Some Aspects of Gastric Disease in the Dog

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Summary

Results of detailed investigation of 100 vomiting dogs, including the use of either or endoscopy and radiology as a means of diagnosis are presented. Endoscopy proved to be better of the two investigative aids as it allowed widespread examination of the gastric mucosa, but it was decided that both methods should be applied as they compliment each other.

Many of the canine patients examined endoscopically were found to have bile present in their stomachs or actively refluxing, which was interpreted as a duodenogastric reflux gastritis in these vomiting dogs. However, a number of control dogs also had bile reflux identified to varying degrees. A number of dogs were found to have peptic ulcers that had been caused or exacerbated by the ingestion of non-steroidal anti-inflammatory drugs. Most were managed successfully by the short term administration of H_2 receptor antagonists and the cessation of non-steroidal anti-inflammatory drug therapy. However, the severity of the degenerative joint disease required the continued use of non-steroidal anti-inflammatory drug with the concomitant use of H_2 antagonist in one dog to prevent the unwanted gastric-side effects. The results of the studies on the vomiting dogs indicated clearly that gastric abnormalities in this species are, as yet, not fully understood and the diagnosis, treatment and prevention of gastric abnormalities warrants further investigation.

The effect on the gastric mucosa of the administration of acid, bile and two non-steroidal anti-inflammatory drugs (aspirin and carprofen) individually and in combination was studied in the rat. It was found that carprofen appeared to cause as much damage as aspirin and that this damage was exacerbated by the concomitant administration of bile. The effect of prior treatment of the rats with famotidine, an H_2 receptor antagonist, or sucralfate, a mucoprotective drug, before challenge with aspirin and carprofen both appeared to effect changes in the mucosa, but that sucralfate appeared to be slightly better in its effect on the gastric mucosa, though this was not statistically significant.

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Dedication

Dr. J.P. Renton who does not understand the phrase - *next week.*

Tommy who never understood the phrase - *can't do.*

My parents for patiently hiding their impatience.

My wife and children who know all too well what PhD means

and who have become understandably sick of it.

Declaration

I, Martin Sullivan, do hereby declare that the work carried out in this thesis is original, was carried out by myself or with due acknowledgment, and has not been presented for the award of a degree at any other University.

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The histology shown in Chapter 2 represents the interpretation of various members of the Department of Veterinary Pathology, reviewed and photographed by Dr. Pauline McNeil of that department.

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1. General Introduction

1.1 Anatomy and Histology

The mammalian stomach is primarily a storage organ where initial digestion takes place. Although not essential, this preliminary digestion is designed to present the small intestine with material with which it can deal more easily, and on which it can perform the major digestive processes. There is some variability between the species as far as the shape of the stomach is concerned and the proportion each of the regions of the stomach occupies. Indeed, in some omnivores such as the pig and rat, there is a non-glandular portion to the stomach that resembles the oesophagus. The stomach, being a dilation of the alimentary canal for accommodating larger volumes of food, has been divided into four or five main regions (Kammeraad 1942, Ellenport 1975, Warwick and Williams 1973).

i] the *non-glandular* or oesophageal part as exemplified by the rat and pig.

ii] the *cardia* - the entrance to the stomach.

iii] the *fundus* - the reservoir area.

iv] the *body* - where much of the mixing of food and secretion takes place.

v] the *pyloric part* divided into the antrum, where hormones or regulatory peptides, such as gastrin, are produced and sensors for gastric emptying are located, and the pylorus which is the sphincter between the stomach and the duodenum.

Unfortunately, there is some confusion regarding the descriptive terms applied to particular regions in different species. For example, the fundus in the rat is non-glandular, whereas in man and the dog it is glandular. In the rat, the body is that part of the stomach between the limiting ridge and the antrum, whereas in the dog and man the body is that part of the stomach between the fundus and the antrum (Berg 1942, Shay and others 1945, Robert 1971). Thus there are comparable regions between species but the same anatomical names are not always applied. However, each comparable area plays the same part in the function of the stomach regardless of species. The stomach is roughly J-shaped with a short margin on the hepatic aspect - the lesser curvature. The lesser curvature starts at the cardia and slopes down the body before taking a very sharp upward angle, called the *incisure angularis*, into the pyloric part. The opposite margin is longer, and is called the greater curvature. The stomach has a dorsal (parietal) and a ventral (visceral) surface, which meet at the greater and lesser curvatures.

The stomach wall consists of four layers or coats:

i] *Serous* - a peritoneal covering of which there are two layers, one on the dorsal and the other on the ventral surface, which fuse to form the lesser and greater omenta.

ii] *Muscular* - consists of an outer longitudinal and inner circular layer. At the cardia, the circular layer forms a weak and ineffectual cardiac sphincter. However, at the pylorus this muscle layer becomes thicker to form the pyloric sphincter. The pylorus represents a physical narrowing, but there is debate over whether there is a zone of high pressure here analogous to the pressure zone in the lower oesophagus (Fisher and Cohen 1973, Kaye, Mehta and Showalter 1976, Winans 1976). However, both muscle coats cover the whole stomach except at the incisure where only the circular layer is present.

iii] *Submucosa* - a thin layer of connective tissue that attaches the muscular and mucosal layers to each other.

iv] *Mucosa* - the epithelium is covered by a thick layer of mucus composed mainly of water and protein. In the empty stomach the mucosa is thrown into a series of folds known as the rugae or *plicae gastrica*, which run in a longitudinal direction. Once the mucus layer is cleared, the mucosal surface can be seen to have a rather granular appearance. This is caused by small raised areas, which are covered in microscopic openings or gastric pits (*foveolae gastricae*). Into these pits a variable number of gastric glands open. The surface of the mucosa including the pits is lined by surface mucous cells.

Embryologically, the endoderm (the innermost of the three germ layers) that will form the stomach consists of a single layer of epithelium. This layer becomes stratified and vacuoles form in the epithelium which burst to form the gastric pits. Evaginations from the pits then develop into gastric glands (Kirk 1910, Kammeraad 1942, Salenius 1962).

1.1.1 Gland Types

There are three types of glands within the mucosa. The anatomical names assigned to these branched tubular glands do not correspond exactly to the anatomical regions they occupy. Indeed, there has been some divergence of opinion on the terminology applied to these glands since their geographical or anatomical location does not quite relate to definitions applied by either histologists or physiologists (Grossman 1958). However, the glands, themselves, have been well defined (Harvey 1906, Berger 1934):

i] *Cardiac glands* are located in a narrow region around the cardia and lie at the bottom of the deep pits found in this region. These glands are coiled with a wide lumen, the walls of which are composed mainly of mucous cells and a few parietal cells. Thus they are concerned with the production of mucus.

ii] *Gastric* or fundic *glands* occupy the fundic and body regions of the stomach, which forms nearly 60% of the mucosa. The function of these glands is the secretion of acid and enzymes into the lumen of the stomach. They are simple tubular glands and comprise a variety of cells. Each gland has three regions, an isthmus at the bottom of the pit, a neck and a base. The isthmus is made up of surface epithelial cells. The neck region is composed mainly of mucous neck cells and parietal cells. As one goes deeper into the gland, the proportion of these two cells changes so that the parietal cell predominates. The mucous neck cell is irregular in shape, with a narrow base and expanded apex. The function of this cell is to produce an acid mucus. The parietal cell is a large pyramidal cell with a pale cytoplasm producing hydrochloric acid (HCl). As the base of the gland is approached, a third cell appears - the chief cell, which is concerned with the production of the enzyme precursor pepsinogen. On contact with the highly acid luminal contents, pepsinogen becomes the enzyme pepsin. This chief cell is also pyramidal but the nucleus lies at the base of the cell and the cytoplasm is filled with granules. At the base of the gland, mixed with chief cells, are a small number of argentaffin cells that produce regulatory peptides such as gastrin (Bloom and Fawcett 1970, Ito 1967). Thus these glands are primarily concerned with the production of acid, pepsin and mucus (Grossman 1958).

iii] *Pyloric glands* the pits in the pyloric region are deep, therefore the glands are short and coiled so that perpendicular sections are rarely seen by light microscopy. These glands are principally involved in the production of gastrin and mucus (Grossman 1958). Most cells in the pyloric gland are mucus secreting, but they also contain a few parietal cells, argentaffin cells, and some chief cells producing proteolytic enzyme (Grossman and Marks 1960).

1.1.2 Parietal Cell

The parietal cell is dominated by a proliferation of mitochondria and canalicular structures. These mitochondria are essential because of the energy requirements needed for hydrogen ion (H^+) secretion. The canalicular structures contain several enzymes, one of which a hydrogen-potassium-adenosine triphosphatase exchanges hydrogen for potassium ions across the apical membrane (Ray and Tague 1978). This enzyme has been referred to as the proton pump (Sachs 1986). The precise source of parietal, and chief cells, is uncertain, but the cells in the neck of the gland are thought to be involved. Hunt and Hunt (1962) suggested that mucous neck cells

transform into parietal cells especially at the neck. It is worth noting that their evidence was derived by damaging the mucosa with compound 48/80, and observing cell division.

However, other workers looking at cell renewal have been unable to find evidence to support this assertion, at least in the normal uninjured mucosa (Messier 1960). Similarly, Messier and Leblond (1960), Lipkin, Sherlock and Bell (1963), MacDonald, Trier and Everett (1964) were unable to find substantive evidence that the parietal cells and chief cells evolved from mucous neck cells, though, they added the caveat that their results had been derived from investigations of mucosa under normal conditions. The lack of agreement appears to have been laid to rest by the work of Matsuyama and Suzuki (1970). They examined regenerating gastric mucosa that had been neonatally grafted subcutaneously to littermate mice and found that immature mucous cells are totipotent and will ultimately transform into mature mucous, parietal, chief and argyrophil cells.

1.1.3 Surface Epithelial Cell (SEC)

The surface epithelial cell is simple columnar, and uniformly lines the entire stomach extending into the gastric pits. The nuclei are located at the base of the cell. The cytoplasm is occupied by dense mucin granules (Leeson 1985), which have been shown ultrastructurally to be densely packed under the apical surface of the cell (Ito and Winchester 1963). The combination of acid and the proteolytic enzyme pepsin in the lumen produces a highly hostile environment. The function of the surface epithelial cell and the overlying mucus is to assist in protecting the underlying cells and so prevent digestion of the gastric mucosa and deeper layers. The mucus is a gel composed of water and mucus glycoprotein, a large molecule with a molecular weight of 2×10^6 (Clamp 1980). The viscosity is some 30-260 times that of water, is alkaline in nature and permeable to ions and small molecules (Davenport 1977). Food and vagal stimulation have no effect on the production of mucus, but starvation and serotonin, cause respectively decreased and increased mucus production (Menguy 1969).

The cells on the luminal surface are extruded as they age, to be replaced by less mature cells migrating along the basement membrane from the isthmus. Messier (1960) and Hunt and Hunt (1962) showed, using autoradiography, that there were two mucous cell types - the surface epithelial cells and mucous neck cells that are independent of each other. The surface epithelial cells are said to be mucus secreting, producing a neutral mucus in contrast to the acid mucus of the mucous neck cell (Leeson 1985).

1.1.4 Scanning Electron Microscopy

The topography of the surface cell has been studied by scanning electron microscopy in a variety of species (Pfeiffer 1970a). This study showed that the apex of the cell is covered by microvilli. The surface epithelial cells are arranged in circles or whorls around the openings of the gastric pits. The apical membranes slightly bulge and where the cells are in contact with each other an intercellular junction is visible. Thus the cells form an integral surface, and rather than giving a flat-sheet appearance, the convexity of individual cells produces the appearance of cobblestones (Dubois and others 1985).

Knowledge of mucus secretion by the surface epithelial cells is scant. Pfeiffer (1970ab) did not find any evidence of mucus secretion by the surface cell. This is in contrast to evidence from transmission electron microscopical studies carried out by Ito and Winchester (1963) and Sedar (1964). Ito and Winchester (1963), studying the fine structure of the gastric mucosa of the bat, found granules beneath the apical surface in close contact with the cell membranes near the bases of adjacent microvilli. Occasional invaginations of the cell membranes were found in the same region suggesting that these were the empty residues of mucous granules which had discharged their contents into the lumen. However, in a number of observations active secretory images were noted suggesting apocrine secretion.

Beneath the cell membrane the mucous granules are densely packed as quite dark structures which on higher magnifications have a stippled appearance. Between the subsurface cluster of mucous granules and the nucleus, the cytoplasm is dotted with occasional mucous granules, Golgi apparatus and smooth endoplasmic reticulum (Lillibridge 1964). Between adjacent cells, tight junctions and desmosomes are evident as dense trilaminar plaques - parts of the junctional complex (Lillibridge 1964, Toner and Carr 1971, and Fawcett 1981). Transmission electron microscopy also shows that there is intimate contact between the basal lamina and the basement membranes (Kelly and others 1979).

Ito (1967) and Zalewsky and Moody (1979) summarised three possible mechanisms of secretion, namely, extrusion of the entire cell containing the mucous granules, fusion of the mucus granule with the apical cell membrane or passage of soluble mucus through the intact cell membrane. The difference of opinion over surface epithelial secretion may be due to Pfeiffer (1970b) primarily examining the surface cells on the luminal surface, these cells being the oldest and therefore, most likely to have ceased production and secretion of mucus.

As these cells age and reach the apices of the gland they are inevitably discarded. By scanning electron microscopy, the degeneration of the normal surface epithelial cell

has been shown to begin as a small pit located in the centre of the convex surface of the cell. This cavitation enlarges and eventually the contents are dissipated. The adjacent viable cells then converge on the site of cell degeneration (Pfeiffer 1970b), thus matching one of the proposed mechanisms for mucus secretion (Ito 1967).

1.2 Cell Renewal

Since cells double their deoxyribonucleic acid (DNA) prior to mitosis, they take up DNA precursors. Therefore, the most frequently used method of determining turnover or renewal is by measuring the uptake by cells of ^{3}H labelled thymidine, which is incorporated into the DNA of rapidly dividing cells (MacDonald, Trier and Everett 1964). They and others, who have used this method have made the assumption that the tritium does not alter the behaviour of such labelled cells. Alternatively, Bertalanffy (1960) used colchinine to measure both the mitotic rate and renewal times in the gastric epithelium of the rat.

The normal turnover rate of the surface epithelial cell is approximately three days. However, variability arises between different parts of the stomach and between species. Indeed, Clark and Baker (1963) demonstrated in the rat a circadian periodicity to the mitotic division of the mucous neck and surface epithelial cells. Under standard conditions of light (6am to 6pm), they found the peak mitotic rate to occur mid-morning, troughing during the night.

In man, the turnover time is between 48 and 96 hours (Lipkin, Sherlock and Bell 1963). In the mouse, it is 24 hours (Creamer, Shorter and Bamforth 1961). In the rat, 72 hours is a consistent finding (Leblond and Walker 1956, Messier 1960, Baker 1964) so that by the sixth day the labelled cells are found free in the lumen (Hunt and Hunt 1962).

1.3 Gastric Secretion

1.3.1 Stimuli

In the normal stomach, the measurement of gastric secretion is awkward because of such added effects as the physical presence of saliva, food, antral secretions and refluxed duodenal content. To overcome this difficulty but still allow the normal stimuli of alimentation, portions of the stomach can be surgically isolated. Examples of these are Pavlov and Heidenhain pouches (Konturek 1974). Thus much of the information relating to the physiological processes of gastric secretion has been gleaned from the use of such pouches in the dog, or the use of isolated perfused portions of gastric mucosa in the rat or frog.

Sight, smell and taste are potent stimulators of acid secretion (Feldman and Richardson 1986). Acid is produced at a low basal rate when the stomach is empty, but when food is experienced acid output is stimulated and there are three distinct phases apart from the basal rate; cephalic, gastric and intestinal (Konturek and others 1976, Konturek, Rayford and Thompson 1977).

1.3.2 Gastric Control

Distension of the stomach will provoke acid production through cholinergic nerve fibres. In the antrum, not only distension, but also the excitation of chemoreceptors will lead to acid secretion (Elwin and Uvnäs 1967). The effect of this stimulation is the release of gastrin from the G-cells in the antrum.

1.3.3 Cellular Control

The actual concentration of acid in the lumen is determined by the rate of secretion, the buffering effect of food and the diffusion of acid into the mucosa. Cells producing hormones are influenced by the luminal contents. As an example, a low antral pH will abolish antral gastrin and stimulate duodenal secretin, and secretin has been shown to affect pyloric motility (Golenhofen and others 1980). Uvnäs-Wallensten (1977) showed that gastrin is released into the lumen of the stomach either directly from the apices of gastrin (G) cells, or through release of gastrin into the interstitial tissue from where it reaches the lumen.

Stimulation of acid secretion at the cell level is thought to occur through three distinct pathways which provide chemical messengers to the parietal cell (Wolfe and Soll 1988).

i] *Neural pathway*: Acetylcholine is discharged from post-ganglionic nerves in the gastric wall.

ii] *Endocrine pathway*: Hormones such as gastrin are released from endocrine cells.

iii] *Paracrine pathway*: Tissue factors such as histamine are released from storage sites to diffuse across intercellular spaces to the parietal cell.

Yet, these three pathways, though distinct, are in fact interdependent. Histamine H_2-receptor antagonists prevent acid secretion not only from histamine stimulation but also from gastrin and vagal stimulation (Wolfe and Soll 1988).

The accepted model of parietal cell secretion of acid is the *pump* model where hydrogen and chloride ions are pumped across the membranes independently. The presence of a potential difference across the mucosa tends to support this. To reach the gastric lumen it must pass through the mucus layer. Recently, a mechanism of

mucus channels that allows acid to reach the lumen without interfering with the mucus layer has been suggested (Rarnakrishnan and others 1992).

1.3.4 Suppression

As a result of the various excitatory pathways, acid floods the stomach mixing with the food and other secretions present. However, the acid flood will eventually produce an environment in the antrum such that when a pH of 3 or less is induced, the acid will act directly on the gastrin cells and halt the release of gastrin (Walsh and Grossman 1975).

As with other secretions produced in the digestive tract, gastric juice includes an inhibitor. In the gastric juice, the inhibitor is directed against acid production. The inhibitor has been identified and named gastrone, although consisting of two components - the active inhibitor is a gylcoprotein with a molecular weight between 1-4 $x10^4$ (Fiasse, Code and Glass 1968).

Somatostatin has been found in the antrum and the pancreas (Arimura and others 1975). In cats which have been given infusions of pentagastrin, acid liberation is suppressed by the administration of somatostatin (Albinus and others 1977). Further evidence for this suppressing action was found by noting the effect of somatostatin antiserum on gastrin secretion in the perfused rat stomach. From this, the authors deduced that somatostatin inhibits gastrin secretion, in the basal state at least (Saffouri and others 1979). Importantly, it would also appear that somatostatin inhibits cell proliferation and the trophic action of gastrin (Lehy, Dubrasquet and Bonfils 1979, Lipkin 1985). Somatostatin, as well as being found in the cat and other small animals, has been identified immunocytochemically in the small intestine of the horse (Kitamura and others 1984).

