin produces acute fundal ulcerations, whereas more prolonged injection by chronic intravenous infusion of aspirin or indomethacin produces more chronic antral ulcers in cats[43].

Drug induced ulceration bears little similarity, with the exception of gastrin (and possibly histamine) in Zollinger - Ellison syndrome, to spontaneous human peptic ulcer disease.

iv) Ethanol

A popular method, recently, of creating gastric mucosal injury, has been instillation of 100% ethanol into the stomachs of rats. Ethanol rapidly penetrates the mucosa and causes endothelial damage in superficial and deep capillaries and venules, resulting in increased permeability and decreased blood flow. This leads to complete circulatory standstill in superficial capillaries, so that the direct damage to surface epithelial cells is compounded by hypoxia and deep haemorrhagic necrosis in 1-5 minutes.[44] This is grossly unphysiological, does not require the presence of luminal H^+, and Silen in a recent editorial review has questioned whether this model has any relevance to human disease. [39]

v) Insulin

High doses of insulin have been used to induce ulcers in the oxyntic gland area of the stomachs of fasted rats.[45] Unlike low doses that stimulate acid secretion, the ulcerogenic dose used, 5IU/kg s.c., in addition to causing profound hypoglycaemia, suppresses basal and stimulated acid secretion and stimulates gastrin secretion and activation of histadine decarboxylase in the gastric mucosa. These ulcers were prevented by prior antrectomy, vagotomy or treatment with cimetidine or omeprazole, but not by Sucralfate.

c) Direct Application of Acid

The importance of timing of exposure to acid was demonstrated by experiments in dogs that had had HCl infused into the stomach (smaller amounts than secreted by normal dogs during digestion) via Mann-Bollman fistulae at a rate of 1ml/minute for 8 hours a day during fasting. The dogs developed chronic gastric ulcers along the lesser curve in about 4 weeks. Administration of food during the period of infusion was protective.

C) HAEMORRHAGIC SHOCK

This produces acute superficial, haemorrhagic, mucosal lesions, distributed mainly in the fundus, with sparing of the antrum. It simulates acute stress ulceration in humans, and is as much a model of inadequate tissue perfusion, as it is of specific gastric mucosal injury.

D) CENTRAL NERVOUS SYSTEM MANIPULATIONS

a) Stressed Rats

Rats are stressed by restraint, and other methods, and these rats develop ulcers (or, more precisely, erosions, which do not penetrate the muscularis mucosae) in the glandular portion of the stomach. Vagotomy and anticholinergic drugs protect the rats from developing these lesions. These lesions frequently bleed but do not perforate.

b) Executive Monkeys

Experimental psychologists set up behavioural situations using operant conditioning techniques and compared the incidence of duodenal ulcers in "executive" monkeys and non-decision-making "worker" monkeys. The advantage of this model is

that it produces duodenal ulcers in primates over several weeks, without surgery, drugs, or dietary changes. The disadvantages are that it requires highly trained investigators, skilled in designing complex schedules of reinforcement and utilizing complex equipment and that the experimental designs are questioned by members of animal rights movements

c) Cysteamine ($HSCH_2CH_2NH_2$)

According to a recent review[36], the cysteamine-induced duodenal ulcer in rats has become the most widely used animal model of duodenal ulcer disease.

Cysteamine is a sulfhydryl compound with a variety of biological effects.[46] It is formed in mammalian tissues by enzymatic hydrolysis of pantetheine[47 48], and plays a role in the biosynthesis of hypotaurine. It has been used clinically to treat paracetamol poisoning and nephropathic cystinosis, and experimentally as a radioprotective agent.

That cysteamine invariably causes the development of acute perforating duodenal ulcers in rats when administered to these rats either orally or parenterally, was a serendipitous discovery by Hans Selye and Sandor Szabo during the course of some experiments to study the effect of hormones on resistance to various toxicants[49].

The ulceration is associated with increased gastric acid output, delayed gastric emptying, and elevated serum gastrin levels, and can be modulated by antacids, anticholinergic drugs, histamine receptor antagonists and vagotomy. This model is similar to human duodenal ulcer disease in virtually all morphological and functional parameters. However, a difference is its frequent association with adrenocortical lesions.

Hormonal changes that follow cysteamine administration include acutely increased serum gastrin levels, prolonged suppression of plasma secretin, transient depletion of somatostatin in gastric and duodenal mucosa, pancreas and hypothalamus and transient elevations of plasma levels of corticosterone, glucagon, insulin and glucose.

Functional changes induced by cysteamine include greatly increased volume of gastric juice, delayed gastric emptying and increased output of alkali. In addition there are important alterations in duodenal motility, which may be related to the abundance of dopamine binding sites in the muscularis propria. The migrating myoelectric complexes are disrupted, the frequency of slow waves is decreased and there are changes in intraluminal pressure and transit time. The net effect is faster transit from proximal to distal duodenum and slower passage from distal duodenum to duodenal bulb.[36]

d) Pantothenic acid deficient Zucker rats

This model has been known for many years but not widely used because of the need for the special strain of rat and the slow development of the lesions, which are associated with villous atrophy, duodenitis and erosions progressing to ulceration. Acid output is first decreased, but in subsequent weeks is increased. The intriguing feature of this model is the biochemical relationship to the cysteamine model, pantotheine being the naturally occurring molecular combination of pantothenic acid and cysteamine, and an intermediate in the pathway of coenzyme A.

e) Other sulfhydryl compounds

Duodenal ulcers were also noted to develop after administration of 3,4-toluene-diamine and propionitrile, although these were less acute and less consistent.

Subsequently n-butyronitrile, a molecule closely related to propionitrile, was identified as a more rapid duodenal ulcerogen with lower toxicity than propionitrile.[50] Propionitrile induces duodenal ulcers that are morphologically identical to those induced by cysteamine but, unlike cysteamine, does not deplete tissue somatostatin or elevate serum gastrin, but does decrease alkaline secretion in the duodenum while only transiently and sporadically increasing acid and pepsin.

Other related chemicals which are weak ulcerogens are acetanilide and 3,4 toluene-dithiol. Mepirazole is another compound which induces acute and chronic duodenal ulcers which are superficial and accompanied by gastric erosions and ulcers.[50]

E) MECHANICAL FACTORS

The observation that ulcers always formed at the point of impact of the ejected acid with the mucous membrane led to experiments aimed to establish whether a mechanical factor was important in determining the site of the ulcer:[38]

in the Mann-Williamson model, straightening out the segment receiving the acid so that the expelled gastric juice runs in the same direction for about 12cm, resulted in the ulcers forming at a point just proximal to where the loop bends acutely, and not just beyond the line of anastomosis

slightly altering the axis of the pylorus in Mann-Williamson model dogs, when one ulcer had already formed, resulted in the formation of kissing ulcers

deliberately making the stoma between pylorus and intestine narrow, after a duodenal drainage procedure, to create a "nozzle-like action", resulted in the ulcers developing more quickly and perforating earlier.

transplantation of vascularized patches of jejunum into the wall of the stomach (Morton), resulted in ulceration of the patch only very rarely.

dogs with complete obstructive jaundice developed duodenal ulcers more rapidly if fed a diet of dog biscuits and bones than if fed milk and syrup

F) ECK FISTULA

Dogs with a simple Eck fistula (side-to-side porto-caval shunt) frequently die from perforated duodenal ulcers. The mechanism, however, is not clear[51].

G) PHYSICAL INJURY

Grossly unphysiological insults such as application of boiling water and concentrated alkali have been used to create gastric mucosal injury. Unlike most in vivo models of gastric mucosal injury, this model does not require the presence of luminal H^+.

H) BILE DUCT LIGATED PIG

a) History

Spontaneous perforating and bleeding oesophago-gastric ulcers in pigs, once an agricultural curiosity, is now a significant problem in the swine industry, with 20 - 50% of pigs coming to slaughter having these lesions[51,52,53]. The ulcers have been attributed to nutritional and environmental factors, as the incidence can be modified by changing the diet and not housing in small enclosures with slanting floors, as was the common practice[54,55].

These ulcers also complicate experimental surgery on pigs, with a histological incidence of 80% and mortality from haemorrhage of 50-60% in survivors of orthotopic liver transplantation, as well as liver autografts [56,57]. This ulceration was

noted to be associated with cholestasis, and ligation of the bile duct reproduced the ulcer with 100% reliability[58,59]. Although bile duct ligation itself is lethal in the pig, highly selective vagotomy done at the same time as bile duct ligation, significantly prolongs survival and completely eradicates the problems of ulceration of the pars oesophagea[60].

b) INVESTIGATION

Arnot studied changes in Heidenhain pouches following bile duct ligation and found increased basal acid secretion, decreased response to histamine, and increased response to pentagastrin at low doses, though not at high doses. He deduced that the increased basal secretion was not likely to be due to increased parietal cell sensitivity or decreased hepatic degradation of pentagastrin, but was possibly due to absence of a circulating inhibitor or increased amounts of a circulating secretagogue. Gastrin levels were measured and found to decrease after bile duct ligation, the lowest levels being noted within 48 hours. These returned to normal by the 5th day after bile duct ligation. Gastrin was thus clearly not the stimulus to the gastric hypersecretion.[58]

Watson found that exogenous gastrin aggravated the oesophago-gastric ulceration following bile duct ligation, and concluded from studies with total gastric fistulae that the hypersecretion was not due to gastrin, calcium, abnormality of liver function or absence of bile from the stomach. He confirmed that bile duct ligation causes greatly increased volume and concentration of acid secretion and also showed that antral secretion was decreased but more alkaline. From this he deduced that the alkali was insufficient to neutralize the acid, or the ulceration was not entirely due to acid hypersecretion, and suggested that bile duct ligation diminished inhibition of acid secretion or stimulated secretion of a "non-gastrin secretagogue" (? entero-oxyntin). Further investigations showed that bile duct ligation was followed by increased serum

levels of several amino acids, infusion of plasma from bile duct ligated pigs into pigs with total gastric fistulae caused gastric hypersecretion (up to 14x greater than plasma from sham operated pigs), infusion of plasma from bile duct ligated and sham operated pigs into rats had similar effects, and electron microscopy of gastric cardia and pars oesophagea revealed degranulation of mast cells in bile duct ligated stomachs but not sham operated stomachs. The conclusion from these studies was that the ulceration of the pars oesophagea of pigs whose common bile duct has been ligated, could be attributed to an active gastric secretagogue.[61]

c) Anatomical features of the porcine stomach

The pig stomach has several anatomical differences from the human stomach. Firstly, there is the pars oesophagea, which is a rectangular, 2x1cm, area of stratified, non-keratinized, squamous epithelium at the cardiac entrance of the stomach, immediately distal to the oesophagus and distinct from oesophageal epithelium. Secondly, the fundus is relatively much larger, and the lesser curve relatively much shorter than those of the human stomach. Then, unlike the arrangement in the human, the common bile duct enters the first part of the duodenum a few millimeters distal to the pylorus on the anti-mesenteric aspect, separate from the pancreatic duct which enters several centimeters further distally on the inner aspect of the duodenal curve, and bile refluxes freely into the stomach. Finally, the pylorus looks very different, having a bulbous structure, the torus pylori, which projects into the lumen from the lesser curve aspect.

d) Porcine gastric mucus

The viscous and gel-forming properties of gastric mucus depend on the polymeric structure of the undegraded glycoprotein, which is a polymer of four

subunits of equal size joined by disulphide bridges located between the carbohydrate-free parts of the protein core of each subunit.[62] The undegraded glycoprotein from human gastric mucus has been shown to have the same size, polymeric structure, and overall properties as that in pig gastric mucus.[63]

2. IN VITRO

Using isolated sheets or sacs of mucosa allows investigators to control the many variables that bedevil in vivo models, and to dissect the intimate intracellular biochemical events that lead to cell death and ulceration. However, a sheet of organized surface cells cannot simulate the interactions between surface and oxyntic cells, and these systems are also hampered by the fact that cells can only be assessed as viable or non-viable, since simple functions attributable to surface cells are almost impossible to measure in these systems.