Using isolated duodenal and distal intestinal pouches, with hydrochloric acid instilled into them, it has been shown in the dog, at least, that there are also acid receptors which are located only in the duodenal bulb or ascending duodenum. The acidification of a duodenal pouch with topical acid caused a pronounced inhibition of the acid secretory response to gastrin, with little effect obtained by acidification of the distal intestinal pouch. The nerves from these receptors would either directly inhibit, or potentiate humoral suppression of acid secretion (Andersson, Nilsson and Uvnäs 1967). In man and dog, secretin, a local intestinal hormone, is a potent inhibitor of acid secretion (Andersson and Grossman 1966, Berstad and Petersen 1970). Shoemaker and Buckner (1976) in considering the secretion as a complex pathway, concluded that secretin acted after histamine in the pathway but before cyclic adenosine monophosphate (AMP). A molecule known as peptide YY was shown by

Taylor (1985) to be released postprandially in the dog. In the same year it was demonstrated that among other actions it inhibited gastric secretion that had been induced by vagal stimulation (Adrian and others 1985). The suppressing effect of secretin (25%) is enhanced in an additive manner by the release of peptide YY (21%) following a meal to 38% (Olsen and Christiansen 1991).

1.3.5 Histamine

Over the years attempts have been made to distinguish a common mediator for the various pathways that lead to acid secretion. The suggestion that mucosal histamine might be the mediator for acid secretion was made as early as 1938 by MacIntosh. Much work since then has confirmed the crucial role histamine has in acid secretion by the parietal cell, particularly the recognition and blocking of a specific H_2 receptor on the parietal cell (Code 1977).

Histamine directly stimulates the parietal cell to produce and release acid into the luminal environment. But it has also been proposed, by no means unanimously, that it is the final common mediator in the pathways of gastrin and acetylcholine stimulated release of acid, since it has been shown that the vagus and histamine have specific and separate receptors (Soll 1977). Fromm, Silen and Robertson (1976) presented findings which showed that histamine decreased antral luminal acid loss that could not be accounted for by active H^+ secretion, and proposed that histamine had caused a spontaneous decrease in antral mucosal permeability. This change in permeability was also noted in the fundus and antrum, where a salicylate provoked increase in permeability was repressed. They drew the conclusion that there were two H_2 receptors, one that governs permeability and the other related to acid secretion, thus bestowing on histamine two roles; as acid secretion mediator and a mediator of permeability.

Further conflicting evidence was provided by Smith and others (1977) found that histamine protected rabbit mucosa from hydrochloric acid, in contrast Hansen, Aures and Grossman (1978) showed that histamine enhanced aspirin induced antral ulceration in the cat. Possible explanations for this disparity are that in the cat the aspirin was given intravenously, and actively secreting stomachs are more resistant to back-diffusion of H^+.

1.3.6 Gastrin

The name gastrin was given to the active principle of antral mucosal extracts, which stimulated acid secretion, by Edkins in 1905. Now gastrin refers to a local hormone that was first purified by Gregory and Tracy (1961), sequenced by Gregory and others

(1964) and synthesized by Anderson and others (1964). The cell of origin was first definitively identified by McGuigan (1968) using immunofluorescence. He considered the cells similar to argentaffin or argyrophil classes of cells and found them in the antral or pyloric region interspersed between other cells of the mucosa. In the dog there is variation in the numbers of gastrin cells found in different individuals (Delaney and others 1978). Gastrin has been shown repeatedly to be an extremely potent stimulator of acid secretion. However, there is a variable response to gastrin by parietal cells (Soll and Berglindhi 1987). Gastrin is not simply involved in acid secretion control. Amongst other factors that need to be taken into account in mucosal healing is the trophic effect of gastrin (Håkanson, Oscarson and Sundler 1986). Takeuchi and Johnson (1979) found that pentagastrin increased fundic mucosal DNA synthesis in rats with gastric ulcers, affording some protection to the gastric mucosa.

1.3.7 Acetylcholine

Acetylcholine, a low molecular weight ester, has been shown to release gastrin from the antrum (Pe Thein and Schofield 1959). Less certain is it s ability to stimulate the parietal cell directly, although there is evidence for (Pesvner and Grossman 1955) and against (Kasbekar 1972). However, electrical stimulation of the vagus in the cat, has been shown to deplete the gastrin pool (Uvnäs-Wallensten, Uvnäs and Nilsson 1976).

There are, therefore, three agents that can stimulate acid secretion by the parietal cell. It is unclear how many receptors there are on the parietal cell. Whether each agent acts synergistically with the others, or thorough one agent pathway such as histamine is also unresolved (Walsh and Grossman 1975), since atropine will inhibit not only cholinergic stimulation of acid, but also that of histamine and gastrin (Konturek, Wysocki and Oleksy 1968). A similar effect is seen with the use of histamine blockers (Gibson, Hirschowitz and Hutchison 1974).

1.3.8 Cyclic Adenosine Monophosphate

From the above, it is clear that there has been uncertainty over the final pathway, with histamine as the favourite final messenger. In 1965, Sutherland, Qye and Butcher ascribed cyclic 3',5'-AMP (cAMP) the role as second messenger in the action of some hormones. Following this work, Harris, Nigon and Alonso (1969) showed that cAMP was involved in the intracellular mediation of gastric acid secretion. They demonstrated that theophylline, a methyl xanthine which inhibits the phosphodiesterase that destroys cAMP, stimulated the production of hydrochloric acid, and that exogenous cyclic AMP stimulated H^+ and Cl^- transport. Similar results also implicated cAMP (Levine 1970). Later, Sjostrand, Ryberg and Olbe

(1978), using *in vitro* preparations of guinea pig gastric mucosa and administering histamine, histamine blockers and the dibutyrl form of cAMP, showed that cAMP is a link between histamine receptors and hydrogen ion output.

1.3.9 Proton Pump

With the identification of the role of cAMP came the concept of such a proton pump, though the possibility of a pump had been speculated on by Hogben (1955). This biochemical pump would pump the hydrogen ions from the parietal cell into the lumen. Ganser and Forte (1973) found a potassium-stimulated adenosine triphosphatase (ATPase) in microsomes of the fundus of a number of species, but not the antral mucosa (Forte, Ganser and Ray 1976). In attempting to characterize the microsomal membrane location of the biochemical pump, Lewin and others (1978) were able to identify potassium stimulated phosphatase activity, but not potassium stimulated ATPase activity. In contrast, Scholes and Lee (1978) considered that an ATPase catalysed an exchange of hydrogen ions for potassium ions, suggesting that this ATPase was located on the membrane vesicles of the parietal cell and was responsible for the transport by the luminal membrane of acid.

Soll (1980a) using aminopyrine uptake as a measure of parietal cell response to stimulation, showed that prostaglandin (PG) E_2 inhibited histamine stimulated aminopyrine uptake. However, this prostaglandin did not block the response to dibutyrl cylic adenosine monophosphate (cAMP). From this he drew the conclusion that, at least in isolated parietal cells, PGE_2 inhibited histamine stimulation of cAMP.

1.3.10 Calcium

Soll (1981) investigated the role of calcium gating in cholinergic stimulation of parietal cell function and found a close link between stimulation of parietal cell function and enhancement of calcium influx by cholinergic agents. The obvious conclusion from this was that if the extracellular calcium was blocked from moving intracellular, then acid production would be stopped. The receptors for the control of secretion have been divided into two groups, those which elicit the production of cAMP, and those which stimulate inositol phospholipid turnover and calcium ion mobilization (Rask-Madsen 1987, Levine, Nandi and King 1990).

1.4 Blood Supply

Measurement of gastric mucosal blood flow formerly presented a number of difficulties, but these were largely overcome by the introduction of the aminopyrine clearance method by Jacobson, Linford and Grossman in 1966. An increased gastric

secretion is attended by an enhancement of mucosal blood flow, but that the converse does not occur was noted by Bynum and Jacobson (1971) and Gerber and Nies (1982). The effect of increased mucosal blood flow, as result of increased acid secretion, has been confirmed when the increased secretion has been stimulated by pentagastrin (Curwain and Holton 1972), histamine (Main and Whittle 1973), and the vagus (Lanciault 1975). This blood flow response is probably due to the direct vasodilator action on arteriolar smooth muscle, local vasodilators and redistribution (Jacobson, Swan and Grossman 1967). It has been shown that suppression of acid secretion by drugs also leads to a fall in mucosal blood flow (Tsukamoto and others 1987). Bennett and Curwain (1977) suggested that aspirin induced bleeding by causing vasocontriction followed by ischaemia. It has also been shown that enhancement of the mucosal blood flow will protect the mucosa against aspirin-induced erosions (McGreevy and Moody 1977) and that aspirin will suppress the mucosal blood flow (Gerkins and others 1977).

1.5 Gastric Emptying

The passage of material from the stomach is determined by the activity of the peristaltic waves pushing ingesta from the fundus and body through to the antrum, a fact recognised radiologically as long ago as 1898 by Cannon. The force of the waves is determined partially by the particle size and constituents of the food, but is primarily a phasic activity (Crider and Thomas 1937, Carlson, Code and Nelson 1966, Code and Martlett 1975, Ehrlein, Pröve and Schweiker 1980). The pyloric sphincter is often thought to prevent the egress of food material that is not of the right constitency from the stomach to the duodenum. However, the pylorus does act, to a certain extent, as a barrier to passage of food because of its physical size, rather than by acting as a physiological bottle-neck (Ehrlein, Pröve and Schweiker 1980), and to minimise to-and-fro movements of chyme across the gastro-duodenal junction (Müller-Lissner and Blum 1984). In the oesophagus there is a high pressure zone in the caudal thoracic area that helps prevent reflux of gastric contents (Frank, Walker and Fordtran 1973, Cohen and others 1976, Corrazziari and others 1978). In man, an analogous pressure area was noted by Fisher and Cohen (1973). Similarly, in normal fasting dogs there is a zone of high pressure at the gastro-duodenal junction (Brink, Schlegel and Code 1985) and this, coupled with the action of the pyloric sphincter should ensure that little material refluxes into the stomach. After a bolus leaves the stomach the pylorus is often not completely closed, hence, it is not possible for the pylorus to prevent reflux of duodenal content if an abnormal orally directed peristaltic wave forms in the duodenal bulb (Ehrlein 1981). The small volume of duodenal content that may enter the stomach is not important because of a number of factors.

The stomach is filled with food at this time thus diluting duodenal content, which post-prandially is a mixture of food, pancreatic juice and bile. The peristaltic activity causes thorough mixing of the refluxed material and gastric emptying is occurring throughout, and the reflux of duodenal contents does not adversely affect gastric emptying (Donovan and others 1984). Thus contact time with the antral mucosa is very short.

The stomach is, therefore, a glandular organ that has an extremely acidic luminal environment where significant concentrations of proteolytic enzyme are present. This combination of acid and proteolytic enzyme means that the stomach lining must protect itself from autodigestion. Failure to do so will lead to cellular damage.

1.6 Mucosal Barrier

The phrase "gastric mucosal barrier" (Silen 1977) is proffered as a physiological concept, which refers to the ability of the mucosa to prevent rapid invasion of hydrogen ions from the gastric lumen into the interstitial space, and the rapid diffusion of sodium ions (Na^+) in the reverse direction. The barrier has, to date, no definite anatomical analogy, though the nearest that can approach the analogy is the mucus layer, surface epithelial cells and their tight junctions acting as a physical barrier. However, a comparison between two barrier breakers, urea and ethanol showed that urea produces damage to the intercellular junctions by distorting the tight junctions through the formation of vacuoules or blebs, whereas ethanol produced cytoplasmic changes within the surface epithelial cells without disrupting the cell membrane (Eastwood and Kirchner 1974). The essential function of this barrier is to maintain a high luminal acid concentration and low pH, and in the process evade digesting itself in the presence of the activated enzyme pepsin.

In 1939, Teorell demonstrated, in the anaesthetised cat, that the acid which is secreted by parietal cells into the lumen could, under certain circumstances, diffuse back into the mucosa. In doing so, he found that when hydrogen ions were absorbed, sodium ions were pumped out into the lumen. He further showed that certain molecules such as weak acids were rapidly absorbed by the mucosa, whereas other molecules such as glycine were not absorbed. He concluded that the gastric mucosa acted as a semi-permeable membrane. He further postulated that a mechanism existed that prevented hydrogen ion back-diffusion by the selective absorption of sodium ions.

In 1946, Hollander, Stein and Lauber in looking at some features of gastric mucus

described the concept of a "mucous barrier". Code and others (1955) actually used the term "*barrier*" in the title of a paper, which showed the resistance of the mucosa to absorption of sodium ions was complete when the gastric mucosa was secreting acid. This work, like that of Teorell (1939), was done in anaesthetised animals, and they noted a difference in the absorption of sodium when the use of pentobarbitone was compared to ether. That a barrier exists to sodium was also confirmed in healthy conscious human subjects where there could be no effect of anaesthesia on absorption (Reitemeier, Code and Orvis 1957). Similar work has shown that the same effect occurs in the dog and therefore pan-species. Code and others (1963), using isotopes of sodium and potassium, demonstrated that acid in the stomach stopped absorption of sodium. However, these studies were concerned with the gastric mucosa as a barrier to intraluminal absorption of sodium ions.

Hollander (1954) in attempting to explain the failure of peptic ulcer patients to prevent the autodigestion of the gastric mucosa postulated a two-component mucous barrier. The first element was the viscous mucus layer that sits on the surface of the epithelium. In the normal unchallenged stomach, the mucus is translucent and contains few cells. When irritated the mucus then contains large numbers of cells (Hollander, Stein and Lauber 1946) or when in the presence of food (Grant 1944). However, Janowtiz, Hollander and Jackson (1951) found, by suppressing stimulation of gastric secretion with atropine, that the alkaline opalescent viscid secretion of mucus was free of cells and concluded that exfoliation was not intrinsic to the process of mucus secretion. Hollander (1954) felt that the mucus, because of its properties of adhesiveness, viscosity and acid neutralizing capacity, retarded the penetration of chemical irritants. The second element was the surface epithelium that under normal conditions provides mucus, but when irritated will rapidly desquamate. Heatley (1959) inferred that mucus safeguarded the epithelium by supplying an unstirred layer on top of the mucosa. The theory propounded was that H^+ ions diffusing from the lumen were neutralised by bicarbonate (HCO_3^-) ions in the mucus layer. The unstirred layer maintained the bicarbonate ions at the mucosal surface and thus avoided the ions mixing with the luminal hydrochloric acid, that this layer was a dynamic barrier with the constant production of mucus, rather than as one might mistakenly imagine, a static layer (Thomson 1984).

In 1969, Menguy reviewed the role of mucus as part of the gastric barrier. Whilst finding that antral acidification stimulated and starvation reduced dramatically mucus production, he was unable, from reviewing the actions of several non-steroidal anti-inflammatory drugs (NSAIDs), to be convinced conclusively that mucus played a major role in gastric mucosal ulceration. He did accept that NSAIDs such as aspirin and indomethacin did reduce mucus secretion.

Davenport (1975) in contemplating a personal decade's work on the barrier was uncertain of the role of mucus in the barrier, but confident that the cell membrane played an imperative role. Indeed his conclusion, arising from experimental work, was that the barrier consisted of the apical membrane and the intercellular tight junctions (Davenport, Warner and Code 1964, Davenport 1967a). Flemstöm (1976) found that there was an alkaline secretion in the immediate vicinity of the luminal membrane of the gastric epithelium in both the antrum and the fundus, which, by raising the border pH, would contribute towards the limitation of damage due to the low intragastric pH .

This uncertainty over the role of the mucus layer was also felt by Silen (1977), considering, in his view, the layer's weak neutralising and buffering power and it's inability to prevent diffusion of acid through it.

Nonetheless it seemed difficult to dismiss mucus - "*the slimy secretion of the stomach lining*" (LaMont and others 1983) - as merely a lubricant. And in 1980 Allen and Garner set out to explain that the role of the mucus layer, bicarbonate ions or mucus would individually have a limited barrier effect, but by integrating these two elements as a single system they could see this system as providing a competent measure of protection. The gastric transport of bicarbonate probably occurring by Cl^-/HCO_3^- exchange at the luminal membranes of the surface epithelial cells. Lowering luminal pH did indeed increase transport of HCO_3^- (Flemström and Garner 1982) as may be caused by bile (Konturek and others 1984a). Further work demonstrated that this bicarbonate transport was not affected by the common local hormones and transmitters such as histamine, secretin and gastrin (Flemström, Heylings and Garner 1982), but could be stimulated locally by prostaglandins (Kauffman, Reeve and Grossman 1980).

In pulmonary surfactant there are amphoteric phospholipids that markedly enhance the water repellency of surfaces when adsorbed to negatively charged membranes (Hills 1982). These same phospholipids have been shown to be present in high concentrations in the gastric juice of the rat and dog (Slomiany and others 1978). In particular, phosphatidylcholine represented 50% of total phospholipids in the rat and dipalmitoyl diglyceride was present in similar concentrations as those found in the lung - this being the major active component of pulmonary surfactant (Wassef, Lin and Horowitz 1979). In 1983, Hills, Butler and Lichtenberger, using the contact angle subtended between a droplet of aqueous fluid and a non-wettable surface as a direct estimation of the hydrophobicity of the non-wettable surface, found that the mean angle in the fundus of the dog to be 85.2°. This angle is similar to that displayed by acid resistant substances such as polyethylene.

In the same year Butler, Lichtenberger and Hills published results which showed that these mucus phospholipids could form an adsorbed layer of surface-active phospholipids beneath the gel phase of the mucus on the luminal surface of the gastric epithelium, with this layer having the property of hydrophobicity (Figure 1.1).

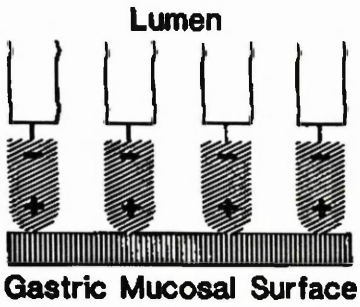


Figure 1.1. Diagram of adsorbed layer of surface-active phospolipids.

Provocative testing for this surfactant has confirmed its presence and ability to protect the mucosa, where acid induced gastric ulceration and bleeding in rats was markedly reduced (Lichtenberger and others 1983). Szelenyi and Engler (1986) showed that ambroxanol, a stimulant of pulmonary surfactant, increased the level of phospholipid content of the mucosa, and also showed that when the mucosa had been pre-treated with phospholipase C and challenged with ethanol, the degree of damage was greater than ethanol used solely. Using fluorescent hydrophobic probes and a phospholipid selective cytochemical stain, Kao and Lichtenberger (1991) showed that the source of the phospholipid in the mucus layer resided in the mucous cells as large infranuclear inclusion bodies. They also noted that these organelles were specific for mucous cells and not a general feature of degenerating cells. Therefore, evidence has been presented that the mucus layer may have a chemical component by producing pH gradients and a physical property of hydrophobicity.