D. SUCRALFATE

Peptic ulcer treatment has for decades been based on the principle of reducing available acid in the gastric juice and since the mid 1970's has been dominated by the H_2-receptor antagonists. However, another group of drugs that had no effect on gastric acid proved to be as effective as the H2-receptor antagonists, cimetidine and ranitidine and came to be known as "cytoprotective agents" or "site-protective agents", because they appeared to strengthen the mucosal barrier.[64,65]

One of these agents is Sucralfate, a basic aluminium salt of sucrose octasulphate which has proved to be significantly better than placebo, and as effective as cimetidine and ranitidine in short term healing of duodenal and gastric ulcers[66,67]. Marks et al have also shown lower recurrence rates of duodenal ulcers after healing with sucralfate than after healing with cimetidine.[68] This phenomenon has been confirmed by Lam et al[69], although disputed by other studies[70,71]. Maintenance therapy in a dose of 2g nocte, also is as effective as H_2-receptor antagonists in preventing relapses of both gastric and duodenal ulcers.[72,73,74] Doses of 1g b.d. have also proved to be effective maintenance therapy for duodenal ulcer patients.[75,76] In addition, Sucralfate protects the gastric mucosa against injury by ethanol[77,78,79] and aspirin[80], and is now regarded as the drug of choice for stress ulcer prophylaxis[28], being more effective than H_2-receptor antagonists and at least as effective but more convenient than antacids. Also, sucralfate has some inherent antibacterial activity[81] and, because it does not increase pH, is less prone than H_2-receptor antagonists and antacids to allow bacterial overgrowth, which by way of reflux of gastric contents predisposes to nosocomial pneumonias in mechanically ventilated patients.[82,83,84]

The effectiveness of Sucralfate was at first ascribed to coating of the ulcer base.[85] Mixed with HCl, sucralfate polymerizes to form a sticky, viscous paste and a

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The effectiveness of Sucralfate was at first ascribed to coating of the ulcer base.[85] Mixed with HCl, sucralfate polymerizes to form a sticky, viscous paste, and a

white paste-like substance was noted to adhere selectively to ulcerated and eroded gastric mucosa, and was thought to prevent diffusion of protons and pepsin. Other properties ascribed to the drug and thought to contribute to its mechanism of action were adsorption of pepsin and adsorption of bile salts.[86]

However, the effectiveness in preventing recurrent gastric and duodenal ulcers, stress ulcers and alcohol-induced gastric mucosal injury, suggests a more complex mechanism involving the mucosal defences. There has been much interest in possible prostaglandin mediation of increased mucus and bicarbonate secretion[78,87] and improved mucosal blood flow, but the effectiveness in preventing aspirin-induced mucosal injury makes it likely that other mechanisms are involved[88], and in a recent study prostaglandin could not be linked mechanistically to increased mucus secretion in rats protected from ethanol-induced gastric mucosal injury by sucralfate. These investigators also noted that although mucus secretion was stimulated by sucralfate, mucus synthesis was not.[89] Currently it is agreed that while prostaglandins may contribute to the protective effects of sucralfate, other mechanisms are also involved.[90,91] It has also been suggested that the protective effect of sucralfate may involve epidermal growth factor (EGF) by binding EGF and carrying it to the ulcer.[92]

Changes in gastric mucus have also been proposed to account for the protective effect of Sucralfate. In healthy human volunteers, Sucralfate tablets were seen to disperse and adhere firmly to a relatively small area of mucosa where striking histological and ultrastructural changes occurred in the surface epithelial cells. Mucus granules were discharged and a thick mucus layer formed over the mucosal surface. Cells separated from the basal lamina, vacuoles appeared in the cytoplasm and nuclei enlarged.[93] The Slomiany group from New York have suggested that the protective effect of sucralfate is due to inhibition of peptic degradation of mucus glycoprotein and binding of sucralfate with the mucus glycoprotein to enhance the viscosity and improve

the capacity of the mucus to retard H^+ diffusion. These investigators extracted gastric mucus from pig stomachs, obtained from the local abattoir, by filling the stomachs with 2M NaCl solution buffered to pH 7.0, and then dialyzing and lyophilizing the instillate. The extracted mucus was subsequently mixed with different concentrations of Sucralfate powder to measure viscosity and hydrogen ion diffusion.[94,95]

II. AIMS

I.

To attempt to verify the claimed effects of Sucralfate on viscosity and retardation of hydrogen ion diffusion of porcine gastric mucin, *in vivo*, using the bile duct ligated pig model

II.

To assess the effectiveness of Sucralfate in the prevention of oesophago-gastric ulceration in the bile duct ligated pig model.

III.

To compare the effects of Sucralfate with those of Misoprostol (an analogue of Prostaglandin E_1) and Famotidine (a long-acting H_2-receptor antagonist) on oesophago-gastric ulceration and mucus viscosity and H^+ diffusion in the bile duct ligated pig model.

IV.

To assess the effects of Sucralfate, Famotidine and Misoprostol on prostaglandin concentrations in gastric mucus, gastric flora, gastric juice pH, and gastric juice pepsin concentration.

III. METHODS

A. SURGERY

Experiments were performed on Landrace X Large White pigs weighing between 20 and 30 kg and aged 8-12 weeks. The pigs were starved (except for water) for 24 hours before surgery. The pigs were assigned to one of two large groups: bile duct ligation or sham. Each of these groups contained four subgroups receiving different test drugs: 0.9% physiological saline; Sucralfate; Misoprostol; and Famotidine. Each subgroup consisted of seven pigs.

Anaesthesia was induced with intravenous thiopentone (2-3 mg/kg) and maintained with O_2 and nitrous oxide administered via a cuffed Portex endotracheal tube.

An incision was made in the neck and the internal jugular vein was cannulated for fluid administration. Midline laparotomy was then performed and a small gastrotomy made in the anterior wall of the distal $^1/_3$ of the stomach. The mucous membrane of the stomach was swabbed for bacteriological culture and gastric juice was collected for subsequent pH and pepsin estimation. The first dose of the test substance was inserted in the stomach through the gastrotomy and a large bore Foley catheter, after being passed through a stab incision in the lateral abdominal wall, was then placed in the stomach and fixed with two purse-string $^2/_0$ silk sutures after inflating the balloon of the catheter and pulling it up to the wall of the stomach. Those pigs allocated to a bile duct ligation group, then had the bile duct ligated and divided. The abdominal wound was then closed and the corked Foley catheter was fastened with a skin suture, as far dorsally as possible to prevent the pigs from biting the catheters.

Thereafter, the pigs were returned to individual cages in a warm environment and were given free access to drinking water. Intravenous *Maintelyte* (SABAX) infusions were continued for approximately 24 hours. During the 48 hours after the

operation, the test substances were administered down the Foley catheters at strictly 6 hourly intervals for Sucralfate (1g), saline(30ml), and Misoprostol(200ug). Famotidine (40mg) was given once daily.

Forty eight hours after the initial surgery, the pigs were re-anaesthetised in similar fashion and total gastrectomy performed before the animals were sacrificed. The pyloric and oesophageal ends of the stomachs were firmly ligated before removal. Gastric juice was again collected for estimation of pH and pepsin. The stomachs were then drained completely and food residue washed out with tap water, before being filled to capacity (2.5-3L) with 2M NaCl-10mM sodium phosphate buffer pH 7.0. After 30 minutes the instillate was collected in glass bottles, and the stomachs were opened along the greater curve to: a) inspect for macroscopic evidence of ulceration; b) excise the pars oesophagea for histology; c) swab the mucous membrane for culture; and d) scrape off residual mucus with a glass slide for prostaglandin assay.

B. MUCUS EXTRACTION

The retrieved gastric instillate was filtered, first through glass wool and then through a bottle top vacuum filter fitted with a Schleicher & Schuell GF92 glass fibre prefilter. The filtrate was then dialysed in No. 4 Spectra/por cellulose dialysis tubing, against distilled water for about two days to remove all the NaCl. Dialysis was done in a refrigerator at 4^{o}C. The dialysate was partially dried in a rotary evaporator and then lyophilised. The dry mucus powder was collected, weighed and stored in glass bottles at -20^{o}C.

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C. PROSTAGLANDIN ESTIMATION

Prostaglandin E was estimated by radioimmunoassay in mucus scraped off the mucosal surface of the stomach after the 2M saline instillate had been drained from the stomach. The scraped mucus was frozen in liquid nitrogen immediately after collection and stored at -20°C.

The mucus sample was thawed and added to 1ml 0.01M phosphate buffered saline pH 7.4 (PBS) and 3ml extraction solvent, 3:3:1 (v/v) Ethyl acetate : Isopropanol : 0.1N HCl in an all glass Potter Elvjhem homogenizer and homogenized. The homogenous suspension was transferred to a glass stoppered centrifuge tube, the homogenizer was then rinsed with 2ml ethyl acetate and then 3ml distilled water, and these were both then transferred to the centrifuge tube, which was then shaken continuously for 15 minutes and then centrifuged for 10 minutes at 3000 r.p.m. The separated aqueous phase was kept for assay of protein. The supernatant phase was transferred to a conical test tube containing approximately 250mg $NaHCO_3$ and 250 mg Na_2SO_4 and well mixed on a vortex mixer. The solvent was then evaporated to dryness under a stream of Nitrogen and the residue dissolved in 1ml of a 60:40 (v/v) benzene : ethyl acetate solvent (Solvent I) while still under N_2. The tube of extract was covered with parafilm and stored at -20°C until chromatography was performed.

To prepare the chromatography column, Silicic acid (SIL-A -200 60-200 mesh) was placed in a shallow dish and heated for at least one hour in an oven at 110°C, then taken out and placed in a beaker containing 10ml Solvent I. The slurry was transferred to a glass column with rinsing, and was washed with 15ml Solvent I, so that the column of Silicic acid had a faint blue tinge and was completely translucent.

The extract was transferred to the column and the tube was rinsed several times with Solvent I which was also added to the column. The column was then eluted with another 5ml of Solvent I. This eluate of approximately 12ml contained Prostaglandins A & B (PGA and PGB) and was discarded. Thirty millilitres of Solvent II (Benzene : Ethyl acetate : Methanol :: 60:40:3 v/v) was then applied to the column and eluted at about 2ml/min. This eluate, which contained PGE, Thromboxane B_2 and 6-keto PGF_{1a}, was evaporated to dryness under a stream of N_2 and then 1ml 0.1% bovine serum albumin in PBS (PBSA) was added while still under nitrogen. Then the sample was transferred to a small stoppered Teflon tube covered with a stream of N_2 and stored at -20°C until required for the radioimmunoassay.

To assay for Prostaglandin E, 0.1ml of sample or standard were pipetted into assay tubes. A zero control, a blank and a total tube each containing 0.1ml PBSA were prepared. One half millilitre rabbit anti-prostaglandin E-BSA serum (Miles-Yeda Ltd. Kiryat Weizman, Rehovot, Israel) were added to all tubes except the total and blank tubes. To these were added 0.5ml buffer. All tubes were incubated at 4°C for 30 minutes, then 0.1ml tritiated Prostaglandin E standard (160Ci/mmole supplied by Amersham Ltd and diluted with buffer to 100 000 degradations per minute/ml) was added to all tubes and they were incubated at 4°C for 60 minutes. Then 0.2ml dextran coated charcoal solution was added to each tube except the total tube, to which 0.2ml buffer was added. All the tubes were mixed on a vortex mixer, incubated at 4 °C for 10 minutes, then centrifuged at 4°C at 3000 r.p.m. for 15 minutes. An aliquot (0.5ml) supernatant was removed and added to 6ml scintillation fluid (Picafluor or Instagel, Packard Instruments Ltd) and counting was done in a liquid scintillation spectrometer.

The fraction bound was calculated by the formula:

$$\% \text{ fraction bound} = \frac{\text{cpm in sample} - \text{cpm in blank}}{\text{cpm in zero ctrl} - \text{cpm in blank}} \times 100$$

The extract was transferred to the column and the tube was rinsed several times with Solvent I which was also added to the column. The column was then eluted with another 5ml of Solvent I. This eluate of approximately 12ml contained PGA and PGB and was discarded. Thirty millilitres of Solvent II (Benzene : Ethyl acetate : Methanol :: 60:40:3 v/v) was then applied to the column and eluted at about 2ml/min. This eluate, which contained PGE, Thromboxane B_2 and 6-keto PGF_{1a}, was evaporated to dryness under a stream of N_2 and then 1ml 0.1% bovine serum albumin in PBS (PBSA) was added while still under nitrogen. Then the sample was transferred to a small stoppered Teflon tube covered with a stream of N_2 and stored at -20°C until required for the radioimmunoassay.

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A standard curve was obtained using a working dilution of the antiserum and plotting % bound vs concentration. Values of unknown samples were obtained from the curve and PGE concentrations calculated allowing for dilutions.

Working dilutions were prepared by first preparing a stock solution of 1mg PGE2/100ml absolute ethanol, then diluting 1ml stock in 100ml PBSA. The first dilution was diluted again 1ml:10ml PBSA to give a concentration of 10ng/ml. Then further serial doubling dilutions were prepared in buffer to give standard 5, 2.5, 1.25, 0.63, 0.31, and 0.15 ng/ml solutions.

D. VISCOSITY MEASUREMENT

Thirty milligrams of the dry mucus powder was dissolved, with constant stirring at room temperature for 12-24 hours, in 1ml 0.1M NaCl-0.05M Na_2HPO_4 buffer (pH 6.0) to which was added 1ul of phenylmethanosulphonyl fluoride (PMSF), an inhibitor of proteolysis. Viscosity of 0.5ml samples was measured with a Brookfield cone/plate digital viscometer, model LVTD, equipped with a CP40 cone and constant temperature (37°C) water bath, at shear rates of 45, 90, 225 and 450 s^{-1}. Several readings were taken at each shear rate and the means calculated. The 1.565° cone used by the Slomiany group was found to be unsuitable for our samples.

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E. HYDROGEN ION BACK DIFFUSION

A Perspex apparatus, similar to that described by Sarosiek et al[96] was constructed (Figure I). The chamber on one side of the centre panel was filled with 0.15M HCl and the other chamber with 0.15M NaCl. The sample port, with a capacity of 150μl, was separated from the two chambers (350ml each) by two millipore membrane discs (pore size 0.45μm). This apparatus was incorporated within a closed chamber which was connected by an inlet and an outlet port to a thermostatically controlled water heater-cum-pump. The test solutions were thus continuously surrounded by circulating water at 37°C.

The samples were prepared by dissolving 30mg dry mucus powder in 1ml 0.15M NaCl with constant stirring for 12-24 hours. One microlitre Phenyl methan sulphonyl fluorid ($C_7H_7O_2SF$) (PMSF) was added to the sample.

The test sample was placed in the sample port first. Then the NaCl and HCl solutions, prewarmed to 37°C, were poured simultaneously into their respective chambers. The solutions were continuously agitated with magnetic stirrers, and kept at a temperature of between 37 and 38 °C.

The pH of the NaCl solution was continuously monitored with an electrode connected to a Radiometer pH meter. Recordings were made of the time taken for each change of 0.1 pH units. The rate of H^+ movement into the NaCl solution was then calculated by dividing the difference in hydrogen ion concentration by the number of seconds taken for the pH to change that 0.1 unit.