The mucus that coats the mucosa is considered to consist of two layers, a so called "*visible*" layer that is insoluble in water and contains glycoproteins. The second "*dissolved*" layer is contains essentially the same components. The mucus layer acts as a partial barrier producing a concentration gradient so that fewer ions reach the cell, representing the first solid line of defence (Allen and Carroll 1985). The chemical and physical properties of the apical membranes and tight junctions that bind the cells together are such that water soluble substances are very slowly admitted. This impermeability is an important factor in the defense of the gastric mucosa against injurious agents (Code 1981). Thus the mucus layer has emerged with an important role in the mucous barrier and consequently its source and production are of some importance. Zalewsky and Moody (1979) and Moody, Zalewsky and Larsen (1981) have demonstrated three possible routes through which mucus reaches the lumen, namely through exocytosis, apical expulsion and by actual exfoliation of an intact surface cell.

In summary then, protection or defence of stomach wall integrity depends on a number of features of the stomach, the main ones being the mucus layer or mucus gel (Menguy 1969), epithelial cells (Code 1981) and blood flow (Konturek and Robert 1982).

1.6.1 Mucosal Restitution

Superficial injury, without deeper mucosal damage and vascular injury, is repaired rapidly by cell migration, so called "mucosal restitution" (Pihan and others 1986). Flaws created in the surface of the gastric mucosa are restored by re-epithelialisation. The cells closing the defect are produced from proliferating stem cells in the neck zone of the gastric glands. Regeneration by mitotic activity requires from one to three days (depending upon species) for differentiation and migration (Leblond and Walker 1956, Messier 1960, Creamer, Shorter and Bamforth 1961, Lipkin, Sherlock and Bell 1963, Baker 1964). Yet it has been known for some time that superficial injury can be repaired rapidly by the stomach in less than four hours (Grant 1945). Clearly there is a disparity in the time sequence and some other mechanism must come into play to rebuild the defences. Mucosal restitution is the phrase that has been coined to describe the process whereby the differentiated and mature surface epithelial cells migrate across the basal lamina to cover the exposed basal lamina, thus re-establishing epithelial continuity (Silen and Ito 1985).

Restitution of frog surface epithelium was demonstrated *in vitro* in 1982 by Svanes and others. *In vivo* work in the rat, using ethanol as the injurious agent, has also shown that restitution occurs (Lacy and Ito 1984). In this study 4ml of absolute alcohol was injected into the gastric lumen for 30-45 seconds and the stomachs fixed at time intervals thereafter. The ethanol destroyed the majority of the superficial epithelial cells without extensive hyperaemia, or causing deeper macroscopic haemorrhagic lesions. The migrating cells flattened themselves and extended feet-like projections so that after 15 minutes the basal lamina was covered, and by 60 minutes the flattened epithelial cells had become a more normal columnar shape. The first evidence of response was within 3 minutes of injury. Similar findings using ethanol have also been reported by Terano and others (1986). Svanes and others (1982) considered the role of two factors, luminal pH and bicarbonate ions, on epithelial restitution using frog fundic mucosa in Ussing chambers. They found that a low luminal pH inhibited, and high nutrient bicarbonate ion concentration supported restitution after damage, histologically, there being a layer of sloughed cells and mucus above the epithelium.

Two factors that have been identified as essential to the whole process are an intact basal lamina, and adequate microvascular perfusion (Morris 1986). This author was sceptical of any direct role being played by prostaglandins in restitution. This partly confirmed the work of Whittle and Steel (1985) who found that low doses of 16,16-dimethyl prostaglandin E_2 did not protect the superficial epithelial cell, though a higher dose of 20μgkg^{-1} did reduce the severity of damage. They felt it more likely that prostaglandins have their main effect on perfusion of the tissue. Grënbech and others (1987) demonstrated that low intraluminal pH had no effect on restitution in anaesthetised cats, but that hyperaemic response is important for satisfactory restitution (Grënbech and others 1988).

Apart from the role in mediating acid secretion Soll (1981), calcium has been implicated in the restitution of the surface cell. Critchlow and others (1982) bathed amphibian gastric fundus in 1M NaCl for 10 minutes. They found uniform disruption of the surface epithelial cells, the integrity being restored in 4-6 hours. If the bathing solution had calcium ions added then recovery was much more rapid compared to calcium deprived solutions. Whether the action of the calcium ions was intracellular or not was not determined. However, calcium is necessary for the migration of undamaged surface cells that move to cover exposed basal lamina and for the formation of tight junctions (Morris 1986). Thus mucosal restitution results in only transient functional impairment, and represents the first line of defence against mild gastric injury (Pihan and others 1986, Haglund 1990).

1.6.2 Mucoid Cap

Morris and Wallace (1981) and Lacy (1985) amongst others have shown that following injury there is extensive mucus secretion. This mucus secretion may have a role in the restitution process by providing a congenial climate for quick cell migration (Morris and Harding 1974, Ito and Lacy 1985, Whittle and Steel 1985). The mucus secretion forms a gelatinous layer with exfoliated surface epithelial cells, and has been termed localized mucus cap. However, mucus secretion is not dependent upon exfoliation or desquamation of cells as has been shown by topical application of acetylcholine to the gastric mucosa. The resultant secretion was alkaline, opalescent and viscid but free of cells (Janowitz, Hollander and Jackson 1951). The cap may be stabilized by fibrin, and a further effect is the presence of bicarbonate in the fluid producing a high pH environment (Svanes and others 1984, Wallace and McKnight 1990). However, the environment created by this cap can be dissipated by acid (Morris and Wallace 1981). They flushed the rat stomach with 40% ethanol and found the released mucus stabilised by a network of fibrin, with underlying cell migration. However, by repeating the experiment with 50mM HCl,

they found the platelet thrombi and fibrin network destroyed by the acid dissipating the adherent mucus coat, leading to irreversible damage of vulnerable cells and devastation of the basal lamina. Dissipation is also caused by the inhibitors of prostaglandin synthesis, such as non-steroidal anti-inflammatory drugs, and by reduction in mucosal blood flow (Wallace and McKnight 1990). Evidence confirming the role of mucus in the barrier was reported by Wallace and Whittle (1986). They tested the hypothesis that mucus provides a microenvironment when the mucosa is damaged. To do this they evaluated the epithelial damage that occurred following exposure to 50% ethanol, with or without mucolytic agents being used. The mucus was removed with either N-acetylcysteine (5%) or pepsin (0.5%). In doing so they showed that the mucoid cap disintegrated, macroscopic damage was greater, there was increased mucosal leakage of albumin, the recovery of transmucosal potential difference was retarded and less epithelium covered the denuded areas.

1.6.3 Mucosal Regeneration

Regeneration is the relatively more long-term recovery of the gastric epithelium to injury, including that caused by daily wear and tear. Regeneration of the epithelium of artificial ulcers has been shown to be quite rapid (Ferguson 1928, Williams 1953, Finckh and Milton 1960). Townsend (1961) reviewing the work done on injury concluded that by 24 hours the blood clot formed after the initial trauma would have a ledge of epithelial cells oval in shape and that by 36 hours that ledge would be wider and the cells flatter. So that by six days following trauma, gland structure would be identifiable with mucous neck, parietal and chief cells clearly discernible. Concurring, Yeomans, St. John and de Boer (1973) discerned that injury by aspirin could produce superficial erosions that would be healed in 24 hours, but that deeper erosions healed more slowly taking on average 5 days. Lempinen, Penttilä and Fock (1968) looking at the regeneration of fundic mucosa histochemically found that the epithelium regenerated rapidly, but at four months the general glandular arrangement resembled antral mucosa and the parietal and chief cells had still not fully differentiated. With scanning electron microscopy, the normal cobblestone appearance is lost and the cells become flatter with obvious gaps between cells (Yeomans 1976). Changes seen, ultrastructurally, of nuclear swelling, chromatin clumping and cytoplasmic oedema were considered to be initially reversible in the light of previous findings (Luft and Hechter 1957, Hingson and Ito 1971).

1.6.4 Prostaglandins

Prostaglandins were found first in the seminal fluid of man (Goldblatt 1933). These molecules are saturated long chain fatty acids, and members of different groups (E,F and I) have been found in the gastrointestinal mucosa (Robert 1974, Robert 1981).

Prostaglandins have been proven to have a number of effects on the gastrointestinal tract such as acid and mucus secretion and mucosal blood flow. The precursor arachidonic acid is released from tissue phospholipids by phospholipases. The prostanoids that are derived from arachidonic acid are all cyclooxygenase products (Rask-Madsen 1987).

Under conditions of maximal histamine stimulation, intravenous PGE_1 was shown to inhibit acid output (Wilson and Levine 1972). However, such drastic stimulation is not required to demonstrate this suppressor effect (Robert, Nezamis and Phillips 1967, Nezamis, Robert and Stowe 1971, Miller 1983). The mode of action in suppressing acid secretion has been demonstrated, in isolated canine parietal cells, to be the prevention of cAMP production (Soll 1980a).

Prostaglandins (PGE and PGF) have been shown to stimulate secretion of gastric mucus, mucin and bicarbonate in a variety of species (Bolton and Cohen 1978, Bolton, Palmer and Cohen 1978, LaMont and others 1983). Bolton and Cohen (1979a) demonstrated that prostaglandins would stimulate fluid production from the non-parietal cells that contained sodium and chloride ions. The movement of sodium ions into the lumen occurred without an equal flux of hydrogen ions moving in the opposite direction - a feature associated with barrier disruption.

Kauffman, Reeve and Grossman in 1980, demonstrated that topical 16,16-dimethyl PGE_2 stimulated bicarbonate secretion, but that topical prostaglandin was more effective than prostaglandin delivered intravenously and also stimulated mucus as measured by glucosamine output. Lichtenberger and others (1983) and LaMont and Szabo (1984) found that 16,16-dimethyl prostaglandin E_2 and prostaglandin $F_{2\beta}$ caused a swift accumulation of hydrophobic phospholipids in the mucosa.

Extensive experimental evidence has also established that various prostaglandins increase gastric blood flow (Main and Whittle 1973, Kauffman and others 1979). In 1980, Cheung exteriorized part of dogs' greater curvatures and measured blood flow by venous outflow α-labelled microspheres. He found that in the resting state dimethyl PGE_2 increased blood flow almost twofold from 4.2 to 7.4$ml min^{-1}$, but in histamine stimulated stomachs the blood flow was reduced from 14.6 to 9.2$ml min^{-1}$. These apparently anomalous results were explained by the fact that the reduction in the actively secreting stomach was due to secretory inhibition. Gerber and Nies (1982) concurred using radioactive labelled spheres and electromagnetic measurement of blood flow.

Apart from local factors causing prostaglandin release, nerve stimulation has been found to cause significant increases in release of prostaglandin, particularly PGE_2 and

$PGE_{2\alpha}$, from the stomach, indicating that there are more distant control mechanisms (Bennett, Friedman and Vane 1967, Coceani and others 1967). Fringes, Lorenz and Oehlert (1985) used PGE_2 to treat rat gastric mucosa challenged with 80mM sodium taurocholate and found that there was increase in mucus production to explain the cytoprotective effect of PGE_2.

Synthetic prostaglandins such as riprostil (a synthetic E_1 analog) have been found to have "*antigastrolesive*", antisecretory and mucus stimulating effects in the experimental rat and also in fistulated dogs (Katz, Shriver and Rosenthale 1987, Katz and others 1987ab). This effect was equally effective whether riprostil was given orally or as a topical preparation.

1.6.5 Cytoprotection

The observation that prostaglandins are able to prevent injury to the gastric mucosa by a number of diverse agents distinct from their acid suppressing effects has been termed "cytoprotection" (Robert 1976). The precise mechanism whereby prostaglandins effect protection and indeed the exact cell of origin has not been defined, but they exert their activity at the site where they are produced and experimentally non-antisecretory doses can afford protection (Miller 1983). However, prostaglandins inhibit basal and stimulated acid secretion, stimulate blood flow, mucus and bicarbonate secretion, thus it may well be that there is no single mechanism involved, but each of these recognised biological actions is cumulative, thus giving cytoprotection (Johansson and Bergström 1982).

Prostaglandins have been shown to effect protection of the mucosa in a wide range of species. Ferguson and others (1973) induced ulcers in the rat by using serotonin, the mode of injury being through intense vasoconstriction with breakdown of lysosomal membranes. They showed that treatment with PGE_1 prevented such ulcer formation and stabilized the lysosomal membranes. Cohen and Pollett (1976) found in man that challenge to the barrier with aspirin or indomethacin was warded off by the use of PGE_2, indicating that prostaglandins are definitely contributory to mucosal integrity.

A similar effect was noted in the dog where mucosal permeability was used as a measure of barrier stability. Here intravenous prostaglandins returned the permeability to normal where there was already established mucosal damage caused by exposure to aspirin (Bolton and Cohen 1979b). Pihan and others (1978) explored the effect of ethanol on mucosal blood flow, which they found to be reduced and correlated well with the extent of haemorrhagic mucosal lesions. Pre-treatment with 16, 16-dimethyl PGE_2 prevented capillary stasis and the development of

haemorrhagic gastric mucosal lesions.

Much of the original work on prostaglandin cytoprotection used macroscopic change or transmucosal potential differences as gauges. Lacy and Ito (1982) looked at the microscopic changes of mucosa damaged by ethanol, but treated with prostaglandins. They noted that, whilst oral PGE_2 prevented the typical haemorrhagic necrotic lesions, destruction of gastric mucosal cells still occurred. Konturek and others (1982a) and Schmidt and others (1985) found that prostaglandins produced almost complete macroscopic protection against injury induced by aspirin/HCl and absolute ethanol respectively. But when the mucosa was examined by light, transmission and scanning microscopy, superficial surface cell necrosis had still occurred, though the deeper damage had certainly been prevented.

From the work that has been published it is clear that prostaglandins will protect against deeper damage accompanied by vascular impairment, but not against superficial mucosal injury, particularly where the damage has been caused by intraluminal exogenous agents, where the effect is in all probability caused by direct chemical or physical damage of those cells (Tarnawski and others 1985a, Szabo and Goldberg 1990). This failure is not complete, as was found by Whittle and Steel (1985) using high doses of 16,16-dimethyl prostaglandin E_2, and by restoration of surface hydrophobicity demonstrated by Lichtenberger, Richards and Hills (1985). Moody, Zalewsky and Larsen (1981), in a review of gastric epithelial protection, broadened the definition of cytoprotection to include other factors that protect, such as the epithelium itself, the overlying mucus and mucosal blood flow.

Local blood flow has been shown to be cytoprotective in experimental erosive gastritis, where injurious agents, which produce a high rate of back-diffusion of H^+ and Na^+ efflux, failed to produce erosive gastritis (Moody and others 1978). The increased blood flow ensures adequate oxygen and energy for the hungry intracellular metabolism. It provides a reservoir of ions to supply the cells with sodium and bicarbonate ions, which act to prevent hydrogen ion back-diffusion. Secondly, blood flow is stimulated by, and allows the circulation of prostaglandins, thus preventing circulatory stasis (Main and Whittle 1973, Moody and others 1978, Cheung 1980, Cloud and Ritchie 1982, Konturek and Robert 1982, Pihan and others 1986).

One interesting phenomenon that has been recognised is that of adaptive cytoprotection. Chaudhury and Robert (1980) examined the effect of 80mM acidified taurocholate on rat gastric mucosa. They found that this agent produced extensive lesions within one hour. However, if 5mM acidified taurocholate was given orally 15 minutes before the 80mM taurocholate, the development of lesions was avoided. Their theory was that prostaglandins stimulated by the 5mM bile and

released before the administration of 80mM bile prevented injury. As confirmation of this proposed mechanism they found that indomethacin would suppress this protective effect. Further, evidence provided by Scheurer and others (1981) indicated that chronic exposure to bile increases resistance of the mucosa to bile, possibly by stimulating prostaglandin production. Support for this possibility comes form the work of Lev, Siegel and Glass (1972), Eastwood (1984), Coleman and others (1987). Lev, Siegel and Glass (1972) found that following chronic administration of aspirin to dogs, areas that were grossly normal had a cuboidal regenerative-type surface epithelium histologically, with a reduction in the amount of intracellular mucin. Eastwood (1984) found that, in the fundus, doses of aspirin and indomethacin would stimulate epithelial proliferation at levels that produced no detectable injury. Similar stimulation of epithelial proliferation was found with chronic feeding of aspirin to rats. No such effect was noted in the antrum (Eastwood and Quimby 1982).

1.7 Injurious Agents

Damage to either the mucus or the surface epithelium allows attack on the physiological integrity of the barrier (Davenport 1975). This can arise in a number of well recognised situations. Examples are burns, spinal shock, administration of NSAIDs and following resection of the pylorus (Fletcher and Harkins 1954, Van Heerden and others 1969, DenBesten and Hamza 1972, Goodman and Osborne 1972, Lucas and others 1972).

Ritchie (1975) found that 5mM taurocholate could be recovered from benign ulcer patients. This concentration of bile was enough in the dog to break the barrier, but not to produce ulceration unless acid and impaired blood flow was present. The importance of mucosal blood flow has been stressed by others (Ritchie and Shearburn 1976, McGreevy and Moody 1977, Kivilaakso, Fromm and Silen 1978, Ritchie and Cherry 1979, Watt, Sloan and Kennedy 1984, Lanza and others 1986, Haglund 1990).

Experimentally produced ulcers often have a characteristic shape, being elongated or focal and follow a linear pattern. This pattern was thought to be related to the presence of mucosal folds. Using a rat gastric chamber and three ulcerogenic treatments (acid pH1, haemorrhagic shock and complete ischaemia), Mersereau and Hinchey (1982) showed that the apex of a fold is more sensitive to injury. They found that the mucosal potential difference at the crest of a fold was lower than at the trough. They postulated that this could be due to the thin peak, the lack of gastric musculature to absorb the acid and a lower potential difference indicating a different blood flow in this region.

1.7.1 Acid

The gastric mucosa, as a whole, must protect itself from a highly acid environment created by the secretion of acid from it s own parietal cells. It is recognised that a feature of barrier disruption is back-diffusion of hydrogen ions. Hydrochloric acid 120mM pH1 added to rabbit fundic pouches caused superficial erosions, and in isolated frog mucosa produced deterioration in potential difference across the mucosa when the mucosa was in the resting state. If acid secretion was stimulated, by histamine, the aforementioned mucosal changes did not occur, and if histamine was suppressed the lesions were again found (Smith and others 1977). These findings indicated that the gastric mucosa could be protected from exogenous luminal acid if the mucosa was actively secreting. The reason postulated for this was a greater alkaline tide during active or stimulated secretion (Fromm, Silen and Robertson 1976). Thus the stomach is able to protect itself by virtue of the fact that acid is usually present in the lumen of an actively secreting stomach.

The lipoprotein layer of the plasma membrane is relatively impermeable to ions, but is extremely permeable to un-ionised and fat soluble chemicals. Thus when acidic drugs are given they are almost always un-ionised (depending upon their pK_a) and move rapidly into mucosal cells by passive diffusion. Once in the cell (pH7) these acidic drugs will become ionised and damage the cell due to build up, because of the cell's inability to diffuse these ions out rapidly enough (Martin 1963). When the mucosa is damaged, acid diffuses into the mucosa and stimulates the release of histamine from the injured cells into the venous circulation in both the rat (Johnson 1966) and the dog (Johnson and Overholt 1967). Histamine stimulates acid secretion, vasodilation and increased permeability of the capillary walls to proteins (Johnson 1968). The mucosa becomes oedematous and interstitial ions filter across the mucosa into the lumen. The effect is that sodium ions, potassium ions and proteins leak into the lumen and hydrogen ions re-enter the mucosa. The hydrogen ion stimulus in the mucosa leads to further acid and pepsin secretion (Johnson 1971, Code 1981).