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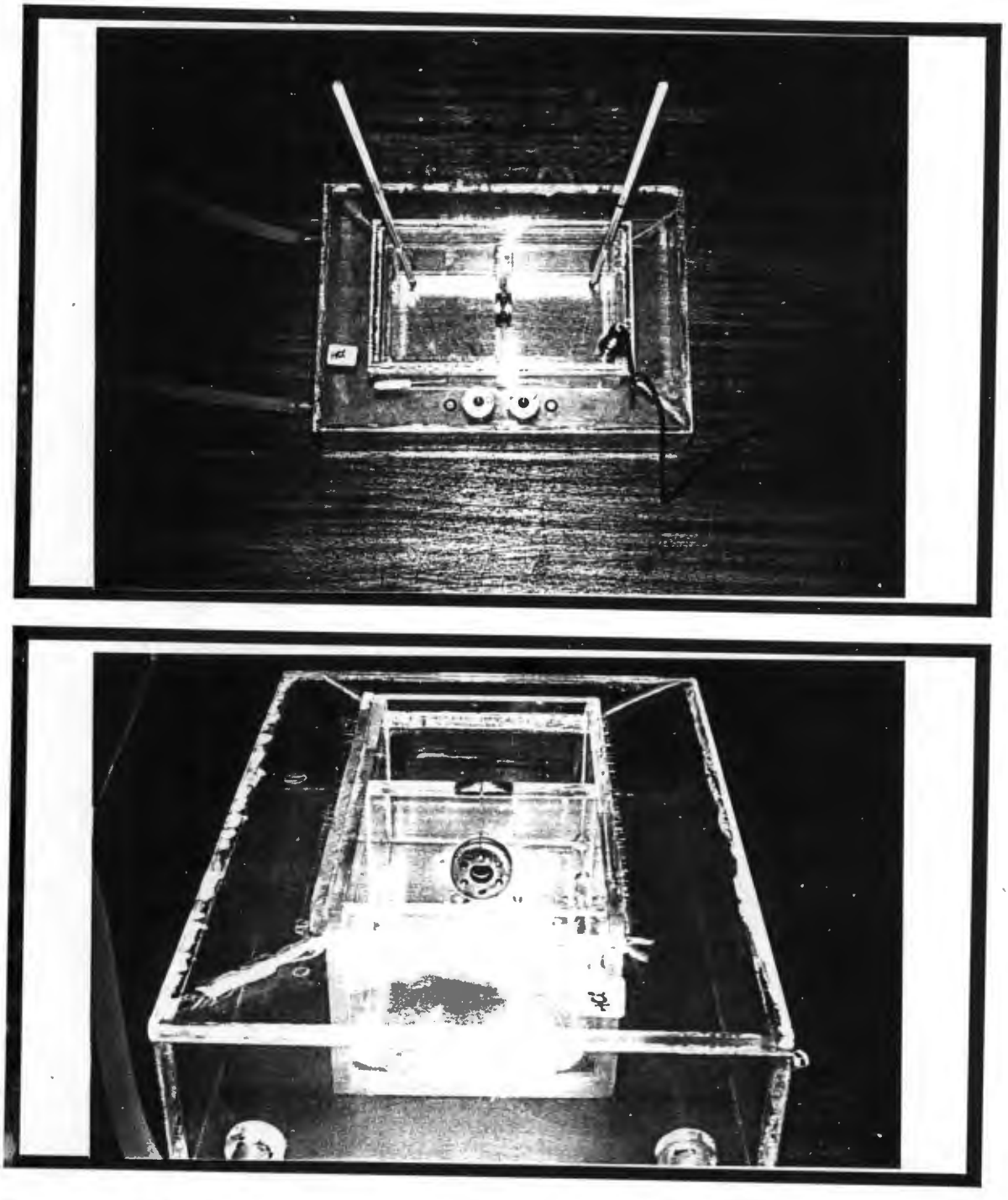
FIGURE I



Photographs of the apparatus for measurng Hydrogen ion diffusion. Two chambers are separated by a panel containing the sample port, which is filled via a small channel drilled down the centre of the panel. The solution chambers are covered with a well-fitting lid which has holes for thermometers on each side and for the pH probe on the NaCl side. The solutions are surrounded on the sides by a separate compartment through which water is continuously circulated by a thermostatically controlled heater pump. On top of the water bath compartment are the stainless steel discs and rubber O rings which hold the millipore filters across the openings of the sample port.

F. CHROMATOGRAPHIC ANALYSIS

Thirty milligrams of dry mucus powder was dissolved in 1ml 0.10M NaCl-0.05M Na_2HPO_4 buffer (pH 6.0), with 1ul PMSF added. Chromatography was done on a 100 x 1.5cm Sepharose 2B column, using the PAS method (Mantle & Allen 1978) for carbohydrate estimation and Biorad method for protein estimation of the fractions

G. PEPSIN

Gastric juice pepsin activity was assayed by the Berstad modification[102] of the method of Anson[101], which uses acidified (pH 1.8) human haemoglobin as substrate. After incubating diluted, acidified, gastric juice samples with haemoglobin for precisely 10 minutes, 0.3N trichloro-acetic acid (TCA) was added to stop the reaction (by precipitating protein). A blank was prepared in similar fashion, except that TCA was added in advance. TCA-soluble split products in the filtrate were read in a spectrophotometer at 280um against the blank and a standard made from crystalline Pepsin at a concentration of 30mg/ml.

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Thirty milligrams of dry mucus powder was dissolved in 1ml 0.10M NaCl-0.05M Na_2HPO_4 buffer (pH 6.0), with 1ul PMSF added. Chromatography was done on a 100 x 1.5cm Sepharose 2B column, using the PAS method for carbohydrate estimation and Biorad method for protein estimation of the fractions

G. PEPSIN

Gastric juice pepsin activity was assayed by the 1970 Berstad modification of the method of Anson (1938), which uses acidified (pH 1.8) human haemoglobin as substrate. After incubating diluted, acidified, gastric juice samples with haemoglobin for precisely 10 minutes, 0.3N trichloro-acetic acid (TCA) was added to stop the reaction (by precipitating protein). A blank was prepared in similar fashion, except that TCA was added in advance. TCA-soluble split products in the filtrate were read in a spectrophotometer at 280um against the blank and a standard made from crystalline Pepsin at a concentration of 30mg/ml.

V. RESULTS

A. INCIDENCE OF ULCERATION

In the 4 sham groups only two pigs (one in the Famotidine group and one in the Misoprostol group) had some macroscopically evident superficial ulceration of the pars oesophagea. Microscopic examination revealed occasional superficial ulceration or inflammation.

In the bile duct ligation groups all pigs treated with NaCl and Famotidine, and 6 out of 7 treated with Misoprostol had oesophago-gastric ulceration. These ulcers were usually deep and in many cases had caused haemorrhage. However, of those treated with Sucralfate, only two of the seven had ulceration on macroscopic observation. These observations are summarized in Table I below. Figure II depicts this graphically.

TABLE I:

NUMBER OF ANIMALS WITH GASTRO-OESOPHAGEAL ULCERS

	SHAMS				BDL			
	NaCl	SCR	Fam	Mis	NaCl	SCR	Fam	Mis
No. of pigs	7	7	7	7	7	7	7	7
Macro:ulcer	0	0	0	1	7	0	7	5
erosion	0	0	1	0	0	2	0	1
normal	7	7	6	6	0	5	0	1
Micro:ulcer	0	0	0	1	3	1	5	3
erosion	1	2	1	1	2	1	1	2
regen.	0	1	0	0	0	3	0	0
normal	4	3	4	3	0	2	0	1
inflam.	0	1	2	2	0	0	0	1

The difference in ulcer rate in the bile duct ligation peptic ulcer model animals given Sucralfate, compared with those given saline, Famotidine or Misoprostol was statistically highly significant ($p<0.01$) and provided clear evidence of a protective effect of Sucralfate.

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normal	7	7	6	6	0	4	0	1
Micro:ulcer	0	0	0	1	3	1	5	3
erosion	1	2	1	1	2	1	1	2
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normal	4	3	4	3	0	2	0	1
inflam.	0	1	2	2	0	0	0	1

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INCIDENCE OF ULCERATION
MACROSCOPIC APPEARANCE

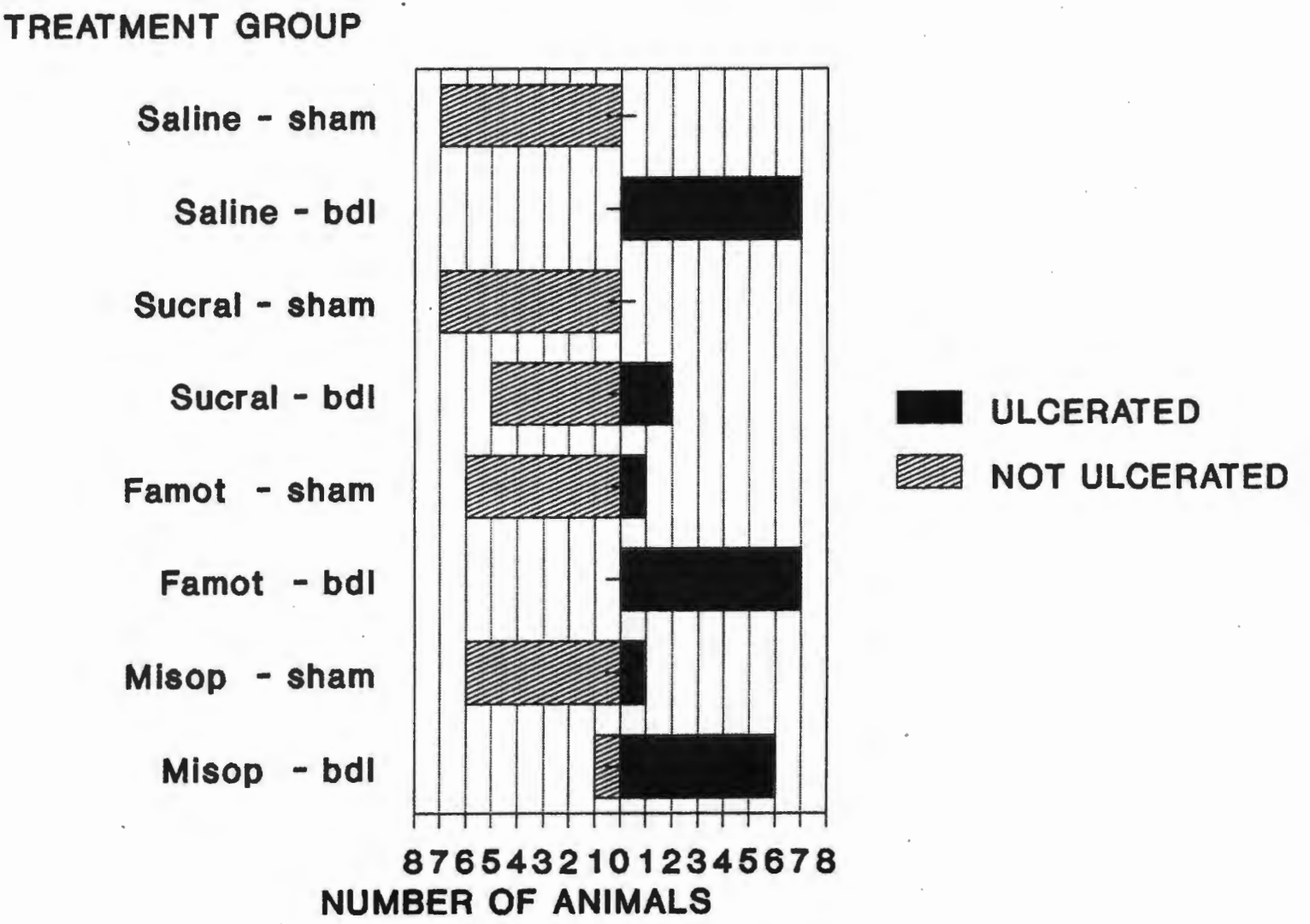

FIGURE II

The details of macroscopic appearances of the stomachs and histological reports of biopsies of the pars oesophagea of individual pigs are listed in tables II and III below.

Table II

SHAMS		
PIG	MACROSCOPIC	MICROSCOPIC
SALINE		
269	No ulcer	Not biopsied
338	No ulcer	Normal
417	No ulcer	Normal
432	No ulcer	Normal
450	No ulcer	Normal
498	No ulcer	Healing erosions of sq. epith.
543	No ulcer	Normal
SUCRALFATE		
268	No ulcer	Not biopsied
349	No ulcer	Normal
415	No ulcer	Erosions of sq. epithelium
447	No ulcer	Healing erosion & regeneration
448	No ulcer	Normal
499	No ulcer	Normal
556	No ulcer	Inflammation in sq. epith. mucosa
FAMOTIDINE		
444	No ulcer	Normal
465	No ulcer	Normal
475	No ulcer	Inflammation in sq. epith. mucosa
495	No ulcer	Focal inflammation
520	No ulcer	Normal
582	No ulcer	Normal
605	Small erosions	Erosions of sq epithelium
MISOPROSTOL		
538	No ulcer	Inflammation in sq. epith. mucosa
558	No ulcer	Normal; mild inflammation
562	No ulcer	Normal
572	No ulcer	Submucosal inflammation
575	Small ulcer	Junctional ulcer
604	No ulcer	Junctional erosions
607	No ulcer	Normal

Table III

BILE DUCT LIGATED PIGS		
PIG	MACROSCOPIC	MICROSCOPIC
SALINE		
271	Deep ulcer	Not biopsied
345	Deep ulcer	Erosions of sq. epithelium
419	Bleeding ulcer	Deep ulcer into m. propria
430	Bleeding ulcer	Erosions of sq. epithelium
452	Bleeding ulcer	Extensive ulcer into m. propria
496	Bleeding ulcer	Flat junctional ulcer
553	Bleeding ulcer	Extensive flat ulcer
SUCRALFATE		
272	Superficial erosion	Not biopsied
347	Superficial erosion	Ulcer in Sq.epith. - to submucosa
418	No ulcer	Normal
429	No ulcer	Normal
449	Erosions in body	Junctional erosions; regeneration
500	No ulcer	Regenerative activity only
550	No ulcer	Regeneration Sq. ep.; Gastritis
FAMOTIDINE		
443	Deep ulcer	Junctional ulcer into m. propria
466	Ulcerated	Flat ulcer & erosion in sq.epith.
478	Bleeding ulcer	Deep ulcer into serosal fat
494	Bleeding ulcer	Flat ulcer in sq. epithelium
521	Ulcers pars & body	Superficial erosions of body
580	Bleeding ulcer	Extensive flat ulcer sq.epith.
603	Ulcerated	Flat ulcer into submucosa
MISOPROSTOL		
548	Ulcerated	Junctional flat ulcer & erosions
559	Ulcerated	Junctional flat ulcer & erosions
563	Bleeding ulcer	Squamous epithelial erosions
569	Erosions-pars&body	Junctional submucosal abscess
576	Huge ulcer	Junctional ulcer into m.propria
606	Superficial ulcer	Squamous epithelial erosions
608	No ulcer	Normal

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B. PROSTAGLANDIN

The mean prostaglandin levels with standard deviations, standard errors of the means and 95% confidence limits for the eight treatment groups are listed in Table IV and the the relationships between mucus prostaglandin levels in the various treatment groups have been depicted in a simple bar graph, Figure III and a notched box and whiskers plot *(Statgraphics)*, Figure IIIa on the following page. Individual prostaglandin levels for each pig are shown in the Appendix.

TABLE IV:

PROSTAGLANDIN IN SCRAPED GASTRIC MUCUS (pg/ml)

		SHAMS	BDL
SALINE	**Mean**	**272.714**	**261.57**
	SD	123.347	212.15
	SEM	46.621	80.187
	95% conf	91.377	157.16
SUCRALFATE	**Mean**	**280.714**	**209.42**
	SD	82.164	127.89
	SEM	31.055	48.338
	95% conf	60.868	94.743
FAMOTIDINE	**Mean**	**214.143**	**237.14**
	SD	86.964	113.26
	SEM	32.869	42.807
	95% conf	64.424	83.902
MISOPROSTOL	**Mean**	**119.714**	**119.29**
	SD	36.468	40.165
	SEM	13.784	15.181
	95% conf	27.016	29.755

Calculated with *Statgraphics 5.0* software using the Mann-Whitney test for comparing two unpaired samples, there was no statistically significant difference in prostaglandin levels between the saline treated sham and BDL groups. There was also no significant difference between the shams given saline and those given Sucralfate, Famotidine, or Misoprostol. The animals given Misoprostol tended to have lower

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and the difference between the bile duct ligation groups given saline and Misoprostol was statistically significant with a two-tailed probability of equalling or exceeding Z = 0.0409.

FIGURE III

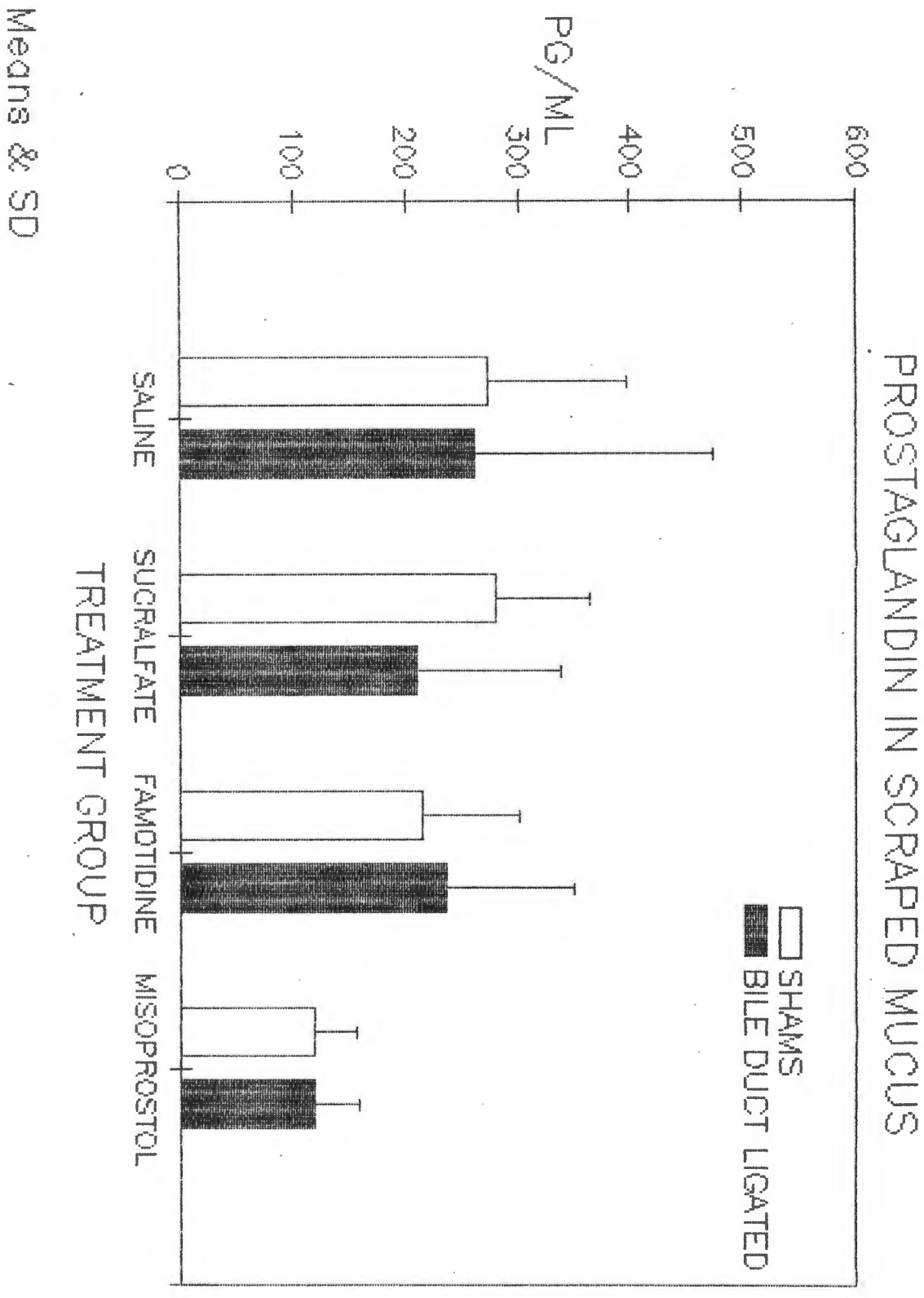
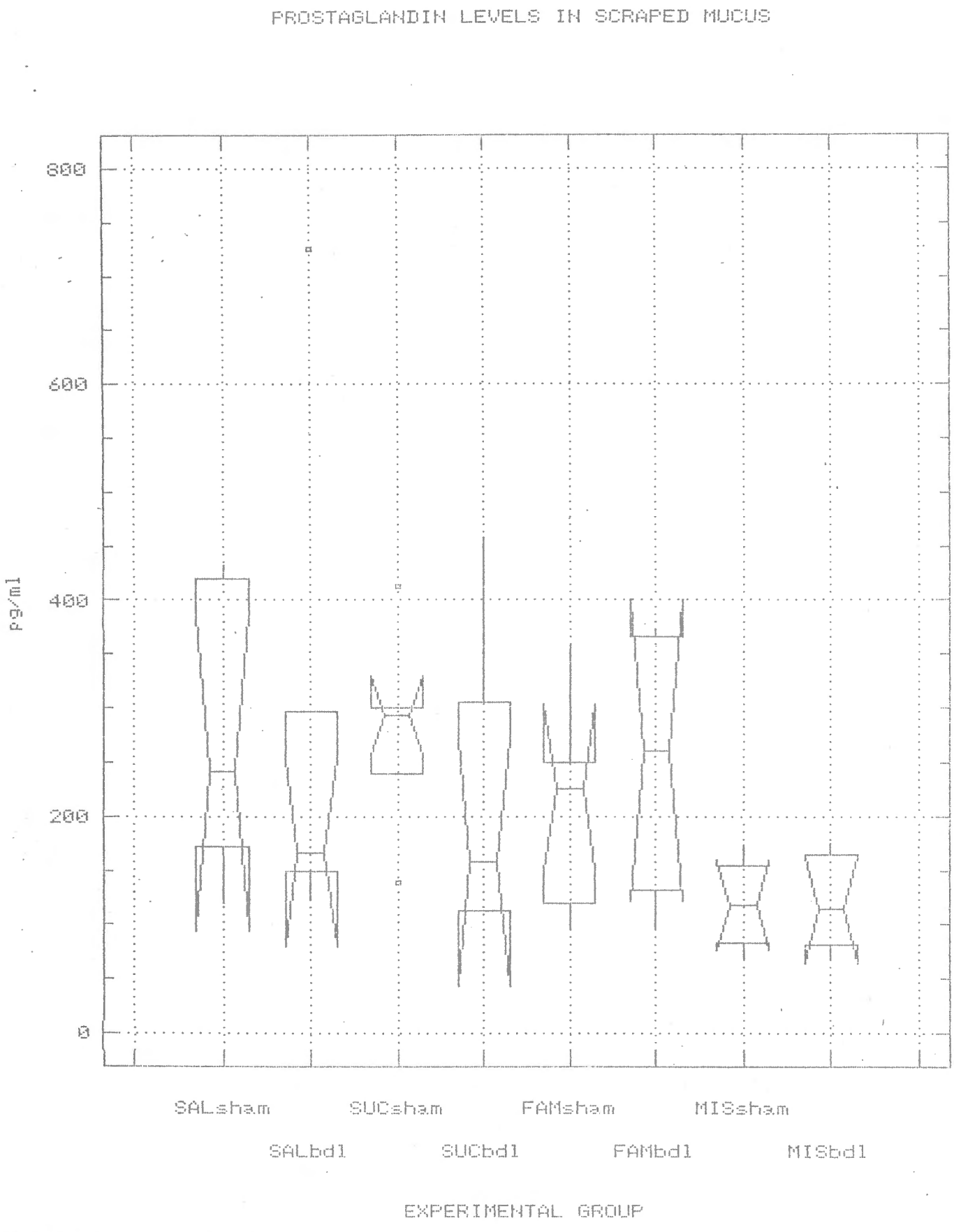
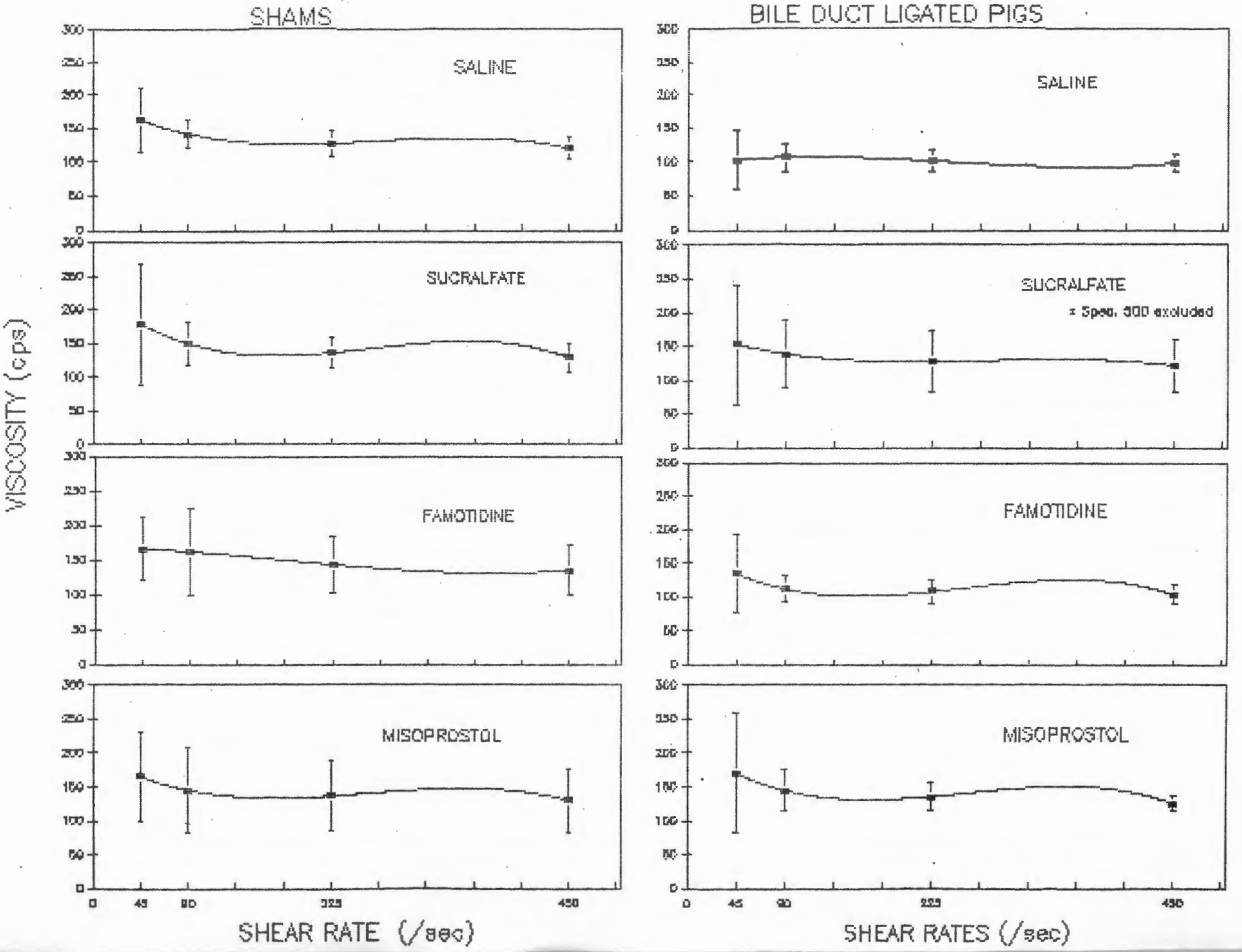