Acid plays a role in accentuating the damage caused by injurious agents, aspirin being a particular example (Brodie and Chase 1967). Rats that were rendered achlorhydric by irradiation did not develop gastric erosions when given large doses ($600mgkg^{-1}$ four times daily) of aspirin subcutaneously (Gottschalk and Menguy 1970). In a complimentary study, Hansen, Aures and Grossman (1978) found that intravenous aspirin had little gross effect in the cat, but when the aspirin was augmented by histamine mainly antral ulcers were induced. In a major review, Flower (1974) recognised that aspirin, amongst other NSAIDs, inhibits prostaglandin biosynthesis. Roth, Stanford and Majerus (1975) demonstrated that aspirin acts as an active-site

acetylating agent for cyclo-oxygenase, thus explaining its anti-inflammatory and anti-platelet action. Fromm (1981) reviewing the role of acid in gastric injury considered that the case had been made, that the presence of acid was required for many injurious agents to produce clinically significant injury, the injury induced by the drugs/agents acting to increase the permeability of the mucosa to its own secreted acid.

1.7.2 Bile

Early evidence that bile was implicated in gastric damage came from work carried out by Smith (1914), and Berg and Jobling (1930) who found that 50% of dogs with Peyton-Rous permanent biliary fistula had gastroduodenal ulcers. In 1959, Borg examined the frequency of bile-stained gastric aspirates in patients with peptic ulcer, found that occurrence increased during the night, peaking at 1-2am in the morning. He speculated that this nocturnal peaking may be due to recumbency and dominant parasympathetic activity during sleep. Similar findings were recorded by Rhodes and others (1969), where the concentration of bile in the stomach due to reflux was higher in ulcer patients. Thus evidence has been found that either bile reflux is a causative factor in peptic ulcer, or if not a primary cause, then a factor that may delay healing.

Hoffman and Small (1967) drew attention to the detergent properties of bile in an extensive review. Then Davenport (1968), Ivey, DenBesten and Clifton (1970) and Werther and others (1970) demonstrated by studying the net ion fluxes, in particular movement of H^+ ions out of the lumen, that in man and animals the mucosal barrier would break after exposure to bile. Davenport (1968) chose bile since it was the detergent most likely to come in contact with the gastric mucosa. He found that bile in sodium chloride, acid or liquid meal damaged or destroyed the mucosal barrier. Confirming earlier work when looking at the effect of acid, aspirin and alcohol Davenport (1969) found that aspirin in neutral solution did not break the barrier, but it would if mixed with 0.001 or 0.01N hydrochloric acid. In one further piece of work Davenport (1970) also showed that lysolecithin would break the barrier when applied to antral vagally denervated pouches. The effect of pH was also studied by Black, Hole and Rhodes (1971), where they noted that the back-diffusion of H^+ was most marked at pH2 and least at pH8. Kuo and Shanbour (1976) accounted for the alteration in ion fluxes that take place by way of increased permeability, the above changes being related to cellular change.

Martin, Marriot and Kellaway (1978) suggested that bile salts decreased the elasticity and viscosity of gastric mucus and that the ulcerogenic properties were related in part to their ability to reduce mucus consistency. Hills, Butler and Lichtenberger (1983) confirmed that this effect was due to bile (and aspirin) eliminating the hydrophobicity

of the mucus layer. Duane and Wiegand (1980) and Duane, Wiegand and Sievert (1982) challenged the gastric mucosa of Heidenhain pouches with 10mM bile and found not only the typical ion fluxes, but also detected an increased phospholipid efflux from the gastric mucosa. However, the result that whilst mucus is solubilized rapidly by pepsin (Allen and Carroll 1985), but unchanged by acid and bile is conflicting.

Cheung, Moody and Reese (1975) examining the effect of various agents on mucosal blood flow found that change in blood flow was directly related to the severity of the injury, and of the three agents used (20mM aspirin, 40mM sodium taurocholate and ethanol) the change in blood flow was most marked with bile. Ritchie (1981a) and Cloud and Ritchie (1982) also showed that topical sodium taurocholate at a low pH increased gastric mucosal blood flow in a dose related fashion. Indomethacin blocked this change and further supplementation with prostaglandins reversed this blocking effect of indomethacin on perfusion, proving that bile-stimulated increased perfusion was mediated by prostaglandins.

Ritchie and Cherry (1979), Harmon, Lewis and Gadacz (1981) and Ritchie (1981b) recognising the ulcerogenic potential of topical bile established that lesion severity was a function of the concentration of H^+ and bile in the lumen. However, Ritchie (1975), Ritchie (1981a) and Cloud and Ritchie (1982) reported topical bile acids not to be ulcerogenic in non-ischaemic mucosa, because of a compensatory increase in blood flow that was related to the induced H^+ loss. Yet Ritchie and Shearburn (1976) found that, in the dog, physiological concentrations of bile salts were sufficient to induce acute mucosal damage. Corroborative evidence was provided by Mann (1976), who noted that bile caused an acute erosive gastritis in rats after one hour.

Eastwood (1974) discovered that taurocholate at 2mM pH1 for 2 minutes produced a reduction in cytoplasmic density and clumping of the nuclear chromatin, when the mucosa was examined by electron microscopy. In 1975, Eastwood found that with bile alone the tight junctions and surface cell membranes remained intact, and confirmed that in the presence of bile, surface cell membranes could be disrupted. Consequently, he concluded that bile salt alters the surface cell membrane in a way that allows either the ingress of the bile salt, or H^+ to cause intracellular damage. Forte, Silen and Forte (1976) recognised large areas of partially damaged cells, ranging from loss of microvilli to complete cell disintegration, but intact junctional complexes. Ritchie (1977) noted consistent transmission electron microscopical findings in barrier damage, namely clumped nuclear chromatin, swollen mitochondria, decreased cytoplasmic and nuclear density, cytoplasmic vacuolization, dilation of the perinuclear spaces and cellular swelling. Yet, admittedly from a

different region of the gastrointestinal tract, *in vitro* ileum of the rabbit, bile appeared to loosen the tight junctions (Fasano and others 1990).

Using scanning electron microscopy to study taurocholate damage Winborn, Guerrero and Hodge (1976) found two types of effect following the application of pure rat bile to the gastric mucosa. Either a massive sloughing with the exposure of the honeycomb lamina propria, or surface cell damage where there was an absence of sloughing were noted. Diserens and others (1984) studied duodenogastric reflux in experimental dogs after diversional surgery and viewed apical cell erosions, exposure of cell contents and cell shells. Again using the scanning electron microscope, Fringes, Lorenz and Oehlert (1985) demonstrated that remnants of surface epithelial cells presented as empty shells, and that the apical membranes of others were covered in mucus vesicles.

Byers and Jordan (1962) implanted part of the fundus, with preserved blood supply, into the gallbladder and looked at the histological changes after the elapse of one to 12 months. They found no histological abnormalities and concluded that pure bile was not an aetiological factor in gastritis, and explained the lack of change as the absence of intestinal juice or adequate gastric secretion. In contrast, Menguy and Max (1970), using the dog as an experimental model, anastomosed part of the antrum to the gallbladder and found that chronic exposure lead to a gastritis with increased rugal folding and occasional polyp formation on these folds. Supporting evidence was the finding that diverting the upper or lower bile duct in the rat produced erosions and ulcers (Kirk 1970).

If bile refluxes, then so also can pancreatic juice. Johnson and McDermott (1974) recovered high levels of lysolecithin from patients with gastric ulcers ($198\mu gml^{-1}$ compared to $18\mu gml^{-1}$ normally). They recognised lysolecithin to be a strong detergent, and also highly toxic to cell membranes. Concluding that lysolecithin was as important as bile in destruction of the gastric barrier. Lawson (1964) noted experimentally that the effects of pancreatic secretions on gastric mucosa were minimal. Rudick and others (1977) were also unable to substantiate this claim, failing to find any change in ion fluxes when the antrum of dogs was exposed to pancreatic juice, or pancreatic juice that had been in contact with the duodenal mucosa for 30 minutes.

Further, bile reflux has also been implicated as a cause of alkaline reflux gastritis in patients following gastric resection for a variety of conditions; Van Heerden and others (1969) - retained gastric antrum, Keighley, Asquith, and Alexander-Williams (1975) - peptic ulcer, Delaney and others (1978), Hoare and others (1978) - gastric remnant, Gaffner, Florén and Nilsson (1984), Watt, Sloan and Kennedy (1984),

Gough (1985) - vagotomy and drainage.

Rhodes and others (1969) looked at bile acids in gastric aspirates from two groups of patients in two situations; a normal and ulcer group following fasting, and after a test meal. In the normal group, fasting bile aspirate from the stomach ranged from 0.01-0.033mM, statistically different compared to 0.03-3.17mM in patients with ulcers on the lesser curvature. Following a test meal, the difference became even more marked with a mean in the normal patients of 0.06mM, and 1.1mM in the ulcer group. Similar findings were reported by Wormsley (1972), with duodenogastric reflux being slight or absent in normal subjects and those with duodenal ulcer, but appreciable in gastric ulcer patients. However, Collins and others (1984) measured total bile acids in gastric juice of patients who had undergone vagotomy and gastrojejunostomy, and found surprisingly wide variations in bile acids in normal and operated patients. They concluded that bile reflux can occur without producing symptoms, but that mucosal changes could be present without producing symptoms.

Delaney, Broadie and Robbins (1975), using the dog as an experimental animal, created tubes of gastric corpus and chronically exposed these to bile, pancreatic juice and pancreatic contents. They found extensive gastritis in all three preparations, but the changes were most marked in those exposed to jejunal contents. Robbins and others (1976) found in man that while bile and pancreatic juices both damage the gastric mucosa, the effects were additive, as would arise when duodenogastric reflux occurred "naturally". In man, partial gastrectomy for duodenal ulcer leads to increased frequency of gastritis, in particular atrophic gastritis (Gjeruldsen, Myren and Fretheim 1968). Chronic exposure caused round cell infiltration into the lamina propria, disorder of the gastric tubules manifested as a "corkscrew" appearance and hyperplasia of the mucous cells leading to a "papillary" configuration (Delaney, Broadie and Robbins 1975, Robbins and others 1976). Confirmatory experimental evidence from the dog was published by Lawson (1981a). Lawson (1981b) found that the Roux-en-Y, which minimises passage of bile into the gastric remnant, prevented the development of gastritis in experimental dogs. Dragstedt and others (1971) using the dog as an experimental animal found histological evidence of gastritis after exposure to bile, but were unable to substantiate the claim from other authors that bile causes peptic ulcers.

Duodenal contents were implicated as presenting carcinogens with an absorptive route when combined with the presence of intestinal metaplasia (seen in atrophic gastritis), since duodenal contents increased lipid solubility and were more active in an alkaline gastric environment (Siurula and Tawast 1956). The incidence of alkaline reflux after diversional surgery has prompted speculation that bile reflux may

be partially responsible for the high occurrence of gastric stump carcinoma (Domellöff 1979 and Domellöff, Reedy and Weisburger 1980). Though finding no evidence of malignant change Watt Sloan and Kennedy (1983) were disturbed by the frequent occurrence of dysplasia in operated patients.

1.7.3 Non-Steroidal Anti-Inflammatory Drugs

From the original discovery by Stone, in 1763, that willow bark reduced fever, salicylate, the active ingredient in willow bark, came to be used extensively. Attention was drawn to the serious manifestations of aspirin as far back as 1930 (Dodd, Minot and Arena). The proliferation of drugs related to salicylate has been marked in the last 15 years and have become to be known as a group - non-steroidal anti-inflammatory drugs (NSAIDs). Many of the new NSAIDs have been associated with serious side-effects in man (Simon and Mills 1980ab). In the veterinary field NSAIDs, as a group, account for a major portion of the drugs dispensed by the small animal practitioner. When new drugs appear on the human market, these are often taken up eagerly by the veterinary profession. Unfortunately because of different species responses, the effectiveness/side-effect balance, as seen in man, is not always mirrored in the species with which the veterinary profession deals. Aspirin (acetylsalycylic acid) is one of the most commonly used NSAIDs, partly because of it's long availability, and secondly because it is obtainable without prescription. The NSAIDs act by suppressing the biosynthesis of prostaglandins (Flower 1974, Roth, Stanford and Majerus 1975, Vane and Botting 1978).

1.7.4 Aspirin

That aspirin damages the mucosa has been recognised for many years in many species; for example in the dog, cat, rabbit and the rat (Barbour and Dickerson 1938, Roth and others 1963, Hurley and Crandall 1964). These last authors found that the mucus coagulated and the mucosa became opacified due to the denaturation of the mucus protein layer and desquamation of underlying cells. Croft (1963) also found exfoliation of cells following the administration of aspirin in man. In 1968, Kent and Allen showed that the biosynthesis of gastric mucus in man was suppressed by aspirin.

Martin (1963) commented that in the gastric lumen all acidic drugs are largely in an un-ionised form. Whereas in the mucosal cell, where the pH is assumed to be 7, the percentage of ionised form will depend upon the pK_a of the acid. In 1964, Davenport showed, using canine vagally denervated pouches, that compounds which are fat soluble cross into the gastric mucosa rapidly. Aspirin dissociates at an acid pH (pK_a 3.5), but being lipid soluble penetrates the apical cell lipoprotein membrane and is rapidly absorbed. Aspirin at pH2 is 95% unionized, fat soluble and diffuses rapidly

across the mucosa. Once inside the cell, it becomes ionised at the higher intracellular pH and is thus able to exert its toxic effects, including bleeding (Davenport 1967a, Garner 1978).

Ample evidence has been provided to show that acid plays a crucial role in the damage that aspirin inflicts to the gastric mucosa. Brodie and Chase (1967) showed that if vagotomised rats were challenged with aspirin and examined 4 hours later, the level of damage was much less than in normal rats challenged with aspirin. They recognised that to induce the typical change in vagotomised rats, 0.1N HCl needed to be added to the stomach of the rats. They also noted that whilst in contact with injurious agents the level of H^+ in the lumen dropped. This feature was due not to acid suppression but to back-diffusion of H^+. In a similar series of experiments confirming the deleterious role of acid, whether it be endogenous or exogenous, Brodie and Chase (1969) demonstrated that the sparing effect of vagotomy was counteracted by the administration of histamine stimulating endogenous acid secretion.

Davenport (1967a) proved that when aspirin diffused across the mucosa, the mucosal characteristics changed with increased fluxes of hydrogen, sodium, potassium and chloride ions. He felt that agents that may be innocuous in contact with the mucosa become toxic if they are able to move into it, and that aspirin would carry hydrogen ions into the cell, or that aspirin was a potent protein precipitor, or enzyme inhibitor. Similar alteration in permeability with back-diffusion of hydrogen ions has been demonstrated in man following aspirin (and also ethanol administration) by Smith and others (1971), which may also affect the alkaline secretion of the mucosal cells (Rees, Gibbons and Turnberg 1984). Equally, aspirin-induced falls in potential difference have been described in man (Murray, Stroltman and Cooke 1974).

Croft (1963) found that in man aspirin caused exfoliation of cells within five minutes of ingesting the drug. The three effects noted by Menguy and Masters (1965) were a local necrosis, mucosal damage and systemic damage. This information was determined from the short term (72 hours) administration of aspirin to young rats. They further noted, histologically, that the amount of Periodic Acid Schiff (PAS) positive material in the mucosa was reduced, indicating that neutral mucoproteins were reduced.

Lynch, Shaw and Wilton (1964) examined the effect of six hour exposure to aspirin on gastric secretion in the cat and found that it was noticeably increased. Grossly, they recorded evidence of bleeding, and histologically there were erosions with the normal cuboidal cells becoming flattened. Taylor and Crawford (1968) demonstrated experimentally that high doses of aspirin produced consistently longitudinal ulceration

in the pyloric part of the stomach. Chvasta and Cooke (1972) looking at the effect of several ulcerogenic drugs on the canine gastric mucosa showed that aspirin produced the highest incidence and the most severe lesions. The aspirin used was mixed in water to produce a solution of 20mM at pH1, which caused bleeding in all dogs. Cohen and MacDonald (1982) studied the damage caused by aspirin to human gastric mucosa and found that prostaglandins were inhibited, but felt that this alone could not explain all the damage present, and found evidence of a direct irritant action causing alteration in mucosal permeability, supporting the conclusions of Roth and others (1963). However, these two proposed methods of action can be linked by the work of Lichtenberger, Richards and Hills (1985) and Kao, Goddard and Lichtenberger (1990), where they discovered that the nonwettable surface of the gastric mucosa is disrupted by aspirin. This disruption would allow direct irritant action, but they found that the nonwettable surface could be preserved by prostaglandins (16,16-dimethyl PGE_2).

Apart from the action on the surface cell, the parietal cell is also affected. Levine, Nandi and King (1990) found that aspirin potentiated acid secretion that had already been stimulated, and that the mode of action was by mobilizing intracellular calcium. They felt that this aspirin-induced augmentation may have been modulated by suppression of prostaglandins.

In the dog, attempts to demonstrate changes radiographically when aspirin has been administered over a short term have not been successful, though the lesions seen at endoscopy of superficial linear erosions and haemorrhage in the antrum and body were confirmed grossly and histologically. The most severe lesions were found at the incisure angularis (Bonneau and others 1972). In man, short courses of aspirin within the therapeutic range will, in normal subjects, produce easily endoscopically recognised damage (Cohen and MacDonald 1982).

Morphologically in mice, Hingson and Ito (1971) established that 20mM aspirin, in combination with various concentrations of hydrochloric acid, produced rapid surface cellular change. Damaged cells were lysed and became exfoliated. Their study, of the fine structural alterations caused by the administration of aspirin/HCl, found nuclear enlargement and chromatin clumping. The cytoplasm became paler and the subsurface mucous granules less uniformly clustered. The apical surface became distorted with blebs of cytoplasm ballooning from the cell. They further noted that the sloughed cells were relatively intact, but trapped in the overlying mucus. The clumping of chromatin and the presence of an agranular space beneath the mucous granules were confirmed by Harding and Morris (1976) and considered characteristic by Eastwood (1985). Work presented by Trabucchi and others (1986) showed that

after one hour exposure to aspirin (200mgkg^{-1}) and acid (0.15N), the mucous cells were difficult to distinguish with transmission electron microscopy (TEM), due to the damage done by the aspirin/acid combination.

Frenning and Öbrink (1971) speculating on the theory that aspirin increased gastric mucosal permeability leading to swelling of cells, due to intracellular accumulation of ionized acids, used scanning electron microscopy to show that feline gastric mucosal cells did indeed become swollen, being more circular than polygonal in shape and accompanied by some incomplete breakdown of intracellular junctions. Harding and Morris (1976), also using scanning electron microscopy, found apical rounding and holes, degeneration and exposure of intracellular contents.