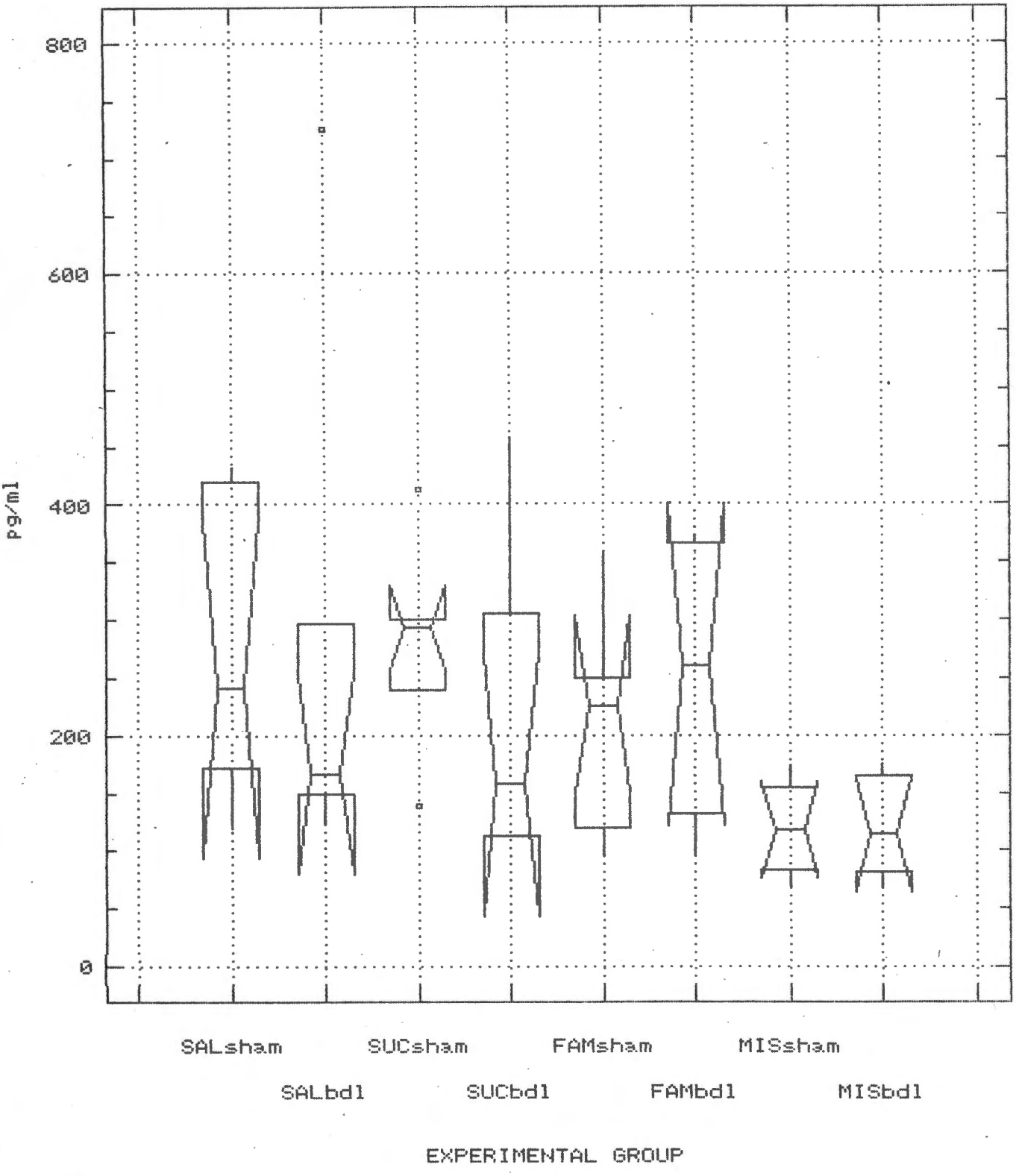
FIGURE IIIa


PROSTAGLANDIN LEVELS IN SCRAPED MUCUS
800
600
400
200
0
pg/ml
SALsham
SUCsham
FAMsham
MISsham
SALbdl
SUCbdl
FAMbdl
MISbdl
EXPERIMENTAL GROUP
Notched box & whiskers

VISCOSITY OF MUCUS



PROSTAGLANDIN LEVELS IN SCRAPED MUCUS



Notched box & whiskers

C. VISCOSITY

Five or six readings were taken on each specimen at each of the shear rates of 450, 225 and 90 per second. Viscosity readings at 45 per second were very inconsistent, so usually only 2 or three readings were taken at this shear rate. Mean values (plus SD and SEM) were calculated for each specimen at each shear rate. The total and mean of all the readings at each shear rate in each group were also calculated.

The mean viscosity was calculated for each specimen at each shear rate. The means of the means were then calculated for each shear rate in each group. These data are listed in Tables V and VI.

TABLE V

	SHAMS			
	SALINE	SUCRALFATE	FAMOTIDINE	MISOPROSTOL
450/sec				
MEAN	**120.14**	**128.05**	**135.18**	**129.51**
SD	15.78	19.89	35.42	45.35
SEM	5.96	7.52	13.39	17.14
225/sec				
MEAN	**127.58**	**135.79**	**143.2**	**136.32**
SD	18.58	23.32	39.67	51.85
SEM	7.02	8.82	14.99	19.6
90/sec				
MEAN	**141.71**	**148.85**	**161.81**	**144.92**
SD	19.4	31.77	62.1	62.6
SEM	7.33	12.01	23.47	23.66
45/sec				
MEAN	**162.4**	**178.19**	**166.38**	**164.85**
SD	47.49	89.27	46.44	65.13
SEM	17.95	36.45	17.55	24.62

TABLE VI

BILE DUCT LIGATED PIGS				
	SALINE	SUCRALFATE	FAMOTIDINE	MISOPROSTOL
450/sec				
MEAN	**97.98**	**170.7(121.3)**	**103.19**	**125.76**
SD	11.78	136.6(39.17)	13.74	11.62
SEM	4.45	51.64(15.98)	5.19	4.39
225/sec				
MEAN	**101.37**	**183.1(127.4)**	**107.34**	**135.67**
SD	15.1	153.1(45.35)	18.08	19.54
SEM	5.71	57.88(18.52)	6.83	7.39
90/sec				
MEAN	**106.86**	**200.95(138.67)**	**111.61**	**145.03**
SD	20.18	171.25(51.04)	19.59	30.66
SEM	7.63	64.73(20.84)	7.4	11.59
45/sec				
MEAN	**102.8**	**264.18(152.2)**	**134.07**	**170.41**
SD	42.49	253.5(89.13)	57.93	88.01
SEM	21.25	117.74(51.46)	23..65	33.27

There was a statistically significant difference (Mann-Whitney) between the viscosity of mucus from saline-treated shams and saline-treated bile duct ligation animals (two-tailed probability of equalling or exceeding $Z = 0.0298$). The mean viscosity of the Sucralfate-treated bile duct ligated pigs was distorted by the mucus of one pig, No. 500, which had very high viscosity readings at all shear rates. On sacrificing this pig it was noted that the bulb of the Foley catheter had obstructed the pylorus and the whole stomach was lined by a white film (presumably Sucralfate, on the surface of, or mixed with the mucus). In the table above, therefore, calculations excluding the readings from this pig are listed in parenthesis. However there was no statistically significant difference between the treatment groups and the saline controls in the sham operated animals, or between Sucralfate and Famotidine bile duct ligation

groups and the saline control bile duct ligation group. Misoprostol, however, did enhance the viscosity of the mucus with respect to the saline controls in the peptic ulcer model ($p = 0.0106$).

FIGURE IV

See overleaf

D. HYDROGEN ION DIFFUSION

The data from the hydrogen ion diffusion experiments is depicted in Figures V and VI.

The graphs were plotted using Sigmaplot version 3.1 software. Rate of Hydrogen ion diffusion (permeability) has been plotted against time in seconds. The dotted lines depict 5th order regression curves of the data from mucus samples from each pig in a particular group. The solid line is a 5th order regression curve of the graph as a whole (i.e. the mean of the seven curves in each graph).

With 0.155M NaCl in the sample port, the permeability curve has an initial fairly steep slope followed by a plateau phase for about an hour and then a steep slope again. With reconstituted mucus samples in the sample port the level of the plateau phase was of a similar magnitude, but was prolonged.

There was very little difference between the shams and bile duct ligated pigs given saline, and very little difference between any of the sham treatment groups. However, it appeared that the rate of H^+ diffusion was, if anything, slower in the bile duct ligated pigs treated with Famotidine, Sucralfate and Misoprostol.

HYDROGEN ION DIFFUSION

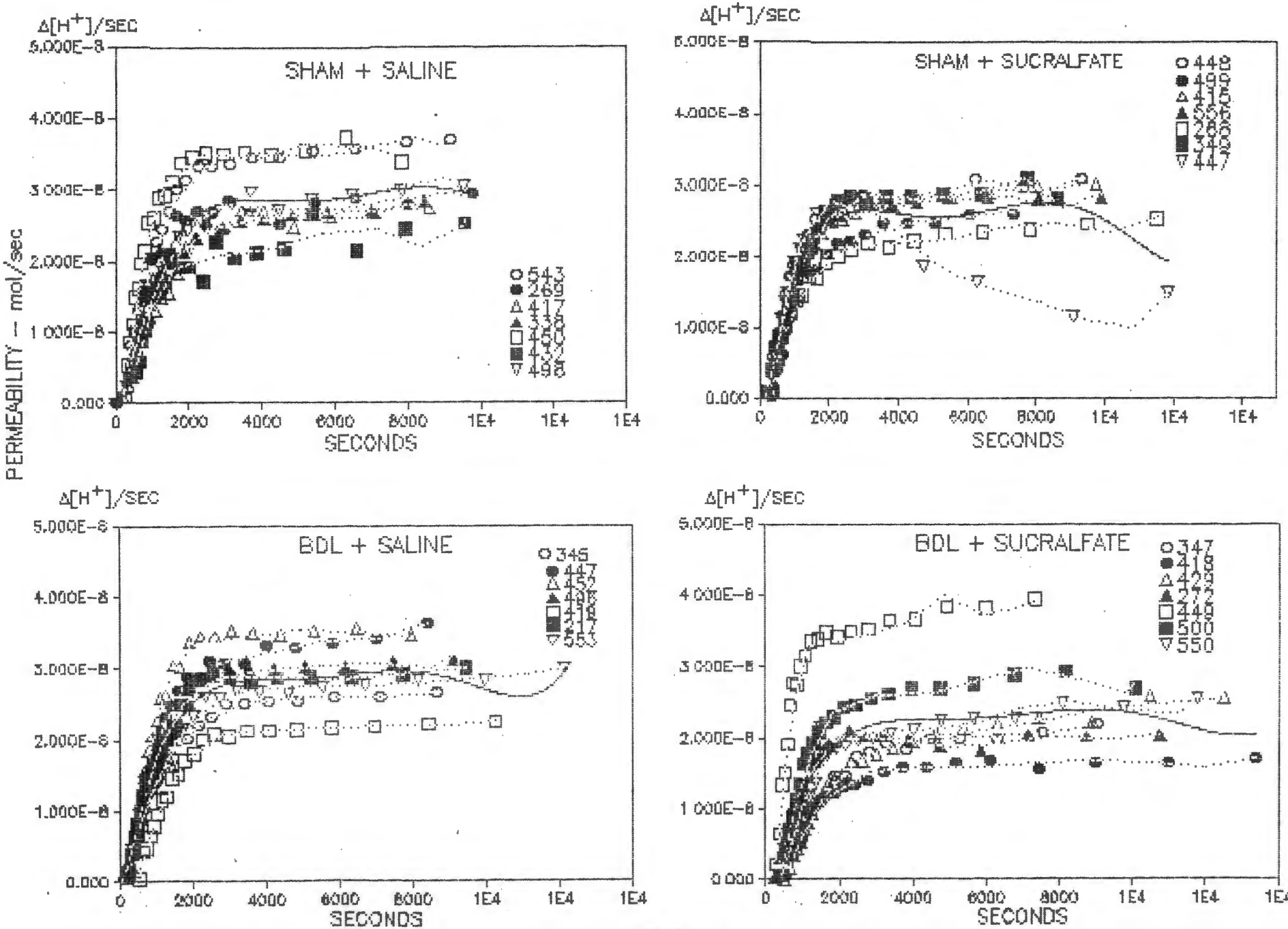


FIGURE V

HYDROGEN ION DIFFUSION

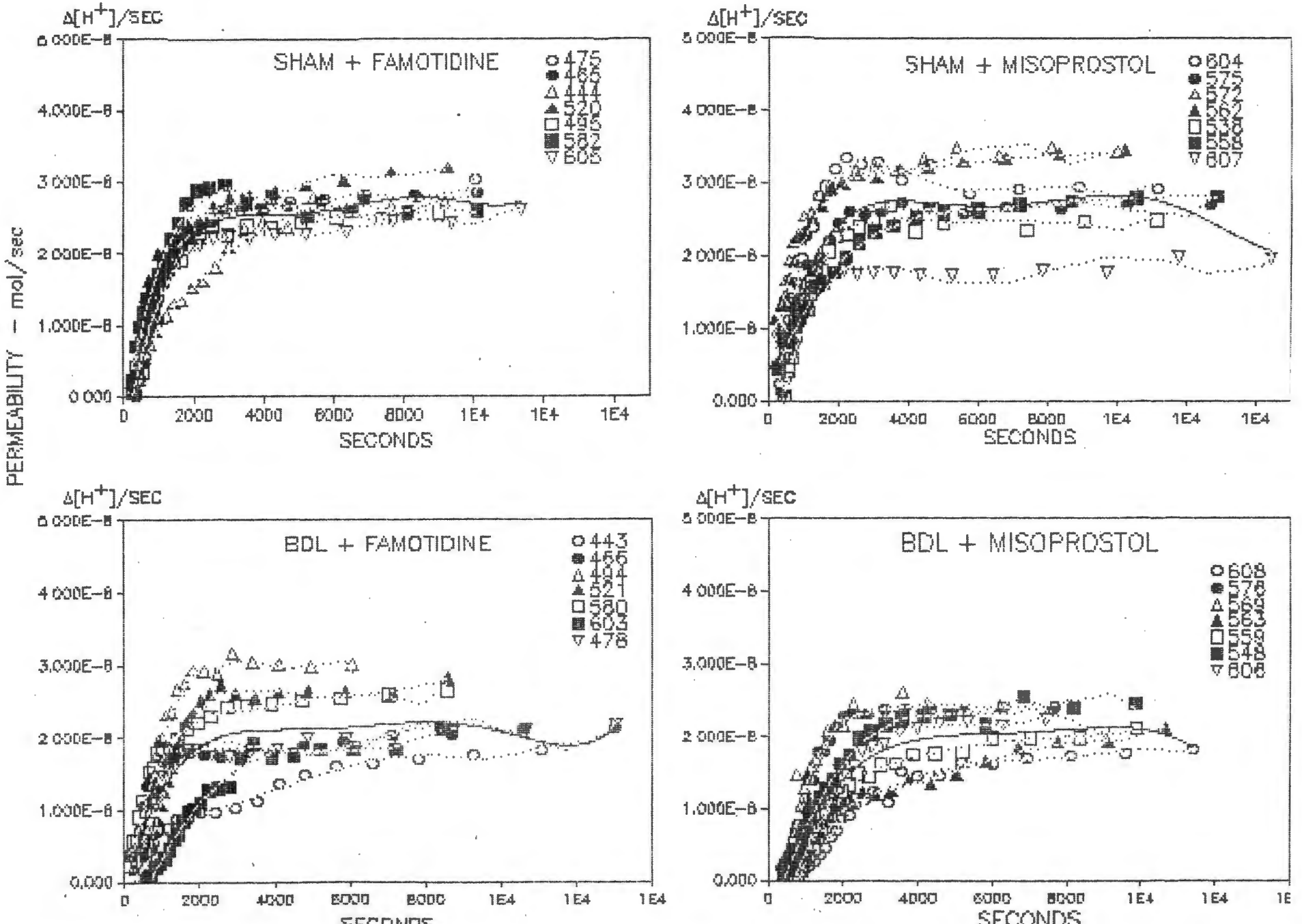


FIGURE VI

E. CHROMATOGRAPHY

On Sepharose 2B gel chromatography, protein (measured by Biorad kit) eluted as two well defined peaks, an excluded peak (V_o) and an included peak (V_i). The former represents mainly intact glycoprotein whilst the latter consists of low molecular weight glycoprotein and protein (e.g. albumin, IgA etc.) The carbohydrate in the eluted fraction was detected by the PAS method (Mantle & Allen 1978) and, as above, the crude mucus eluted as two well defined peaks. The included peak was the result of interference from the high protein content

The mean percentages and standard deviations in the V_o fractions by the PAS and Biorad methods are listed in Table VII and depicted graphically in the bar chart in figure VI. Individual values can be found in the appendix

TABLE VII

		SHAMS		BDL	
		PAS	BIORAD	PAS	BIORAD
SALINE	**Mean**	**37.86**	**38.86**	**23.86**	**24.29**
	SD	11.91	14	3.53	6.99
	SEM	4.5	5.29	1.34	2.64
SUCRALFATE	**Mean**	**39.41**	**45.71**	**28.71**	**36.29**
	SD	15.64	19.37	10.66	20.72
	SEM	5.91	7.32	4.03	7.83
FAMOTIDINE	**Mean**	**24.43**	**33.14**	**22.43**	**22.14**
	SD	3.46	17.34	3.41	9.81
	SEM	1.31	6.56	1.29	3.71
MISOPROSTOL	**Mean**	**26.71**	**19.86**	**22.43**	**18.43**
	SD	2.06	8.36	3.6	5.68
	SEM	0.78	3.16	1.36	2.15

Calculated with Statgraghics version 5.0, using the Mann-Whitney test for comparison of unpaired samples, there was a significantly lower percentage, with both

PAS and Biorad methods, of V_o (intact mucus glycoprotein) in the bile duct ligation control group than in the sham operated controls. The 2-tailed probability of equalling or exceeding Z was 0.0298 for the PAS data and 0.047 for the Biorad data.

In the sham groups, those treated with Misoprostol had significantly lower percentages of Vo (PAS and Biorad) than the saline controls. The famotidine treated animals also tended to have lower percentages of Vo than controls and Sucralfate treated animals and the difference with the PAS data was statistically significant.

CHROMATOGRAPHY — % INTACT GLYCOPROTEIN

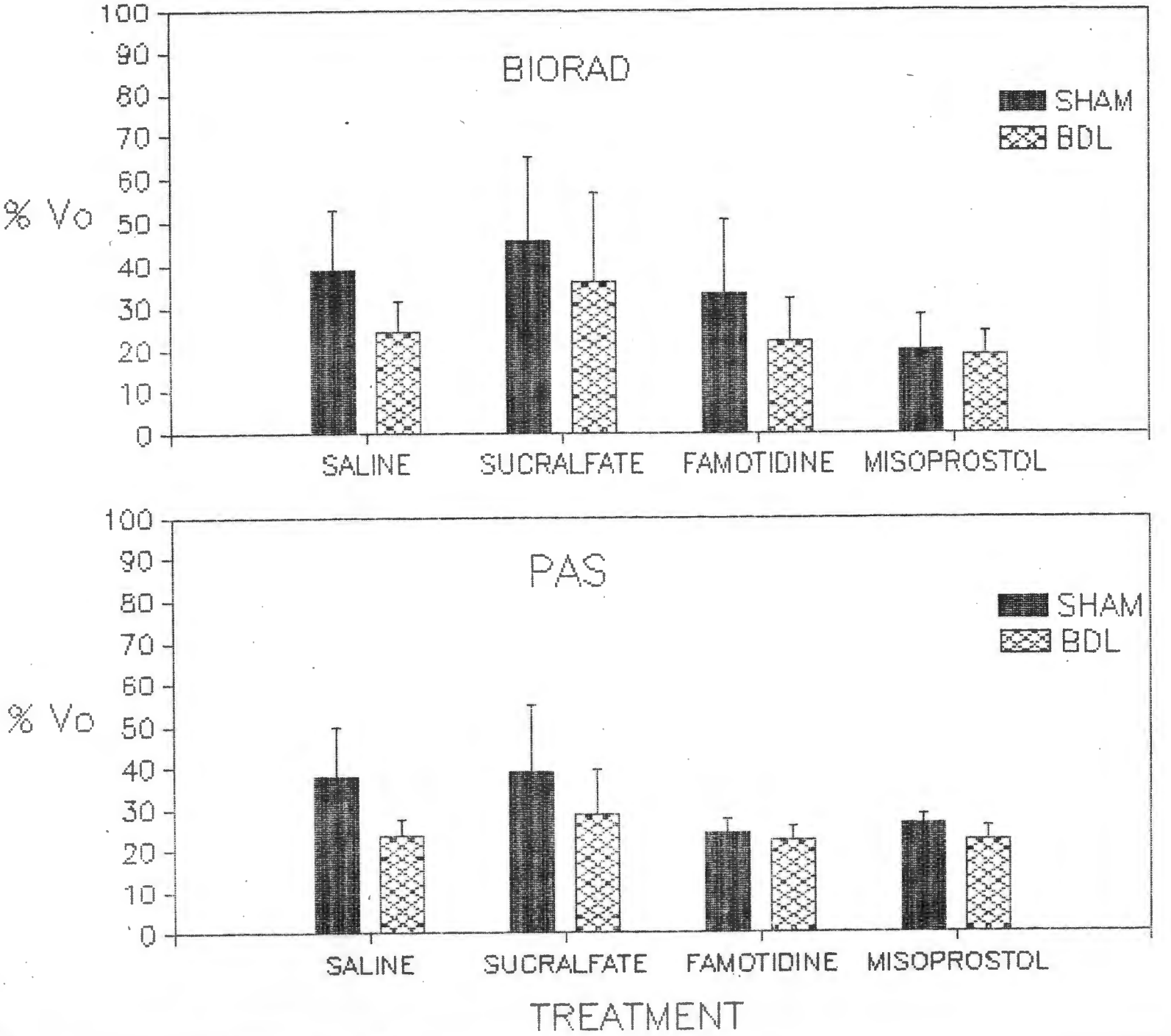


FIGURE VII

F. PEPSIN AND PH OF GASTRIC JUICE

The pH of the gastric juice was measured at the initial gastrotomy, before any treatment, in 43 pigs. The **mean pH was 3.55** (range 6.84 - 1) with standard deviation (SD)= 1.564 and standard error of the mean (SEM)= 0.238.

Gastric juice pepsin activity was measured in 37 pigs before treatment. The **mean pepsin was 61.9 mg%** (5-138) with SD = 31.7 and SEM = 5.2.

Mean pepsin and pH values of gastric juice at the time of sacrifice after treatment are displayed in Tables VIII and IX and depicted graphically in Figure VIII

TABLE VIII

SHAMS								
	NaCl		SCR		FAM		MIS	
	pH	Peps	pH	Peps	pH	Peps	pH	Peps
Number	5	5	5	7	7	7	7	
Mean	3.8	64.1	3.47	43.6	4.32	54.9	3.25	37
SD	.84	31	1.7	21.5	1.06	36.3	1.6	18.4
SEM	.38	13.9	.76	9.6	.4	13.7	.6	6.9
95% conf	.74	27.1	1.49	18.8	.78	26.9	1.18	13.6
99% conf	.97	35.7	1.96	24.8	1.03	35.4	1.56	18

There appeared to be some reduction in pepsin in the shams after treatment with each of the three drugs, but the differences were not statistically significant. Bile duct ligation, on the other hand, tended to cause an increase in gastric juice pepsin, but this was also not statistically significant.

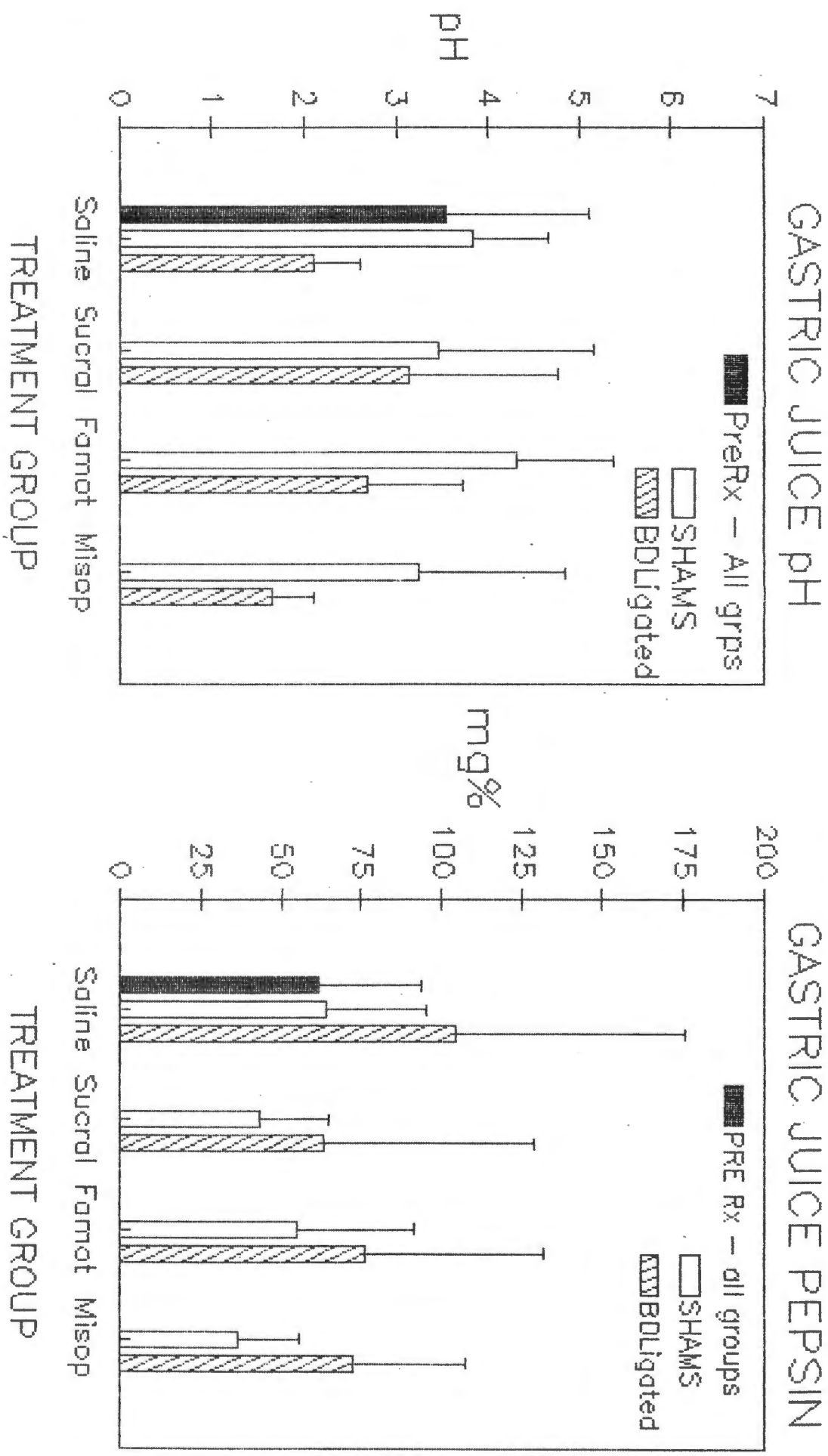
Gastric juice pH did not change after treatment with Sucralfate or Misoprostol but did increase a little after treatment with Famotidine. After bile duct ligation there was a decrease in gastric juice pH of saline controls compared with pre-treatment pH,

which approached statistical significance. (Mann-Whitney test for comparing two unpaired samples; *Statgraphics version 5.0*)

TABLE IX

BDL								
	NaCl		SCR		FAM		MIS	
	pH	Peps	pH	Peps	pH	Peps	pH	Peps
Number	4	4	5	5	7	7	7	7
Mean	**2.11**	**104.2**	**3.16**	**63.0**	**2.7**	**76.2**	**1.66**	**72.1**
SD	0.5	71.5	1.61	65.4	1.04	55.2	.44	35.3
SE	0.25	35.8	.72	29.3	.39	20.8	.17	13.3
95% conf	0.49	70.1	1.41	57.4	.77	40.9	.33	26.2
99% conf	0.64	92.3	1.86	75.5	1.02	53.8	.43	34.4

FIGURE VIII


GASTRIC JUICE pH
pH
0
1
2
3
4
5
6
7
PreRx – All grps
SHAMS
BOLigated
Saline
Sucral
Famot
Misop
TREATMENT GROUP
GASTRIC JUICE PEPSIN
mg%
0
25
50
75
100
125
150
175
200
PRE Rx – all groups
SHAMS
BOLigated
Saline
Sucral
Famot
Misop
TREATMENT GROUP

G. BACTERIOLOGY

From Tables X & XI, it is clear that the stomach of the pig is usually colonized with a variety of organisms, the most frequently isolated in this study being Escherichia coli (E.coli), Enterococci and Diphtheroids. Yeasts were also often cultured. These cultures were of swabs taken of the stomach at gastrotomy and no nasogastric tube had been inserted prior to surgery. Of 48 stomachs swabbed at the initial gastrotomy, 19 yielded E. coli, 7 Diphtheroids, 6 Staphylococcus epidermidis, 8 Enterococcus, 4 Candida albicans and 5 "other yeasts". Usually the growth was light, but occasionally there was a moderate or heavy growth. *Helicobacter pylori* was not cultured in any pig stomach in this series.