In contrast, Pfeiffer and Weibel (1973), using ferrets as the experimental animal, demonstrated that cell disruption occurred in localised regions, and importantly intracellular junctions remained intact; and with transmission electron microscopy, partial disappearance of microvilli and lack of ground substance between the apical mucous granules were evident, these changes leading to generalized cellular disintegration.

Rats receiving a single dose of 120mgkg^{-1} aspirin *per os* were found histologically to have superficial lesions with desquamation of surface epithelial cells, mainly in the corpus after 30 minutes. These lesions were found to have healed within 24 hours. Similar changes were seen in chronically aspirin-dosed rats (Yeomans, St. John and de Boer 1973). With electron microscopy, repair was visible as flattened surface epithelial cells, which appeared to be derived from adjacent surviving cells, with intercellular gaps (Yeomans 1976). Ohno, Ohtsuki and Okabe (1985) administered aspirin by gastric intubation and found apical erosions of the surface epithelial cells after 10 minutes exposure, using scanning electron microscopy.

1.7.5 Indomethacin and Piroxicam

Chvasta and Cooke (1972) compared the effect of several ulcerogenic drugs on canine fundic mucosa, using a Heidenhain pouch. Indomethacin was found to break the barrier, as measured by Na^+, H^+ and Cl^- fluxes after 30 minutes exposure at pH1 or pH7.3, in contrast to aspirin that required an acidic environment. Similar work by Mann and Saachdev (1976) compared the ulcerogenic effects of aspirin, ketoprofen, ibuprofen and naproxen on the rat gastric mucosa. They found that all four NSAIDs caused erosions, but they were most marked with aspirin with little to choose between the other three.

Thus aspirin is not unique amongst the NSAIDs in its ability to cause toxic damage to

surface epithelial cells and induce disruption of the gastric mucosal barrier. Indeed, indomethacin has been shown to induce acid secretion by significantly inhibiting prostaglandin synthesis (Levine and Schwartzel 1984), presumably by acting in the same manner as aspirin and mobilizing intracellular calcium.

1.7.6 Effect of Combinations

It has already been shown that acid enhances the damaging effects of many barrier breakers. Davenport (1967b) having noted the fact that gastric mucosa, if damaged by salicylates, became more permeable, wondered what would happen if bile was present in such a situation. He found that with luminal concentrations of taurocholate at 10, 20 and 40mM, bile could be found in the venous blood of dogs with Heidenhain pouches, the mucosa of which had been damaged by aspirin. He concluded that bile salt absorption from the stomach was the result of passive diffusion through large pores rather than by active absorption. Guth and others (1976) found that bile duct ligation reduced the effect of aspirin in inducing gastric mucosal lesions, and postulated that this effect was through inhibition of acid secretion.

1.8 Protective Drugs

1.8.1 Histamine H_2 receptor Antagonists

In 1972, Black and others published a paper on the definition and antagonism of H_2 receptors. Recognising that H_1 antagonists did not inhibit gastric acid secretion, they defined two types of receptor and demonstrated the suppressive action of burimamide on acid secretion. The fact that acid was suppressed indicated that histamine was a local common mediator, but also that the actions of gastrin were coupled to those of histamine. Gibson, Hirshowitz and Hutchison (1974) using metiamide reported that histamine blockers of the H_2 variety suppressed histamine, gastrin and cholinergic stimulated acid secretion. Cimetidine was the first of a range of H_2 antagonists to be released for general use (Brimblecome and others 1975, Burland and others 1975). Cimetidine is an analog of histamine that has a bulky side chain substituted for the ethylamine moiety. It retains the imidazole ring of histamine, which has been replaced in more recently released H_2 blockers, ranitidine and famotidine (Ostro 1987, Brunton 1990). Code (1977) found that the H_2 receptor on the parietal cell was a major controller of parietal cell hydrogen ion output and that cimetidine attached to this receptor blocking the pathway of activation. In the same year Hirschowitz and Hutchison reported that cimetidine inhibition of H^+ stimulation was competitive, and that pepsin secretion was also suppressed. However, Code (1977) was unable to find evidence that gastrin was a histamine liberator, and Soll (1980b)

reported that gastrin-stimulated acid was not suppressed by cimetidine as gastrin acted at another receptor site.

Rees and others (1977a) found that sodium taurocholate increased ionic fluxes in Heidenhain pouches, but if the animals were given histamine antagonists (H_1 and H_2) this flux change could be prevented. This action was due to the suppression of histamine release, obviating the normal vascular response. However, earlier work reported by O'Brien and Carter (1975), with metiamide, and Kenyon, Ansell and Carter (1977), with cimetidine did not support this potentially cytoprotective effect. They could find no evidence of restoration of normal ion fluxes in dogs that had the mucosal barriers disrupted by 5mM or 20mM sodium taurocholate and which had been treated with cimetidine. The argument being that whilst H_2 antagonists blocked acid they did not block other actions of H_1 receptors. Microscopic damage was similarly unchanged by pre-treatment with cimetidine when rat mucosa was challenged with absolute ethanol, where extensive damage occurred to the superficial and pit cells (Lacy 1986, O'Brien and others 1990). Despite this, it s role in mucosal injury would be to reduce the amount of H^+ available to exacerbate a barrier damaged by other agents (Carmichael, Nelson and Russell 1978).

Support for this rationale came from MacKercher and others (1977). Here the induced damage to surface epithelial cells by aspirin was cut dramatically by the administration of cimetidine. The consequence of suppressing acid secretion was twofold; firstly, the suppression of acid caused the intraluminal pH to rise dissociating the aspirin and preventing it s absorption by surface epithelial cells; secondly, by suppressing acid, any damage done by aspirin, which is lipid soluble, was minimised because of the lack of H^+ ions available for back-diffusion. One further possible mode of protection would be the inhibition of increased ionic permeability. The evidence for this action is incomplete as both H_1 and H_2 blockers were required to achieve this type of effect (Rees and others 1977a), yet seemingly supported by the results produced by Kauffman and Grossman (1978), demonstrating that the action of PGE_2 and cimetidine, in protecting the mucosa from aspirin ulceration, was not by influencing acid and pepsin output. Support, again using aspirin, for this contention came from Guth, Aures and Paulsen (1979), and with bile from Levine, Sirinek and Pruitt (1979). Noburhara and Takeuchi (1984) providing further evidence for this benefit, found that cimetidine abolished the inhibitory effect of indomethacin on the gastric alkaline response by increasing luminal bicarbonate ion concentration and positively influencing potential difference recovery, accelerating the re-establishment of mucosal integrity.

On the other hand, Grønbech and others (1987) showed that whilst cimetidine

treatment of damage caused by topical sodium chloride increased mucosal blood flow, it alone did not affect restitution, but would do so in the presence of pentagastrin. They concluded that low pH did not prevent restitution, which is somewhat at odds with those supporters of the mucoid cap, which is dissolved by acid (Morris and Wallace 1981). In contrast, Tsukamoto and others (1987) showed that blood flow was decreased by cimetidine at 5 or 20mgkg^{-1}.

When ulcers were produced in the dog by injecting acetic acid subserosally, the rate of healing of the established gastric ulcers did not seem to be affected by the administration of cimetidine, though those in the duodenum healed faster (Okabe and others 1978).

Although cimetidine was the first of the commercial H_2 antagonists, ranitidine is now more widely used (Bohman, Myren and Larsen 1980, Woodings and others 1980), and possibly in the future famotidine (Smith and others 1985). All three suppress basal and stimulated acid secretion (Ostro 1987). Konturek and others (1981a) investigated the cytoprotective effect of ranitidine and compared it to prostaglandins. They found that, even at doses that did not suppress secretion, ranitidine was cytoprotective in the rat against a challenge of aspirin and acid. They also noted that prostaglandin levels did not change, thus the effect of ranitidine was not due to endogenous prostaglandin, or secretory inhibition. In contrast, Konturek (1985) considered that the healing effect (as opposed to acid suppression) of cimetidine, amongst others, was due to the mediating release of endogenous prostaglandins.

1.8.2 Mucus Stimulants

Sucralfate is an aluminium salt formed from sucrose octasulphate and polyaluminium hydroxide. Extensive polymerisation and cross-linking of the sucralfate occurs at pH less than four. The formed polymer is a gel that is sticky and viscid (Nagashima 1981a). It adheres to epithelial cells and ulcer craters (Spiro 1982, Brogden and others 1984). The postulated mechanisms for the protective action of sucralfate are that it forms a barrier on eroded mucosal surfaces, deactivates and binds pepsin, and also binds bile, actions that are pH dependent (McGraw and Caldwell 1981, Nagashima 1981b, Samloff and O'Dell 1985). Other further actions hypothesized to explain the efficacy of sucralfate are that the aluminium ions act to buffer the hydrogen ions as they permeate the sucralfate/mucus layer (Samloff 1983), and stimulate the output of bicarbonate ions by the gastric mucosa (Guslandi 1985). One other suggested mechanism of action (apart from prostaglandins) may involve endogenous sulphydryls (Vergin and Kori-Linder 1990).

However, Danesh, Duncan and Russell (1987) tested the effect of two different pH

environments on the protective action of sucralfate in the rat. The injurious agents chosen were aspirin alone, and aspirin combined with bile. They discovered that sucralfate reduced erosions regardless of pH.

There is evidence that sucralfate stimulates prostaglandins in the gastric mucosa conferring a cytoprotective property, and that indomethacin (inhibiting prostaglandin synthetase) abolishes this sucralfate protection (Hollander and others 1984, Ligumsky, Karmski and Rochmilewitz 1984, Konturek 1985, Coleman and others 1987, Stern, Ward and Hartley 1987). Contrary evidence has been provided by Maclaurin, Watts and Palmer (1985), that whilst indomethacin damage was exacerbated in the guinea-pig by bile salts, sucralfate prevented this damage completely. Corroborative evidence for this protective action against bile, in the face of indomethacin, was published by Romano, Razandi and Ivey (1990), suggesting that there is indeed a protective action that is not dependent on prostaglandins as the mediators. Morris and others (1989) and O'Brien and others (1990) found that pre-treatment with indomethacin made no difference to the protective properties of sucralfate, that sucralfate interacted with the unstirred layer and with the epithelial cells to promote release of mucus and speculated that it may also affect either inflammatory mediator release or synthesis. Tasman-Jones and others (1989) considered that sucralfate did not form a layer on top of the mucus but interacted with the whole layer, and specifically with the superficial portion of mucus, retaining it when it would normally be lost.

Harrington, Schegel and Code (1981) reported that sucralfate enhanced mucus secretion. It has also been shown, using a viscometer, to increase the viscosity of mucus in a concentration related fashion, and also to reduce the permeability of mucus to H^+ (Slomiany and others 1985). In an attempt to define precisely what exactly sucralfate did to the mucus layer Slomiany and others (1989) investigated the effect of five days ingestion of sucralfate in the rat. The features and the results obtained are listed below;

Factor	Increase
Mucus gel dimension	8%
Mucin content	63-81%
Viscosity	190%
H^+ retardation	9%
Hydrophobicity	60%

Their results show that the beneficial effects of sucralfate on the mucus layer are based largely on the increased mucin content, viscosity and hydrophobicity. The mode of action for these features was through sucralfate affinity for proteins, and thus interaction with the proteins of the gastric mucus, which has been proved using ^{14}C

labelled sucralfate (Steiner and others 1982, Slomiany, Laszewicz and Slomiany 1986).

Sucralfate is effective in the treatment of, and recurrence of, peptic ulcer disease in man (Hollander 1981, McHardy 1981, Marks and others 1981). However, apart from it s property of correcting chronic injury, where it binds to the proteinaceous ulcer base and fabricates a barrier over the ulcer site (Garnett 1982, Nakazawa, Nagashima and Samloff 1981), Okabe and others (1983) have shown that it has the ability to protect the mucosa against acute injury. Harrington, Schegel and Code (1981) found that it minimised hydrogen ion back-diffusion, and plasma leakage (Nagashima and others 1983). Nonetheless, a number of reports indicate that it has no antacid properties (McGraw and others 1981, Garnett 1982).

Nagashima and others (1983) and Kuwayama, Miyake and Matsou (1987) compared sucralfate and cimetidine as protectors in the face of ethanol challenge. They found that 200mgkg^{-1} sucralfate produced a 90% inhibition of erosion development compared to controls. Tarnwaski and others (1985b) agreed, and found that H_2 antagonists actually increased the mucosal damage.

Hollander and others (1985) and O'Brien and others (1990) established in rats that pre-treatment with sucralfate would prevent deep mucosal necrosis caused by ethanol, but not superficial disruption. The consequence of this protection from deeper mucosal damage was that restitution occurred rapidly. Those rats used by Hollander and others (1985) were examined between 15 minutes and 4 hours after ethanol instillation. There was a statistically significant reduced drop in potential difference across the mucosa when the ethanol group was compared to the sucralfate group. Histologically, disruption and desquamation of the surface epithelium, notable oedema, as well as leukocyte infiltration were found in both control and sucralfate pretreated rats. They concluded that sucralfate protected the mucosa morphologically and functionally. The restraint of the drop in potential difference by sucralfate suggested to these authors that sucralfate enhanced the mucosal defence mechanisms, perhaps via prostaglandins. However, work by Coleman and others (1987) suggests that any prostaglandin stimulation is of short duration. Interestingly, Shorrock and Rees (1989) found indomethacin had no effect on the secretory response engendered by sucralfate. In the presence of sucralfate the output of bicarbonate ions increased, but returned to basal levels once sucralfate had been removed. In contrast, luminal PGE_2 only rose after sucralfate had been withdrawn. Their conclusion being that prostaglandins and bicarbonate played a significant role in the sucralfate protection.

Work carried out in man has proved that sucralfate decreases the absorption, but not

the eventual bioavailability of naproxen and other non-salicylate NSAIDs. This indicates that sucralfate can be given to ameliorate the gastrointestinal effects of NSAIDs without interfering with their clinical usefulness (Pugh and others 1984, Caille and others 1987, Caldwell and others 1987). Indeed giving aspirin to human volunteers, Tesler and Lim (1981) found that sucralfate afforded complete protection to 8/12 and partial to 3/12 human "guinea-pigs". Further, measurement of serum salicylate levels showed that there had been no inhibition of absorption.

Importantly, sucralfate is stable when confronted with exogenous injurious agents such as aspirin (Okabe and others 1983). It has already been stated that sucralfate is recognised for it s ability to bind to bile. The clinical effectiveness of this property for treating alkaline reflux oesophagitis was shown by Weiss and others (1983), and post-surgical reflux gastritis (Corsini and others 1984).

1.8.3 Adenosine Triphosphate/Calcium Blockers and Others

Prior to the development of H_2 blockers, antacids were the main method of controlling gastritis and ulcers. Antacids are basic compounds that neutralise acid in the stomach. In the presence of food the action of this group of substances is extended to about two hours, compared to total clearance in 30 minutes on an empty stomach. Apart from the ability to neutralise intraluminal acid, aluminium hydroxide, one of the main members of this group, has been shown to chelate bile salts (Clain and others 1977), stimulate mucoprotein secretion (James and Marriott 1982), and decrease pepsin concentration (Sepelyak, Feldkamp and Regneir 1984), and by acting as be cytoprotective (Konturek and others 1991a). However, one other mode of action, which is cytoprotective, is the recognised ability to stimulate output of prostanoids (Berstad and others 1987). Gasbarrini and others (1990) were able to show healing of erosive gastritis and ulcers with high doses of a suspension of aluminium-magnesium hydroxide and an increase in levels of 6-keto-$PGF_{1\alpha}$, presumably causing an increase in mucosal blood flow and thus cytoprotection. Of some concern was the drop in levels of PGE_2, which these authors put down to lesion improvement. Other drugs such as carbenoxolone have been shown to protect the gastric mucosa against bile for example, but have significant side-effects (Calcraft and others 1973).

Recently introduced is omeprazole, which interferes with the hydrogen ion pump (Lind and others 1983). Omeprazole acts as a H^+,K^+-ATPase inhibitor (Ekman and others 1985, Mårdh 1986). Therefore, it quashes the production of acid (Wallmark 1986), and unlike cimetidine it does not affect mucosal blood flow (Tsukamoto and others 1987). At a neutral pH it is inactive, and requires an acid environment to transform into molecules that can enzyme-bind (Clissold and Campoli-Richards 1986,

Sachs and others 1988). Not only is it able to suppress acid production, but has also been shown to have a protective action (Mattsson 1986).

1.9 Aim of Present Work

The first part of the study will involve a partially retrospective analysis of the endoscopic findings in dogs referred to Glasgow University Veterinary School for gastroscopic evaluation, compared with a prospective gastroscopic examination of a group of normal dogs, anaesthetised for problems unrelated to the upper gastrointestinal tract.

The experimental work using the rats aimed to study a number of features of damage to specific parts of the gastric mucosal barrier and to observe the effect of protective drugs on the action of these injurious agents. The action of aspirin and bile alone, and in combination, on the surface epithelial cell will be investigated and compared to the action of carprofen, a new non-steroidal anti-inflammatory drug, alone and in combination, also on the surface epithelial cell.

The efficacy of pre-treatment with protective drugs sucralfate and famotidine will be compared in the face of challenge from aspirin/bile and carprofen/bile combinations.

2. A Study of Vomiting Dogs

2.1 Introduction

The type of food presented to the canine stomach is similar to that consumed by the human and many of the drugs used to treat abnormalities of the stomach of the dog are derived from, or identical to, those used in the treatment of gastric complaints in man. Thus it has been assumed that many of the abnormalities affecting this region of the alimentary tract in the human can occur in the canine. This assumption has been substantiated to some extent with the increase in our knowledge of the variety of conditions known to occur in the dog because of the technology now applied in small animal clinical practice (Else and Head 1980, Strombeck and Guilford 1991).

The spectrum of conditions currently recognised to affect the stomach of the dog include foreign bodies lodged in the stomach, bacterial and viral gastritis, non-specific gastritides, hypertrophic gastropathies, peptic ulceration and gastric neoplasia (Strombeck and Guilford 1991).

The stomach can be involved in pathological processes that also affect other regions of the alimentary tract. On the other hand, the stomach can also be affected by conditions that are primarily associated with other areas of the body, but which cause a secondary response in the stomach -- the clinical signs of which may be the first indication that something is amiss. An example of this is the gastrin secreting islet cell tumour of the pancreas causing hypergastrinaemia (Jones, Nicholls and Badman 1976, Johnson 1989). In man hypergastrinaemia is commonly associated with hypertrophic gastritis (Lewin and others 1984, Clark and others 1986). However, this sequence of events has not been reported to occur as frequently in the dog where hypertrophic gastritis is common (Walter and others 1985, Sikes and others 1986), but hypergastrinaemia is not (Happé and others 1980, Happé, van der Gaag and Wolvekamp 1981, Breitschwerdt and others 1986, Zerbe and others 1989). Thus there appear to be some differences in the two syndromes between the two species, and this might equally apply in other conditions of the canine stomach. Obviously, therefore, abnormal conditions of the stomach of the dog warrants further investigation especially as, as already stated, much of the treatment presently used in veterinary practice is based on that given to humans.