Cultures of swabs taken at the time of sacrifice are obviously bedevilled by contamination, as the gastrostomy had been present for 48 hours. However, it is still valid to make comparisons of different treatments in the sham and bile duct ligated groups and comparison of sham with bile duct ligation for each treatment, assuming that the potential for contamination was the same in all groups.

There was virtually no difference in the cultures of those pigs having sham or bile duct ligation when treated with saline, except that two of the shams had moderate growths and one had a heavy growth, whereas only one bile duct ligated pig had a moderate growth and all others were light growths.

Of 5 shams and 5 BDLs treated with Sucralfate and cultured, there were 4 organisms cultured in the BDL pigs and 6 in the shams; there were 3 moderate growths and no heavy growths in the BDL pigs, and among the positive cultures from the shams there was one heavy growth and the other 5 were light.

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TABLE X

SHAM OPERATED PIGS		
	GASTROTOMY	SACRIFICE
SALINE		
269	Not cultured	Not cultured
338	Not cultured	Not cultured
417	No growth	2+ E.coli; 2+ Enterobacter; 2+ yeasts
432	2+Candida; 1+Bacillus	1+ Candida; 1+ Enterobacter sp.
450	No growth	2+ E.coli; 1+Diphtheroids
498	No growth	3+ Non-fermenting Gram neg bac;1+ E.coli; 1+ß-haem.Strep(grpA)
543	No growth	2+ mixed organisms
SUCRALFATE		
268	Not cultured	Not cultured
349	Not cultured	Not cultured
415	1+ Yeasts	1+ Yeasts
447	2+ E.coli; 1+Enterococ	3+ Enterobacter sp.
448	1+ Enterococcus	1+ Staph. epidermidis
499	1+ Yeasts	3+ Non-fermenting Gram neg bac; 1+E.coli; 1+ ß-haem.Strep(grpA)
556	No growth	2+ mixed orgs
FAMOTIDINE		
444	1+ E.coli; 1+ Enterococ	2+ Klebsiella sp.
465	No growth	1+ Citrobacter diversus; 2+ Diphtheroids
475	1+ Diphtheroids	1+ Klebsiella; 1+ Candida
495	1+ E.coli	2+ E.coli; 2+ α-haemolytic Strep; 1+ Yeasts (not C. Albicans)
520	1+ Staph. epiderm.; 1+ Bacillus sp.	1+ Staph. aureus; 1+ E.coli; 1+Enterobacter sp. 1+ ß-haem. Strep.(not grp. A)
582	Yeasts (not C.alb)	3+ Klebsiella sp.; 3+ E.coli
605	1+ Bacillus sp.; 1+ Proteus mirab; 1+ α-haem. Strep	1+ Klebsiella sp.; 1+ E.coli; 1+Enterococci; 1+ Proteus mirabilis
MISOPROSTOL		
538	1+ E.coli	3+E.coli; 3+ Enterobacter sp.; Yeast; 1+ ß-haem. Strep.(grp.G)
558	1+ Staph. epidermidis	3+ E.coli; 3+ Enterobacter sp.;3+ Pseudomonas aeruginosa
562	1+ Klebsiella sp.	3+ E.coli; 1+ Enterobacter sp.; 1+ ß-haem. Strep. (grp. C)
572	1+ E.coli	1+ E.coli; 1+ Acinetobacter sp.; 1+ Staph. aureus
575	No growth	1+ E.coli; 2+ Micrococcus sp.
604	1+ Candida albicans 1+ Diphtheroids 1+ Staph. aureus	1+ Candida albicans
607	3+ E.coli; 1+Diphther 1+ Staph. epiderm.;	2+ E.coli; 2+ Klebsiella sp.

Swabs were taken from the stomachs of all the pigs treated with Famotidine and Misoprostol. From the pigs treated with Famotidine, there was a total of 15 isolates in the bile duct ligation group and 18 in the sham group. In the bile duct ligation group only one growth was heavy and one moderate, whereas in the sham group five were moderate and two heavy. From the pigs treated with Misoprostol, there was a total of 21 isolates in the bile duct ligation group and 18 in the sham group. However, all the growths in the bile duct ligation group were light, whereas in the shams there were 6 heavy and 3 moderate growths.

TABLE XI

BILE DUCT LIGATED PIGS		
	GASTROTOMY	SACRIFICE
SALINE		
271	Not cultured	Not cultured
345	Not cultured	Not cultured
419	1+ E.coli	2+ Enterobacter sp.
430	2+Diphtheroids; 1+Enterococci; 1+E.coli 1+ Staph. aureus	1+ Candida albicans
452	3+ E.coli; 1+ Enterococ	No growth
496	No growth	1+ E.coli; 1+ α-haem. Strep.; 1+ yeasts (not C. albicans); 2+ Non-fermenting Gram neg bac
553	1+E.coli; 1+Klebsiella sp.	1+ E.coli
SUCRALFATE		
272	Not cultured	Not cultured
347	Not cultured	Not cultured
418	1+ Diphtheroids	2+ E.coli
429	1+ Bacillus sp.	2+ Clostridium sp.
449	No growth	No growth
500	1+ Enterococ; 1+yeasts	2+ E.coli; 1+ α-haem. Strep.
550	1+ Staph.epiderm.; 1+ Enterobacter sp 1+ E.coli; 1+ Proteus	2+ mixed organisms

All the pigs treated with Famotidine and Misoprostol had swabs taken. From the pigs treated with Famotidine, there was a total of 15 isolates in the bile duct ligation group and 18 in the sham group. In the bile duct ligation group only one growth was heavy and one moderate, whereas in the sham group five were moderate and two heavy. From the pigs treated with Misoprostol, there was a total of 21 isolates in the bile duct ligation group and 18 in the sham group. However, all the growths in the bile duct ligation group were light, whereas in the shams there were 6 heavy and 3 moderate growths.

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452	3+ E.coli; 1+ Enterococ	No growth
496	No growth	1+ E.coli; 1+ α-haem. Strep.; 1+ yeasts (not C. albicans); 2+ Non-fermenting Gram neg bac
553	1+E.coli; 1+Klebsiella sp.	1+ E.coli
SUCRALFATE		
272	Not cultured	Not cultured
347	Not cultured	Not cultured
418	1+ Diphtheroids	2+ E.coli
429	1+ Bacillus sp.	2+ Clostridium sp.
449	No growth	No growth
500	1+ Enterococ; 1+yeasts	2+ E.coli; 1+ α-haem. Strep.
550	1+ Staph.epiderm.; 1+ Enterobacter sp 1+ E.coli; 1+ Proteus	2+ mixed organisms

FAMOTIDINE		
443	2+ E.coli	1+ Klebsiella; 1+ Enterobacter sp.
466	1+ E.coli; 1+Enterococ	1+ Citrobacter freundii; 1+ Diphtheroids
478	1+ E.coli; 1+Enterococ	1+ E.coli; 1+ Proteus sp.; 1+ Bacillus sp.; 1+ Enterococci; 1+Diphtheroids
494	1+ E.coli; 1+ Yeast	3+ E.coli; 2+ Enterococci
521	No growth	1+ ß-haem. Strep. (not grp. A)
580	1+ Strep.mit.; 1+E.coli;	1+ E.coli; 1+ grp.A ß-haem. Strep
603	1+Diphther;1+E.coli; 1+ Candida albicans	1+ E.coli
MISOPROSTOL		
548	1+ Staph. epiderm.; 1+ Candida albicans	1+ Staph. aureus; 1+ E.coli; 1+ Enterococci; 1+ Enterobacter
559	1+ Staph. epiderm.;	1+ ß-haem. Strep. (grp A); 1+ Enterococcus; 1+ E.coli; 1+Enterobacter sp.
563	1+ E.coli	1+ Staph.aureus; 1+ Enterobacter; 1+ α-Haemolytic Strep.
569	No growth	1+ Staph. aureus; 1+ E.coli
576	No growth	1+ E.coli
606	1+ Diphtheroids	1+ Staph. aureus; 1+ E.coli; 1+ Clostridium; 1+ Acinetobacter; 1+ß-haem. Strep
608	1+ E.coli; 2+ anaerobic G+ bac.	1+ E.coli; 1+ ß-haem. Strep

TABLE XII

SUMMARY OF CULTURES AT SACRIFICE

SHAMS				
	SALINE	SCR	FAM	MIS
No. pigs cultured	5	5	7	7
Total no. isolates	11	7	18	18
Heavy growths	1	1	2	3
Moderate growths	3	0	5	6
BILE DUCT LIGATED PIGS				
	SALINE	SUC	FAM	MIS
No.pigs cultured	5	5	7	7
Total no. isolates	8	5	15	21
Heavy growths	0	0	1	0
Moderate growths	2	3	1	0

V. DISCUSSION

A. THE BILE DUCT LIGATED PIG MODEL

It is acknowledged that the bile duct ligated pig is not an ideal model of chronic peptic ulceration, the ulcer being located at the oesophago-gastric junction in squamous epithelium, which is clearly different from the human situation. However, no ideal model of human peptic ulcer disease has yet been found and, with the possible exception of the cysteamine model, all other models have little similarity to the naturally occurring chronic peptic ulcer diseases of humans and are useful only in trying to dissect isolated aspects of the pathogenesis to attempt to understand the pathophysiology in its entirity. Advantages of this pig model are that (a) it is a naturally occurring phenomenon of economic significance to the pig farming industry, that can be reproduced with 100% reliability by ligating the common bile duct; (b) it is associated with gastric hyperacidity which appears to be hormonally mediated by either an endocrine or paracrine means, with additional vagal influence; (c) the gastric mucus of pigs is similar to that of humans; and (d) the volume of mucus that can be retrieved from a pig stomach allows individual analysis of mucus of experimental animals rather than pooling mucus from several animals which would be necessary if other (smaller) models were used. Furthermore, as this study set out to verify the claimed *in vitro* effects of Sucralfate on pig gastric mucus, it is appropriate to use a pig model for the *in vivo* study.

B. THE METHOD OF MUCUS COLLECTION

Acknowledged investigators in the field of mucus research disagree on the optimal method of mucus collection for scientific analysis. The majority hold the

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B. THE METHOD OF MUCUS COLLECTION

Acknowledged investigators in the field of mucus research disagree on the optimal method of mucus collection for scientific analysis. The majority hold the

opinion that the surface mucus is the relevant mucus and that the mucus glycoprotein is exclusively responsible for the physico-chemical propertes of mucus. This school believes, therefore, that only the purified mucus glycoprotein that has been extracted from surface mucus scraped off the epithelium, should be studied. The Slomiany group have a different philosophy, based on their data which suggest that intracellular mucus, which differs from surface mucus particularly in its lipid content, also contributes to the gastric mucosal barrier, and the protein, lipid and other glycoproteins associated with the mucus glycoprotein do contribute to the physico-chemical properties of mucus.

The method of instilling hypertonic saline into the stomach followed by filtration and dialysis is employed by Slomiany group to collect both surface and intracellular mucus. The possibility that the high salt concentration may diminish entanglement of mucin molecules is clearly an important objection to this method of collection. The other major criticism of the Slomiany group's methods is that they do not purify the mucus glycoprotein because of their belief that mucus should be studied as a whole rather than being dissected into components.

In this study the methods of the Slomiany group have been followed because the objective was to verify their *in vitro* experiments with an *in vivo* model. The study was designed to include several controls in the form of sham-operated animals and saline treated animals, so that, despite the reservations, the comparisons between various treatment groups were felt to be valid

C. THE EFFECTS OF SUCRALFATE ON GASTRIC MUCUS

1.PREVENTION / HEALING OF ULCERATION AT THE PARS OESOPHAGEA

The most striking, and obviously significant, observation made in this study was the beneficial effect of Sucralfate in preventing the ulcers, which hitherto had only been prevented by highly selective vagotomy. Because of the study design of sacrificing after 48 hours, it is not possible to comment on whether Sucralfate prevents oesophago-gastric ulcer formation or whether it allows rapid early restitution of the mucosa after ulceration commences. However, it is in line with the clinical experience with stress ulceration, and certainly suggests a more complex mechanism of action than simply acting as a physical barrier in an ulcer crater. A subsequent study in our laboratory has shown prevention of ulceration after seven days.

2. EFFECTS ON MUCOSAL PROSTAGLANDIN

Hollander *et al* suggested that the mechanism by which Sucralfate heals and prevents ulcers may be by stimulating prostaglandin release[77].

Prostaglandins are thought to enhance mucosal defences by stimulating bicarbonate secretion, increasing mucus secretion, improving mucosal blood supply and accelerating surface epithelial restitution. Prostaglandin analogues, such as Misoprostol, also inhibit acid secretion.