During the past ten years dogs, with stomach complaints were investigated by clinical examination, radiology, gastroscopy and gastric biopsy in an attempt to reach a diagnosis and also to identify the cause of the condition with special reference to any previous treatment given to the animals, not just as treatment for the stomach

complaint but for any other disease suffered by the patient. Finally, the outcome of treatment once a diagnosis was reached was monitored.

2.2 Material & Methods

2.2.1 Control Animals

The control group consisted of 30 German Shepherd dogs. There were 17 males and 13 females ranging in age from 2-10 years, with a mean of 5 years. These animals had been referred for management of anal furunculosis, and had no history or clinical evidence of gastric disease.

2.2.2 Clinical Cases

Details were collected from 100 dogs referred over a 10 year period to Glasgow University Veterinary School with chronic vomiting (Figure 2.1). Initial clinical details recorded included breed, age, sex and duration. Subsequently, these animals were divided into three groups: "no diagnosis", non-neoplastic and neoplastic.

2.2.3 Radiological Investigation

The dogs were originally not sedated for the contrast studies, but following and to comply with the Ionising Radiations Regulations (1985) the dogs were given acepromazine at a dose of $0.05 mgkg^{-1}$ 30 minutes before the start of the study. No narcotic analgesics were given to accentuate the effect of the sedative for handling and positioning. The barium[a] was administered as a liquid via a short oesophageal tube at a dose of between $5\text{-}10 mlkg^{-1}$. The animal was positioned in dorsal recumbency for most of the examination, but right and left lateral positions were also used. The examination was monitored with either image intensification or serial films. The features looked for were evidence of:

delayed emptying	>90 minutes
intraluminal filling defects	large radiolucent areas in the contrast filled stomach
mucosal irregularity	loss of the normal smooth rugal indentations
retention of contrast	presence of small areas of contrast after lumen has emptied
immotile gastric wall	lack of peristaltic waves
intramural outpouching	contrast pouching out from the line of the mucosal/contrast interface

2.2.4 Haematological Investigation

Blood (10ml) was obtained by venipuncture from the jugular vein using 10ml plastic

a: Micropaque Standard - 100% barium sulphate, Nicholas, Slough, U.K.

syringes and 21 gauge hypodermic needles. A 2.5ml sample of this blood was placed immediately in a plastic ethyl diamine tetracetic acid blood tube to prevent coagulation. Parameters looked at were red blood cell numbers, haemoglobin concentration, haematocrit, mean haemoglobin content, white blood cell numbers and changes in the proportion of different white blood cells. Where considered appropriate, platelet and reticulocyte numbers were evaluated and a blood film examined.

2.2.5 Biochemical Investigation

The remainder of the extracted blood (7.5ml) was put immediately into a heparanised container and submitted for biochemical estimation. In most cases the parameters evaluated were blood urea, creatinine, phosphate, serum alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein.

2.2.6 Endoscopy

The endoscopic examination was undertaken with either an Olympus *GIF Type P2* paediatric panendoscope (1m long 9mm diameter) or latterly an Olympus EVIS 200 videoendoscope *GIF XQ200* (1m long 10mm diameter), both equipped with round cup forceps[b]. After a standard overnight fast with no water restriction the dogs were premedicated with acepromazine at a dose of 0.05mgkg^{-1}. They were then anaesthetised by induction with 2.5% sodium thiopentone at approximately 10mgkg^{-1}. Once induced the animals were intubated with endotracheal tubes of appropriate size and maintained on a halothane/nitrous oxide/oxygen mixture using a variety of circuits depending upon the animals' size. The dogs were placed in left lateral recumbency before introduction of the endoscope into the stomach. First, the gastric contents were evaluated. The presence of hair, foam and bile reflux were noted. The presence of foam or bubbles was graded in accordance with system proposed by McNally, Maydonovitch and Wong (1988):

+	=	no foam or bubbles
+ +	=	minimal
+ + +	=	moderate
+ + + +	=	abundant obscuring mucosal surface

b: Olympus endoscopic forceps FB-1K (GIF type P2) and FB-25K (GIF XQ200), KeyMed, Southport, U.K.

The presence of food was graded as being absent/trace, occasional lumps or near complete mucosal coating. The presence of a bile pool (yellowish green) was graded subjectively on the basis of the apparent volume present on a scale of 4, where 0=none and 3=large pool. It was not possible to remove fluid for most of the study period, but latterly an Olympus suction pump[c] was available and the bile could be withdrawn from the stomach (Figure 2.2). Where bile reflux (bright yellow) from the duodenum was observed it was described as:

+	=	minimal
+ +	=	moderate
+ + +	=	severe

The colour of the antral mucosa was recorded. Inability to examine the whole mucosal surface was noted if the volume of foam or fluid was excessive.

Second, the stomach surface was divided into fundus, body, antrum and pylorus for the purposes of localising change, which was defined as single, focal or generalised. Specific change looked for included:

mucosal inflammation	reddened mucosa
follicular speckling	where the mucosa develops red areas and gives the mucosa a "*measles*" like appearance
mucosal hypertrophy	where the rugal folds were subjectively wider and deeper and tended not to disappear on insufflation
erosions	shallow ulcer-like lesions <0.5cm in diameter
ulcers	≥1cm in diameter. The size of the ulcers were measured by passing a graduated flexible polythene tube through the biopsy channel of the endoscope

c: Olympus KeyVac II Endoscopic suction pump, KeyMed, Southport, U.K.

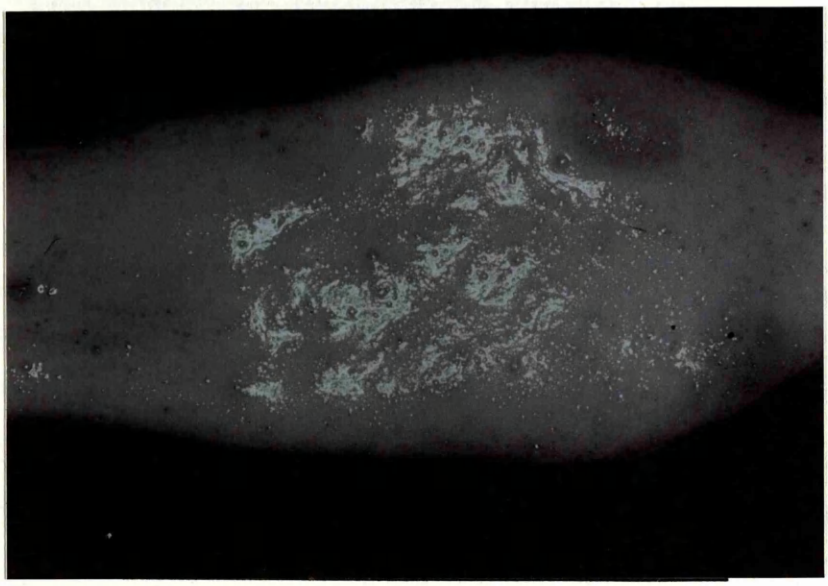


Figure 2.1. The *"Foamy Boke"*. Vomited saliva and gastric juice.

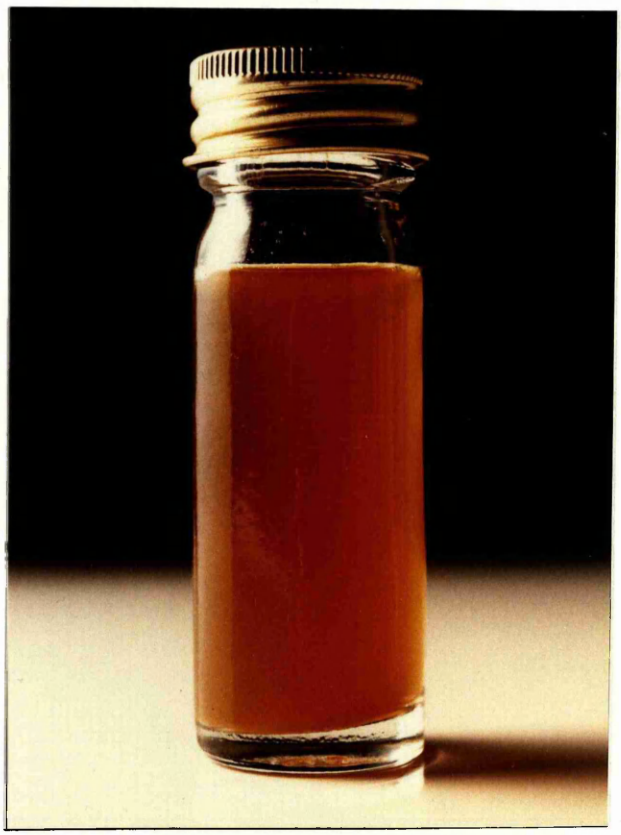


Figure 2.2. Sample of aspirated bile from a dog with duodenogastric reflux.

2.2.7 Pathology

The samples were obtained by

1] endoscopic biopsy forceps - the forceps were passed through the biopsy port, down the biopsy channel and pushed firmly against the area of interest by driving both the open forceps and endoscope forwards. The angle of approach was preferably between 45-90° to the mucosal surface. The jaws were closed, the endoscope withdrawn a little and the forceps retracted into the biopsy channel.

2] laparotomy - when the stomach was exposed for a surgical procedure full-thickness samples could be obtained.

3] *post-mortem* examination - where a specific lesion had been identified the sample was from that area, where the change was generalised a representative sample was taken, usually, from the body of the stomach on the dorsal face.

Samples were placed in fixative (4% buffered normal formalin (BNF)) immediately after harvesting and immersion fixed in 4% BNF for a minimum of 48 hours. The sections were cut at 3-5μm and stained with haematoxylin and eosin (H&E). Further details applicable to this and other chapters are supplied in Appendix 9.

2.2.8 Treatment and Outcome

The animals from the "no diagnosis" group were not treated, other than to advise avoidance of scavenging. In the non-neoplastic group the animals were treated according to the diagnosis reached thus therapeutic regimes including antibiotic therapy, H_2 blocker therapy, antiemetics, corticosteroids, surgery and altered diet were used. Those animals with neoplasia were nearly all destroyed shortly after diagnosis was reached.

2.3 Results

2.3.1 Details of Animals in Each Group

"No diagnosis" = 18	Non-neoplastic	=60	Neoplastic	=22
	chronic gastritis	9	carcinoma	17
	hypertrophic gastropathy	9	lymphosarcoma	3
	peptic ulcer	9	leiomyosarcoma	1
	gastroenteropathy	9	APUDoma	1
	erosive gastritis	6		
	duodenogastric reflux	5		
	follicular gastritis	3		
	atrophic gastritis	2		
	foreign body	2		
	pyloric stenosis	2		
	gastric dilation	2		
	parasitic ulceration	1		
	piroxicam toxicity	1		

Table 2.1. Dogs grouped by diagnosis in a series of 100 vomiting dogs.

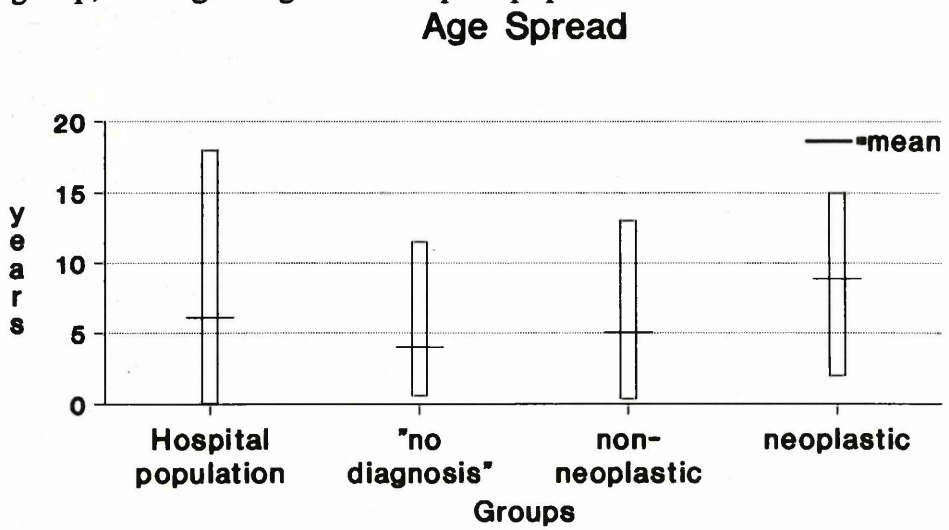
2.3.2 Age and Duration of Clinical Signs

The minimum and maximum age of the animals presented differed little between the first two groups of animals. However, in the neoplastic group the mean age was about twice that of the other two groups (Table 2.2, Figure 2.3). The youngest dog recorded with a tumour was a 2 year-old West Highland White terrier with a neuroendocrine tumour or APUDoma.

The average length of time that the vomiting had been present was very similar for the "no diagnosis" and non-neoplastic groups. However, there was considerable variation between the conditions comprising the non-neoplastic group from the dogs with peptic ulcer (3.83 weeks) to the dogs with gastroenteropathy (40.78 weeks) (Table 2.3). The maximum time and the average time that vomiting had been present prior to referral was much shorter for those dogs that had neoplasia (Table 2.2, Figure 2.3).

	age (years)			duration (weeks)		
	min	max	mean	min	max	mean
hospital population	0.04	18.00	6.10			
"no diagnosis"	0.50	11.50	4.00	2.00	182.00	19.17
non-neoplastic	0.38	13.00	5.09	0.40	156.00	20.40
neoplastic	2.00	15.00	8.98	1.43	52.00	11.47

Table 2.2. Age and duration (mean and range) of clinical signs in 100 vomiting dogs by group, with age range from hospital population.



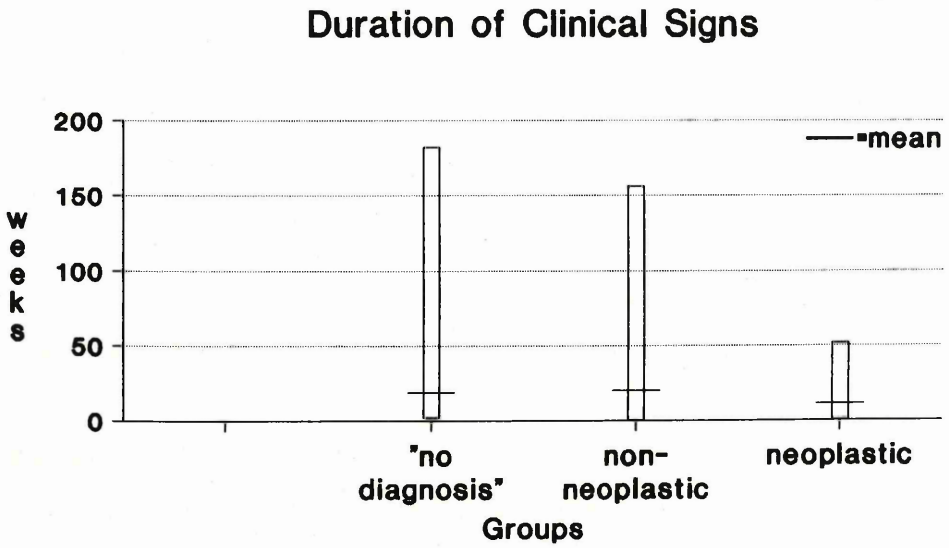


Figure 2.3. Bar charts of age of animals and duration of signs in 100 vomiting animals.

Condition	no	Number M	C	F	S	Mean Age years	Duration weeks
None Diagnosed	18	7	2	7	2	4.00	19.17
Chronic gastritis	9	4	0	2	3	4.45	17.17
Hypertrophic gastritis	9	3	1	2	2	5.21	21.77
Peptic ulcer	9	6	0	1	1	6.40	3.85
Gastroenteropathy	9	5	0	2	1	4.96	40.78
Erosive gastritis	6	2	0	4	0	5.67	33.83
Duodenogastric reflux	5	3	1	1	0	6.07	15.00
Follicular gastritis	3	2	0	1	0	4.19	3.33
Atrophic gastritis	2	1	0	1	0	2.00	20.00
Foreign body	2	1	0	1	0	3.50	21.00
Pyloric stenosis	2	2	0	0	0	0.46	12.00
Gastric dilation	2	2	0	0	0	5.00	8.50
Parasitic ulceration	1	0	0	1	0	4.00	104.00
Piroxicam toxicity	1	1	0	0	0	10.00	0.60
Gastric carcinoma	17	12	0	3	2	9.68	12.44
Lymphosarcoma	3	2	0	0	1	7.33	7.67
Leiomyosarcoma	1	0	0	1	0	15.00	52.00
APUDoma	1	0	0	0	1	2.00	52.00

(M = male, C = castrate, F = female, S = speyed, no = number)

Table 2.3. Age, sex and duration of 100 vomiting dogs by condition.

2.3.3 Breed

Only three giant breeds were found in this review which matches the typical hospital population over a similar period (Table 2.4). In the "no diagnosis" group 6/18 were terrier type dogs and 5/18 were Labradors. The number of Labradors found matched the percentage found in the hospital population. A wide variety of breeds were found in the non-neoplastic group with no breed preponderance. In the neoplastic group 5/22 were Rough collies and 4/22 were Labradors. The number of Rough collie dogs was more than would be found in the general hospital population.

2.3.4 Sex

The distribution of males to females in the general hospital population was 0.96:1. The ratios for male to female for the three groups were "no diagnosis" 1:1, non-neoplastic 1.3:1 and neoplastic 1.75:1. Within the non-neoplastic group only those animals with peptic ulcer had a ratio (3:1) that differed markedly from the whole group (Table 2.3). For those dogs with gastric carcinoma the ratio was 2.4:1. The

reproductive status of the males and the females across the groups was broadly similar with few of the male dogs being castrate and a third of the females being neutered.

Breeds	No diagnosis	Non-Neoplastic	neoplastic	10 year hospital population (%)
Cross	0	9	2	7.96
Labrador	5	4	4	10.26
Retriever	0	3	1	4.10
Boxer	2	3	0	2.99
Border collie	0	0	1	4.07
Rough collie	2	2	5	2.47
Shetland collie	0	2	0	2.34
Yorkshire terrier	2	0	0	2.58
Border terrier	1	0	1	0.35
Cairn terrier	1	2	0	1.78
Jack Russell terrier	0	4	0	1.95
Scottish terrier	1	0	0	0.80
Staffordshire Bull terrier	0	0	1	0.70
West Highland White terrier	1	4	2	3.14
Irish setter	1	2	1	1.58
German Shepherd dog	1	4	3	10.43
Cocker spaniel	1	1	0	3.21
Springer spaniel	0	4	0	2.52
Tibetan spaniel	0	2	0	0.07
German Shorthaired pointer	0	2	0	0.37
Bull Mastiff	0	1	0	0.78
Beagle	0	1	0	1.14
Bernese Mountain dog	0	1	0	0.12
Corgi	0	1	0	0.85
Doberman pinscher	0	1	0	1.91
Dalmation	0	1	0	0.42
Great Dane	0	1	0	1.03
Deerhound	0	0	1	0.19
Miniature Schnauzer	0	1	0	0.09
Poodle	0	1	0	2.70
Rottweiler	0	1	0	0.70
Weimaraner	0	1	0	0.21
Whippet	0	1	0	0.41
Total	18	60	22	

Table 2.4. Breeds encountered in each group of vomiting dogs and numbers of these breeds found in hospital population over 10 years.

2.3.5 Haematology

The range of haematological findings are displayed in Table 2.5. Only 17/100 cases had abnormal findings recorded. In the "no diagnosis" group 4/18 had abnormal findings. One Labrador had a hypochromic microcytic anaemia due to iron deficiency. Of the other three dogs, one had an elevated white blood cell count mainly due to neutrophils, the other two had mild eosinophilia (7% and 9%)

Two animals from the neoplastic group had abnormal haematological findings. One animal with lymphosarcoma had target cells and reactive lymphocytes, and the dog with an APUDoma had a normochromic normocytic anaemia.

The other 11 cases were from the non-neoplastic group and no particular diagnosis showed consistently abnormal results. Only 3/6 dogs with eosinophilic gastroenteropathy had elevated circulating eosinophils. Of the cases of hypertrophic gastropathy, one had target cells, Howell-Jolly bodies, anisocytosis and polychromasia and the second some evidence of anaemia. Three of the nine cases with peptic ulcer had anaemia, as did the dog with piroxicam toxicity. One dog with chronic gastritis had a relatively normal picture with only polychromasia noted. Only one animal with duodenogastric reflux had abnormal features indicating some blood loss. The sole case of duodenal ulcer had evidence of blood loss, but also an elevated white blood cell count of 21.2 $x10^9L^{-1}$.

Condition	RBC	Hct	Hb	MCV	WBC	N	L	E
units	$10^5 mm^{-3}$	%	$g100ml^{-1}$	fl		$10^9 L^{-1}$		
Normal	6-8	35-40	11-14	65-75	6-12	3-11.8	1-4.8	0.01-1.25
"no diagnosis" (18)								
mean	**7.96**	**44.12**	**15.19**	**69.17**	**12.01**	**9.45**	**1.74**	**0.50**
min	5.31	28.20	8.70	54.00	7.20	4.82	0.22	0.07
max	17.30	53.20	18.90	76.00	22.20	20.65	4.66	2.00
Chronic gastritis (9)								
mean	**9.45**	**48.83**	**17.68**	**68.25**	**7.50**	**77.75**	**18.13**	**2.17**
min	6.49	44.70	16.00	66.00	5.30	46.00	5.00	0
max	18.40	53.60	19.50	71.00	10.00	95.00	48.0	5.50
DGR (5)								
mean	**4.71**	**30.37**	**11.25**	**61.67**	**13.25**	**10.40**	**2.17**	**0.24**
min	3.32	14.00	3.70	43.00	5.50	3.96	0.82	0.03
max	5.48	40.90	14.00	74.00	21.20	19.93	5.09	0.64
GEP (9)								
mean	**6.34**	**44.71**	**15.97**	**71.51**	**9.91**	**7.26**	**1.54**	**0.58**
min	4.02	29.00	10.50	67.60	7.70	4.70	0.54	0.08
max	7.56	52.40	18.30	72.00	13.30	11.30	3.19	1.46
HG (9)								
mean	**5.52**	**38.03**	**13.90**	**69.67**	**8.33**	**6.05**	**1.64**	**0.19**
min	4.64	33.30	12.40	68.00	4.60	2.76	0.51	0.02
max	6.03	41.30	15.20	72.00	14.20	11.36	4.12	0.57
Peptic ulcer (9)								
mean	**3.64**	**25.40**	**10.10**	**74.67**	**3.10**	**2.14**	**0.55**	**0.03**
min	1.77	16.80	4.90	54.00	1.50	0.69	0.08	0
max	5.68	40.50	15.60	96.00	32.80	28.37	13.78	0.66
Erosive gastritis (6)								
mean	**5.95**	**43.70**	**15.43**	**73.67**	**8.78**	**6.76**	**1.14**	**0.62**
min	5.60	40.60	13.80	72.00	5.50	3.9	0.66	0.11
max	6.18	45.80	18.90	75.00	13.30	11.04	1.86	1.6
Neoplasia (22)								
mean	**6.47**	**44.88**	**16.65**	**69.83**	**11.72**	**9.90**	**1.15**	**0.26**
min	5.24	32.50	11.10	63.00	6.30	4.72	0.44	0
max	7.82	54.90	19.80	73.00	17.90	15.93	2.06	1.61

min = minimum, max = maximum, RBC = red blood cells, Hct = haematocrit, Hb = haemoglobin, MCV = mean corpuscular volume, WBC = white blood cells count, N = neutrophils, L = lymphocytes, E = eosinophils, DGR = duodenogastric reflux, GEP = gastroenteropathy, HG = hypertrophic gastritis.

Table 2.5. Haematological values of vomiting dogs based on condition (only those diseases with >4 cases are included and the neoplastic group are treated as one).

2.3.6 Biochemistry

A summary of the biochemical values examined are shown in Table 2.6, these are grouped according to diagnosis.

Condition		SAP	AST	ALT	TP
units		UL-1	UL-1	UL-1	gL-1
Normal		0-105	0-40	0-40	50-78
None diagnosed	**mean**	**125.70**	**29.40**	**76.30**	**65.30**
	minimum	54.00	21.00	31.00	54.00
	maximum	257.00	48.00	250.00	85.00
Chronic Gastritis	**mean**	**186.20**	**56.20**	**58.40**	**57.40**
	minimum	51.00	27.00	29.00	41.00
	maximum	554.00	129.00	121.00	73.00
Duodenogastric reflux	**mean**	**99.75**	**26.30**	**32.50**	**59.30**
	minimum	37.00	20.00	22.00	55.00
	maximum	264.00	29.00	42.00	68.00
Gastroenteropathy	**mean**	**164.00**	**38.30**	**50.80**	**61.40**
	minimum	74.00	24.00	26.00	55.00
	maximum	343.00	68.00	79.00	69.00
Hypertrophic gastritis	**mean**	**425.00**	**39.50**	**64.50**	**70.50**
	minimum	128.00	29.00	55.00	70.00
	maximum	722.00	50.00	74.00	71.00
Peptic ulcer	**mean**	**268.30**	**24.70**	**27.00**	**57.30**
	minimum	67.00	18.00	18.00	50.00
	maximum	620.00	34.00	34.00	64.00
Erosive gastritis	**mean**	**87.40**	**23.80**	**43.00**	**56.20**
	minimum	46.00	17.00	24.00	40.00
	maximum	156.00	34.00	79.00	60.00
Neoplasia	**mean**	**90.50**	**34.25**	**64.00**	**65.15**
	minimum	32.00	18.00	20.00	49.00
	maximum	217.00	73.00	210.00	80.00

SAP = serum alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, TP = total protein.

Table 2.6. Serum biochemical values of vomiting dogs based on condition (only those diseases with >4 cases are included and the neoplastic group are treated as one).

Twenty six animals had abnormal biochemical findings. These deviations from normal were largely restricted to elevation in liver enzymes particularly ALT, with a few cases demonstrating hypoproteinaemia. Six of the 18 "no diagnosis" cases had elevated liver enzymes. Alanine aminotranferase was higher than normal in all six, two of these also had mildly raised serum alkaline phosphatase, and one had raised AST. Three dogs with neoplasia had abnormal biochemical findings. Two had

elevation of all three enzymes but no evidence of hypoproteinaemia, in addition one dog with lymphosarcoma was hypoproteinaemic.

The remaining 17 dogs fell into the non-neoplastic category and there was no apparent pattern as regards disease, though 5/9 with chronic gastritis had abnormal liver enzymes. ALT was the most commonly elevated enzyme in 10 dogs, alkaline phosphatase was higher than normal in seven dogs with three >500UL^{-1}, and AST in five.

In none of the 100 cases was there electrolyte imbalance, or elevated urea, creatinine and phosphate levels.

2.3.7 Radiology

The radiological features are summarised in Table 2.7.

Condition	no	delayed emptying	poor peristalsis	intraluminal filling defect	mucosal irregularity	contrast retention	ulcer
None Diagnosed	18	0	0	0	0	0	0
Chronic gastritis	9	0	0	0	0	0	0
Hypertrophic gastritis	9	9	7	5	0	4	0
Peptic ulcer	9	1	0	0	3	3	6
Gastroenteropathy	9	0	0	0	0	0	0
Erosive gastritis	6	0	0	0	0	0	0
Duodenogastric reflux	5	0	0	0	0	0	0
Follicular gastritis	3	0	0	0	0	0	0
Atrophic gastritis	2	1	0	2	2	2	0
Foreign body	2	0	0	0	0	0	0
Pyloric stenosis	2	2	0	0	1	0	0
Gastric dilation	2	2	2	0	0	0	0
Parasitic ulceration	1	0	0	0	1	1	1
Piroxicam toxicity	1	ND	ND	ND	ND	ND	ND
Gastric carcinoma	17	1	0	10	10	3	13
Lymphosarcoma	3	0	0	1	2	1	1
Leiomyosarcoma	1	1	0	1	0	1	1
APUDoma	1	0	0	1	0	0	1

ND = not done, no = number

Table 2.7. Some radiological features found in 100 vomiting dogs.

No abnormalities were detected on radiological investigation of the "no diagnosis" category and in most animals from the non-neoplastic group. The exceptions were the hypertrophic gastropathies, atrophic gastritis and one case of duodenogastric reflux. In those cases of hypertrophic gastropathy there was exaggeration of the rugal folds which were wider than the inter-rugal areas and in a few cases there was also pyloric outflow obstruction evident (Figures 2.4-2.8). Similar changes were obtained from the studies of the two cases of atrophic gastritis, one of which had a large intraluminal filling defect (Figure 2.9). In the case of duodenogastric reflux there was marked reflux of the liquid barium through the pylorus during the examination, a feature not noted in the other cases. Only six of those animals with peptic ulcer had the ulcers detected radiographically and the other three had a non-specific mucosal irregularity and mucosal retention of contrast. The peptic ulcers were seen as small ulcers at the edge of the contrast rather than *en face*. The ulcers were evident as small mushroom-like outpouchings of contrast in the mucosal wall. In only one dog was the classical Hampton line seen, though in all cases there was a lack of a tall shoulder that indicates marked stomach wall thickening (Figure 2.10). In 50% of the tumour cases the radiographic studies were diagnostic. These were all gastric carcinomas where narrowing of the pyloric canal, poor motility of the antral wall, mucosal irregularity and ulceration were all noted (Figure 2.11). In some there were equivocal findings, which were inadequate to justify making a diagnosis. In the lymphosarcoma cases no abnormalities were detected.

Figure 2.4. Hypertrophic gastritis. Lateral abdominal radiograph. The rugal hypertrophy has produced large intraluminal indentations in the contrast.

Figure 2.5. Hypertrophic gastritis. Lateral abdominal radiograph. Semi-annular thickened rugal fold at the pyloric sphincter.

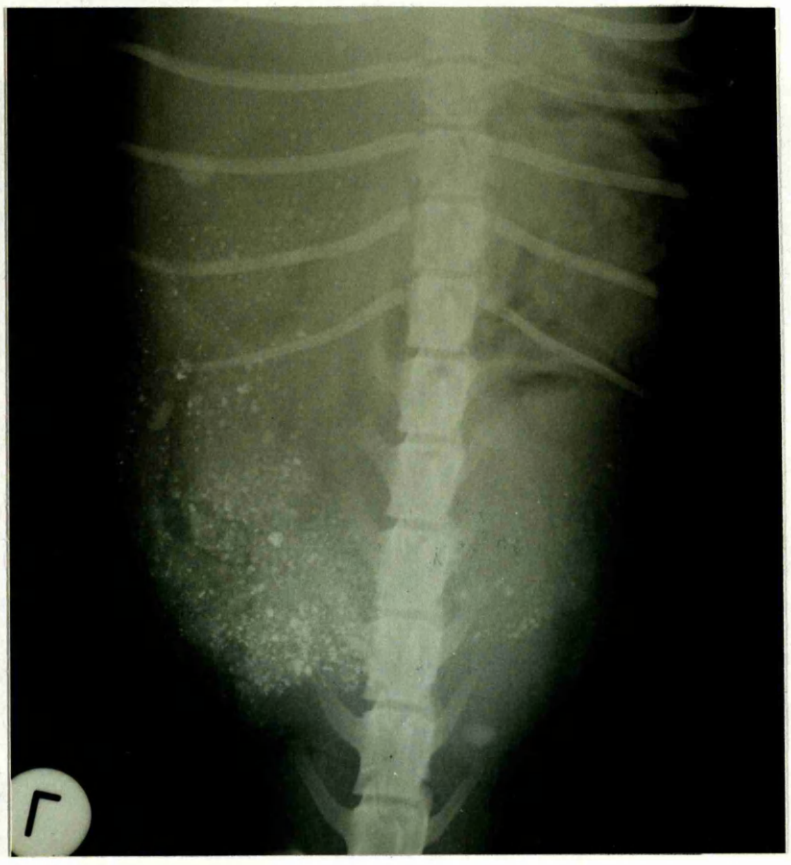


Figure 2.6. Hypertrophic gastritis. Ventro-dorsal abdominal radiograph. There is gross distension of the stomach, which is extending caudally to fill the abdominal cavity. The stomach contains a large volume of ingesta and retained mineralised material - the *gravel sign*.

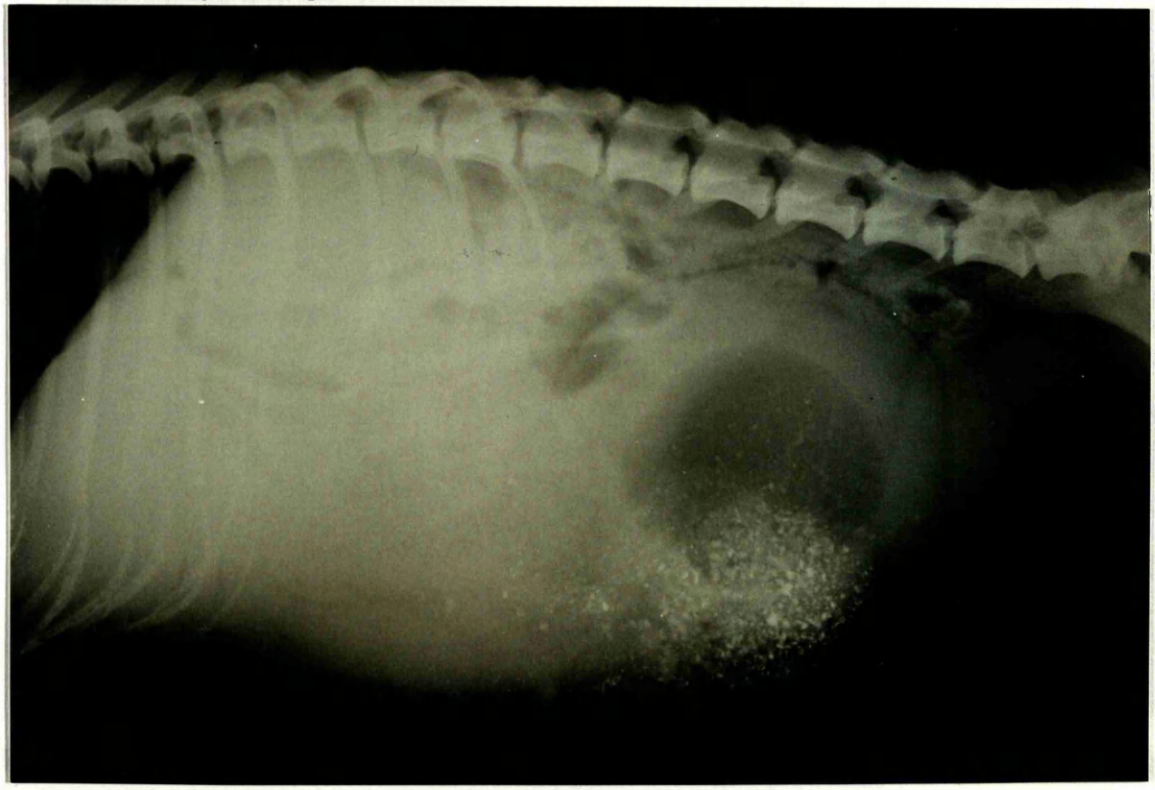


Figure 2.7. Hypertrophic gastritis. Lateral abdominal radiograph. There is caudal displacement of the pylorus, and also the *gravel sign* indicating chronic partial obstruction.

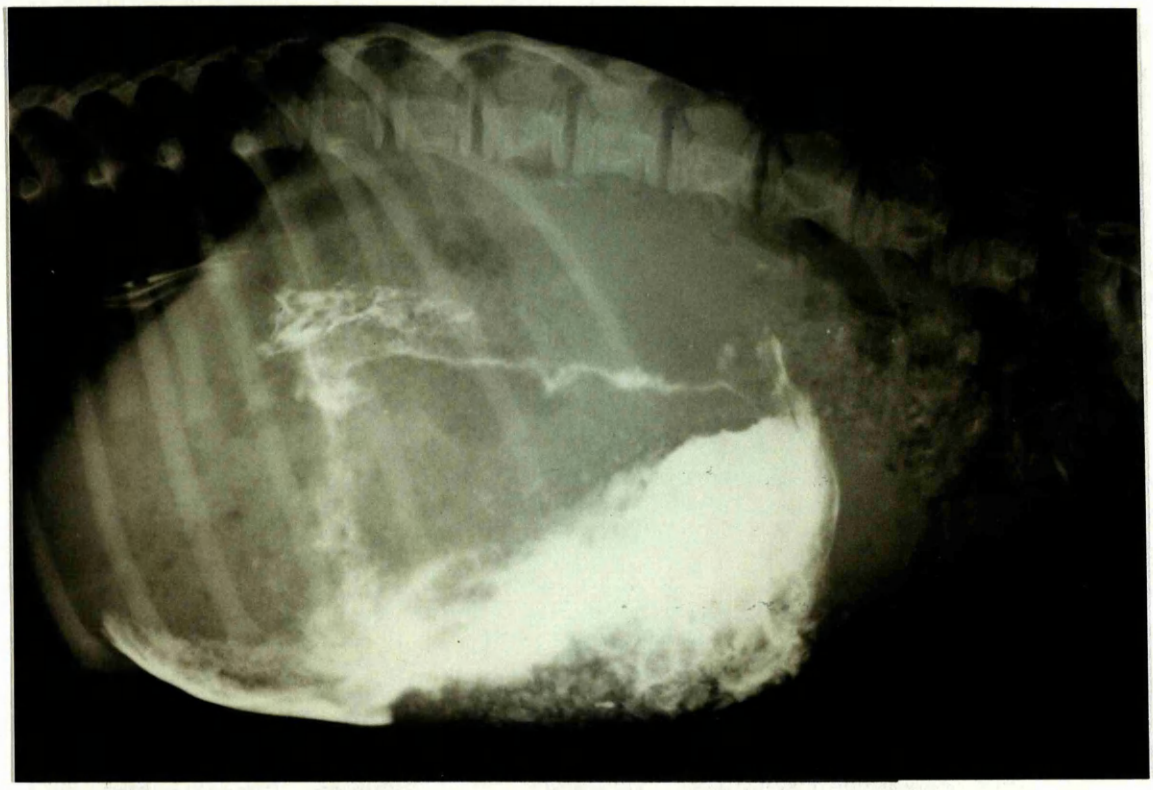


Figure 2.8. Pyloric stenosis. Lateral abdominal radiograph. Following the administration of barium, the stomach is seen to be grossly distended with barium and retained ingesta.