Prostaglandins are ubiquitous molecules which are notoriously difficult to study because they are released after any injury to cell membranes. This is why many deductions about prostaglandins, including those of Hollander *et al* in this context, are inferred from indirect circumstantial evidence where a known effect is prevented by a

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drug (e.g. Indomethacin) which is known to inhibit prostaglandin. It is well known, of course, that anti-prostaglandin drugs readily produce gastric ulceration themselves, so inferrential deductions such as these with respect to the mechanism of action of Sucralfate, must be viewed with circumspection.

As an adjunct to studying the effects of Sucralfate on mucus it was decided to study prostaglandin E in scraped mucus to see if there were any differences between the various treatment groups.

There was a very wide scatter of prostaglandin levels. There was no significant difference detected between the saline controls having sham operations or bile duct ligation, which suggested that Prostaglandin secretion/release was not an important response to injury in this peptic ulcer model. It was interesting that endogenous prostaglandin appeared to be suppressed by Misoprostol. There was possibly a trend to slightly lower prostaglandin levels in the bile duct ligation groups treated with saline and Sucralfate and certainly no stimulation of prostaglandin by Sucralfate

While the study design allowed for comparison between different groups, the method of collection of material for assaying prostaglandin remains a difficult problem. Simply handling the stomach in the process of performing the gastrectomy may be enough to release prostaglandins and certainly distending the stomach with hypertonic saline and then scraping residual mucus off the epithelium is a major insult to the epithelial cells. Whether surface mucus is the best place to measure prostaglandin is also questionable, but taking epithelial biopsies for measuring prostaglandin, produces similar cell trauma and is therefore open to the same criticism

EFFECTS ON MUCUS VISCOSITY

Viscosity is the measure of the internal friction of a fluid. This friction is apparent when a layer of fluid is made to move in relation to another. The force required to cause this movement is called **shear** and **shear stress** is the force per unit area (dynes/cm^2). **Shear rate** is the speed at which intermediate layers of fluid move with respect to each other and the unit of measurement is the "reciprocal second" (sec^{-1}). Viscosity is calculated by dividing shear stress by shear rate and the fundamental unit is **poise**. (1 poise or 100 centipoise (cps) = 1 dyne.sec/cm^2 which is equivalent to 10 Pascal-seconds)

Newtonian fluids have a viscosity, at a given temperature, which is independent of shear rate, the relationship between shear stress and shear rate being a straight line. However most fluids are non-Newtonian and viscosity changes as the shear rate is varied. Therefore the viscometer model, spindle and speed all have an effect on the measured viscosity. Non-Newtonian fluids may exhibit one of several types of flow behaviour viz pseudoplastic, dilatant or plastic. Pseudoplastic behaviour is decreasing viscosity with increasing shear rate, whereas dilatant behaviour is increasing viscosity with increasing shear rate. Plastic flow implies that the fluid requires a certain force to be applied before any flow is induced and once this yield force is exceeded and flow begins, the fluid may exhibit Newtonian, pseudoplastic or dilatant flow characteristics. Thixotropic and rheopectic behaviour refers to changes in behavior with passage of time, the former being a decrease in viscosity with time (at constant shear rate) and the latter an increase with time.

Viscosity implies the existence of laminar flow, the movement of one layer of fluid past another with no transfer of matter from one to the other. Beyond a certain maximum speed, transfer of mass occurs and flow becomes turbulent, resulting in erroneously high viscosity readings. The point at which turbulence occurs depends on

the velocity at which the layers move, the viscosity and specific gravity of the material and the geometry of the spindle and sample container of the viscometer.

Factors which will affect the rheological properties of fluids are the temperature, shear rate (with non-Newtonian fluids), measuring conditions, time (with thixotropic and rheopectic fluids), previous treatment of the fluid (eg. pouring, stirring, mixing etc.), composition and additives and the state of aggregation and shape of particles in emulsions and dispersions. Measuring conditions which may influence viscosity readings are the viscometer model, the spindle/speed combination, the sample container size, the sample temperature and preparation technique, the ambient atmosphere and the homogeneity of the sample.

It is clear from the foregoing discussion that measurement of viscosity requires very carefully controlled conditions. The many intermediary steps used to prepare the reconstituted mucus samples may have compromised the accurate measurement of viscosity.

Despite the fact that mucus was collected and reconstituted by the same methods described by the Slomiany group, the readings obtained with the type of cone used by these investigators were excessively high so the viscometer had to be fitted with a different cone and the plate was reground accordingly.

The mean viscosities of all samples were higher at shear rates of 45 and 90 /second, than at 225 and 450 /second indicating that the mucus behaved as a non-Newtonian fluid. The standard deviations of readings were, however, also much larger at the lower shear rates, suggesting that these readings were less reliable than those at 225 and 450/sec.

Comparing mucus viscosity in the saline controls, it is apparent that viscosity is reduced in the peptic ulcer model. Misoprostol appeared to prevent the loss of viscosity in the peptic ulcer model. Sucralfate was not shown to have any significant effect on the viscosity of mucus in shams or the peptic ulcer model

EFFECTS ON RATE OF HYDROGEN ION DIFFUSION

Hydrogen ions diffused across the reconstituted mucus samples from the HCl solution to the NaCl solution, at a more or less constant rate after an initial lag phase, as can be seen from the 5th order regression curves plotted in figures V & VI

The curve depicting diffusion across the mucus from saline-treated bile duct ligated pigs was slightly steeper than that for shams treated with saline, suggesting that mucus from bile duct ligated pigs might be a little less efficient at retarding H^+ diffusion. However, there was no difference in rate of H^+ diffusion between the differently treated sham operated pigs.

Hydrogen ion diffusion experiments in this study have not convincingly shown any significant retardation of H^+ diffusion and certainly, there were no significant differences between the various groups studied.

EFFECTS ON MUCUS DEGRADATION

There was a slightly greater percentage of intact mucin in mucus from Sucralfate treated sham operated animals than shams given physiological saline. There was significantly less intact mucus in saline treated animals subjected to bile duct ligation than in those having sham operations, suggesting that there is increased degradation of mucus in the ulcer model. Treatment with Sucralfate appeared to inhibit

degradation of mucin (or stimulate secretion) in the ulcer model, as the difference in percentage of intact mucin between shams and bile duct ligation groups treated with Sucralfate was less than with the respective saline treated groups. Treatment with Famotidine or Misoprostol, if anything, appeared to favour degradation of mucin. This may well have been related to the greater bacterial overgrowth noted in these groups. It was also notable that bacterial colonization appeared to be inhibited in the animals given Sucralfate. However, bacterial overgrowth does not seem to be the only factor, because in the bile duct ligated groups there was not much difference between the treatment groups in the number of moderate to heavy growths.

EFFECTS ON GASTRIC ACID AND PEPSIN ACTIVITY

The average pH of gastric juice was higher than would be expected in normal human stomachs. This is probably due to the free reflux of bile into the stomachs of pigs. Famotidine treated animals had the highest mean pH but the differences between groups was not statistically significant. There was also no significant difference in levels of pepsin activity between the different sham operated treatment groups

Ligation of the bile ducts significantly reduced the gastric juice pH in saline treated animals and there was a corresponding increase in pepsin activity. The differences in gastric juice acidity and pepsin activity between shams and bile duct ligated animals was less notable in the other treatment groups

Wide scatter of readings with large standard deviations from the means made it impossible to draw any conlusions from these data. It must be noted that the method used to measure pepsin activity was simple and, perhaps, in the light of the current knowledge of the complex nature of gastric pepsin, rather crude.

VI. CONCLUSIONS

The bile duct ligated pig is a useful experimental peptic ulcer model, particularly for the study of mucus physiology.

Sucralfate proved to be a highly effective agent in the prevention of peptic ulceration in this model. However, no definite conclusions have been reached about its possible mechanism of action. It did seem that Sucralfate may inhibit the breakdown of gastric mucin. It also seemed to inhibit bacterial colonization and gastric juice pepsin activity and one could speculate on how these observations inter-relate. The possibility that Sucralfate promotes the physical attributes of mucus, could not be verified in this study and this study could not substantiate the claims that Sucralfate may exert its therapeutic effect by stimulating gastric mucosal prostaglandin release.

This study did not address the question of Sucralfate binding to growth factors such as epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), which have been shown to stimulate angiogenesis in and greatly accelerate healing of experimental ulcers, as well as having high affinity for Sucralfate.[97,98,99] It has been suggested that Sucralfate, by its affinity for these growth factors inhibits their degradation by gastric luminal acid and by its affinity for damaged mucosa concentrates the growth factors at the ulcer site.[100]

It should be stressed that all aspects of measurement in this study were complicated and also readily influenced by a number of factors. This, together with the variability of any biological model of healing and the small numbers of animals would account for the lack of positive findings. Nevertheless, it should also be borne in mind that subsequent studies and the wealth of clinical data demonstrate a beneficial effect of

Sucralfate treatment on healing of ulcers. Possibly the principal mechanism is not included among any of those measured in this study and remains to be discovered.

APPENDIX

APPX 1: PROSTAGLANDIN E LEVELS IN SCRAPED MUCUS

	SHAMS		BDL	
	Pig	pg/ml	Pig	pg/ml
SALINE	269	172	271	725
	338	241	345	124
	432	420	419	167
	417	432	430	150
	450	331	452	297
	498	193	496	213
	543	120	553	155
SUCRALFATE	268	299	272	158
	349	301	347	459
	415	293	418	150
	447	282	429	112
	448	413	449	305
	499	239	500	170
	556	138	550	112
FAMOTIDINE	444	214	443	283
	465	226	466	366
	475	358	478	261
	495	235	494	374
	520	250	521	149
	582	96	580	131
	605	120	603	96
MISOPROSTOL	562	116	563	175
	558	154	548	114
	538	173	559	164
	572	83	576	81
	575	69	569	67
	604	118	606	130
	607	125	608	104

APPX 2a: MUCUS VISCOSITY DATA

SHAM OPERATED PIGS				
PIG No.	45 /sec mean	90 /sec mean	225/sec mean	450/sec mean
SALINE				
269	200.46	139.21	127.47	117.58
338	186.75	170.26	151.53	137.96
417	109.65	117.71	96.289	94.37
432	228.73	164.48	133.81	127.06
450	151.63	124.9	123.19	110.82
498	161.91	141.35	145.29	138.57
543	97.66	134.07	115.48	114.62
SUCRALFATE				
26	97.66	143.06	124.13	119.93
349	92.52	123.87	110.85	104.75
415	236.44	172.19	163.11	155.95
447	323.82	205.086	157.284	139.166
448		117.58	113.85	110.17
499	178.19	156.77	158.31	148.85
556	140.49	123.36	120.96	117.5
FAMOTIDINE				
444	195.32	269.85	161.1	135.182
465	150.77	141.35	139.4	130.864
475	173.475	162.424	157.113	149.266
495	235.155	221.02	215.366	204.778
520	97.66	118.734	113.697	111.195
582	123.36	98.517	94.918	94.165
605	188.895	120.79	120.79	120.79
MISOPROSTOL				
538	138.78	119.08	106.23	101.34
558	97.52	98.09	108.797	107.426
562	156.77	137.17	125.16	120.72
572	149.06	143.92	131.07	125.93
575	209.03	119.505	117.706	109.482
604	113.94	113.94	113.08	111.024
607	288.87	282.7	252.2	230.62

APPX 2b: MUCUS VISCOSITY DATA

BILE DUCT LIGATED PIGS				
PIG No.	45 /sec mean	90 /sec mean	225/sec mean	450/sec mean
SALINE				
271		82.24	99.97	96.89
345	119.933	106.226	94.405	92.623
419	147.775	147.004	132.201	122.075
430		107.3	89.69	89.54
452	95.947	110.938	106.227	102.389
496		100.23	100.059	95.604
553	47.545	94.062	87.037	86.763
SUCRALFATE				
272		124.22	122.625	119.59
347	111.367	141.86	119.39	111.74
418		96.632	92.52	89.29
429		126.958	125.59	122.975
449	90.81	104.86	89.73	88.61
500	600.095	574.652	517.427	470.104
550	254.43	237.468	214.338	195.32
FAMOTIDINE				
443		114.793	109.585	109.739
478	111.795	99.716	92.863	92.726
494	97.66	96.118	95.09	93.342
466	119.505	97.66	94.919	93.548
521	125.93	135.182	130.727	124.799
580	99.37	95.947	94.062	91.389
603	250.15	141.864	134.154	116.78
MISOPROSTOL				
548	158.055	121.818	125.416	121.612
559	138.78	141.35	123.19	117.81
563	241.58	184.53	169	142.04
569	131.07	126.358	120.276	112.258
576	79.67	139.208	126.444	120.893
606	109.65	111.795	126.885	123.96
608	334.08	190.18	158.483	141.76

APPX 3: CHROMATOGRAPHY DATA

SHAM OPERATED			BILE DUCT LIGATED PIGS		
PIG	PAS V0(%)	BIORAD V0(%)	PIG	PAS V0(%)	BIORAD V0(%)
SALINE					
269	39	54	271	28	28
338	50	48	345	26	32
432	14	18	419	27	22
417	41	37	430	24	16
450	32	23	452	23	16
498	44	52	496	21	33
543	45	40	553	18	23
SUCRALFATE					
268	14	37	449	21	20
349	64	82	272	23	40
415	51	50	347	31	53
447	37	18	418	31	10
499	40	41	429	14	21
448	31	41	500	47	69
556	37	51	550	34	41
FAMOTIDINE					
444	23	17	443	20	12
465	23	33	466	20	27
475	25	29	478	23	29
495	23	68	494	29	30
520	31	39	521	24	33
582	26	29	580	19	9
605	20	17	603	22	15
MISOPROSTOL					
558	27	12	548	29	25
538	26	16	563	20	10
562	27	20	559	18	18
572	25	25	569	23	12
575	26	17	576	24	19
604	25	13	606	23	21
607	31	36	608	20	24

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