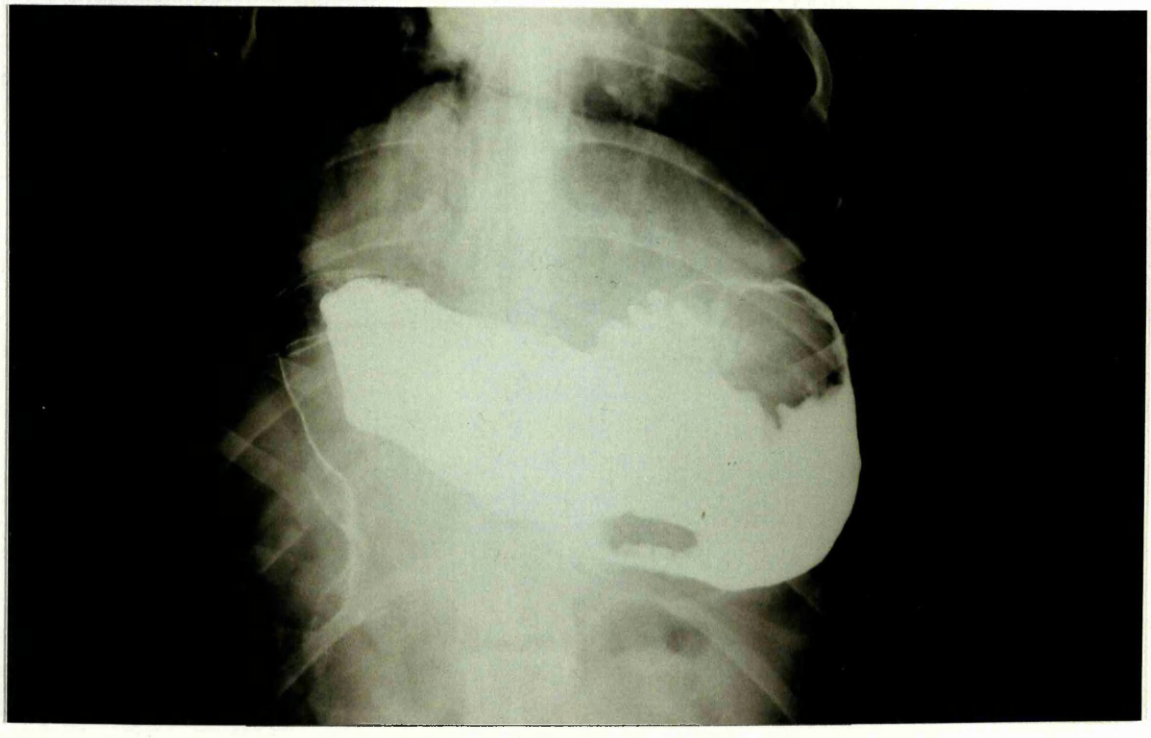


Figure 2.9. Atrophic gastritis. Dorso-ventral abdominal radiograph. A large filling defect in the contrast filled stomach is present. This is most obvious in the fundus with an undulating margin extending to the greater curvature of the body of the stomach.

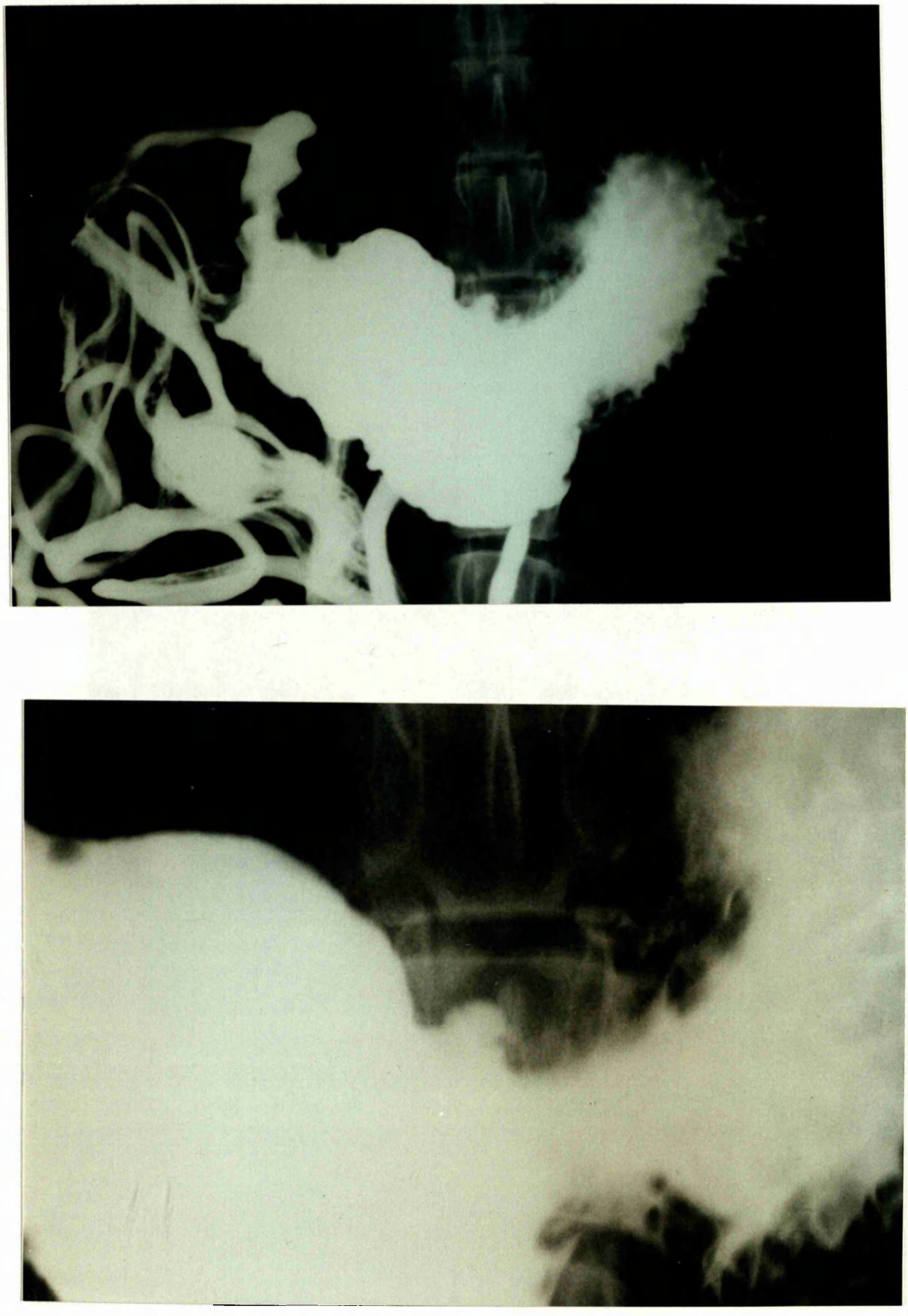


Figure 2.10a & 2.10b. NSAID induced peptic ulcer. Dorso-ventral abdominal radiograph. The barium contrast illustrates a small outpouching of contrast in the mucosal wall with a low shoulder of surrounding mucosa. The ulcer has a radiolucent ring midway down the ulcer - the *Hampton line*.

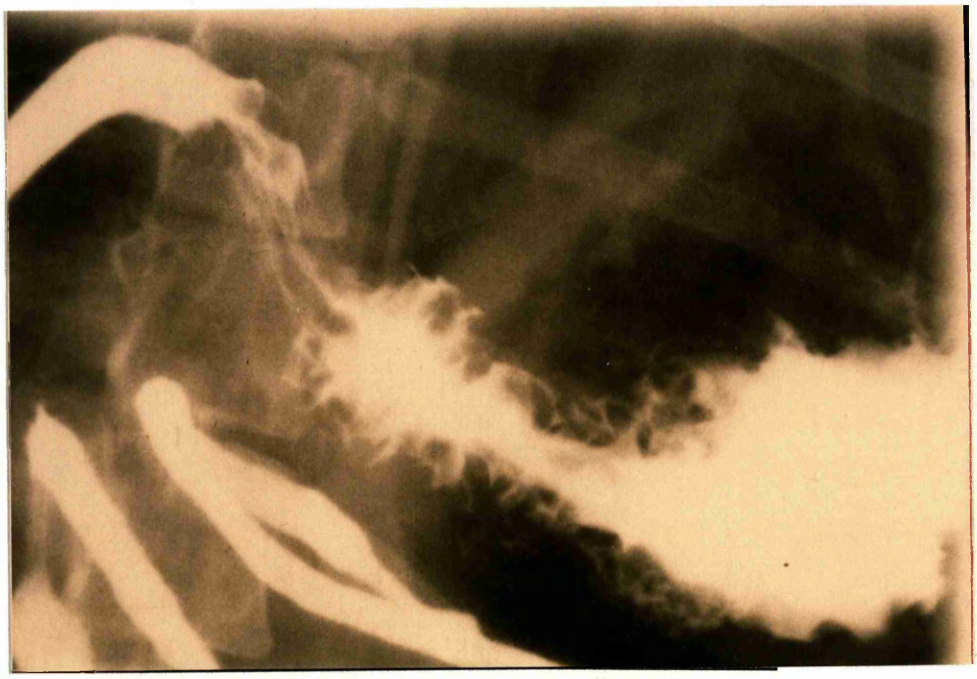


Figure 2.11. Gastric carcinoma. Dorso-ventral abdominal radiograph. The lumen of the pylorus and antrum is narrowed with an extremely narrow lumen with an intense corrugated pattern to the contrast/mucosa interface caused by ulcerated mucosa.

2.3.8 Endoscopy

The contents found in the stomachs and their effect on endoscopy are summarised in table 2.8 for both control German shepherd dogs and those vomiting dogs.

Group		Controls	"no diagnosis"	Non-neoplastic	Neoplastic
number		30	18	60	22
food	-none/trace	19	16	52	22
	-lumps	7	0	4	0
	-coating mucosa	4	2	4	0
hair		7	4	15	2
white foam		11	1	7	3
bile pool	score 0	19	18	35	20
	1	6	0	4	2
	2	5	0	10	0
	3	0	0	11	0
total		11	0	25	2
bile reflux	score 0	22	22	42	20
	1	8	0	0	1
	2	0	0	8	1
	3	0	0	10	0
total		8	0	18	2
antrum red		7	0	11	2
mucosa obscured		7	0	11	2
	-foam	4	0	3	0
	-fluid pool	3	0	8	2
	-blood	0	0	2	0

Table 2.8. Gastric luminal contents found at endoscopy in the control dogs and 100 vomiting dogs.

Despite starvation, the stomachs of four control dogs (13.33%) and six (6%) vomiting dogs retained sufficient food to line the whole mucosal surface (Figure 2.12). Insufflation of air did allow windows of mucosa to be created to permit areas of the gastric mucosa to be viewed. In seven of the control dogs (23.33%) occasional lumps of food were found in the body and antrum, in comparison to only four of the vomiting dogs. The presence of hair (23.33% and 22%) in small volumes of adherent fluid did not impair appraisal.

The presence of white froth or foam in 11 animals in both groups (36.66% and 11%) obscured the mucosal surface. This hindrance to the evaluation of the mucosa could, to a certain extent, be overcome by insufflating the stomach. However, in four

control animals it rendered appraisal impossible, and insufflation had the drawback of largely ablating the rugal folds, thus they could not be adequately assessed (Figures 2.13 and 2.14).

Bile was recognized in the stomachs of 11 (36.66%) of the controls dogs and appeared as a greenish yellow pool of fluid. In the vomiting groups no pooling was seen in those animals where "no diagnosis" was reached but pooling was present in 41.67% of those belonging to the non-neoplastic group. In five control dogs the volume was sufficiently large to form a puddle in the dependent portion of the stomach (score 2) (Figure 2.15). The volume of the bile pool in 11/25 of the non-neoplastic group was greater that in the controls (score 3). By turning the dogs from left to right lateral recumbency, the area submerged in bile could be examined satisfactorily. In only one dog, a control, was both white foam and bilious fluid noted.

No animals from the "no diagnosis" group were noted to have bile reflux during the endoscopic examination. Actual reflux of bile from the duodenum was visible when the antrum and pylorus were examined in eight dogs (26.67%) from the control group compared to 18/60 (30%) from the non-neoplastic group. The reflux was seen as bubbling yellow fluid emerging from the pyloric canal. In six control dogs and 11/18 non-neoplastic dogs, the reflux gave the antral mucosa a red hue, either due to inflammation or to coating of the mucosa and altering the reflected cold-light. Existing luminal bilious fluid together with definite duodenal reflux of bile was observed in three control dogs and 16 of the non-neoplastic dogs.

The mucosal surface was obscured by luminal contents in 7/30 control dogs and 13/100 vomiting dogs. In the controls dogs this was due to the presence of foam (4) or the fluid pool (3), whereas in the non-neoplastic group it was more likely to be due to fluid (8) as opposed to foam (3). In two dogs it was due to the overwhelming presence of blood.

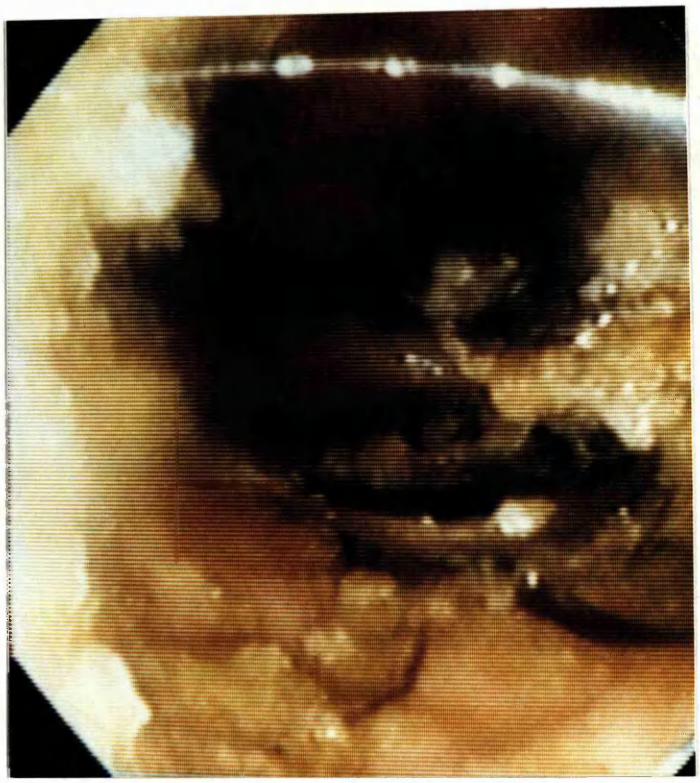


Figure 2.12. Endoscopic view of a filled-food antrum obviating adequate examination.

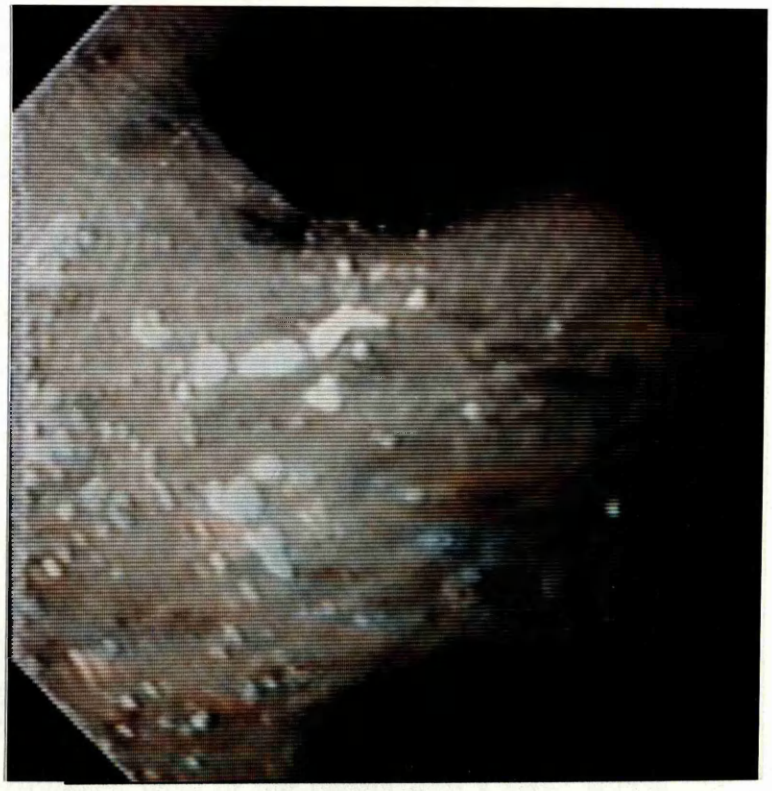


Figure 2.13. Froth in the stomach of a control dog.

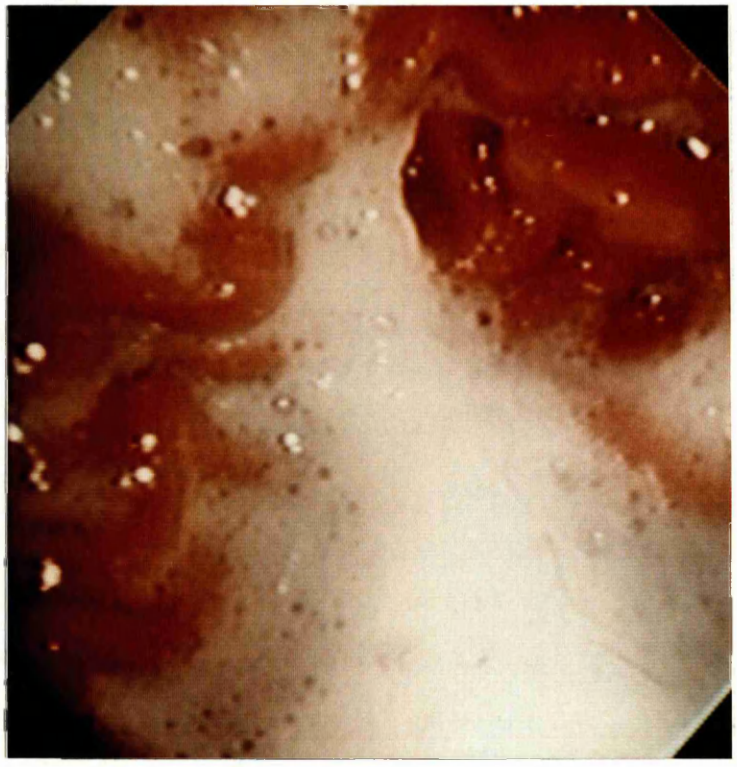


Figure 2.14. Froth partially coating the rugal folds of a dog with gastritis.

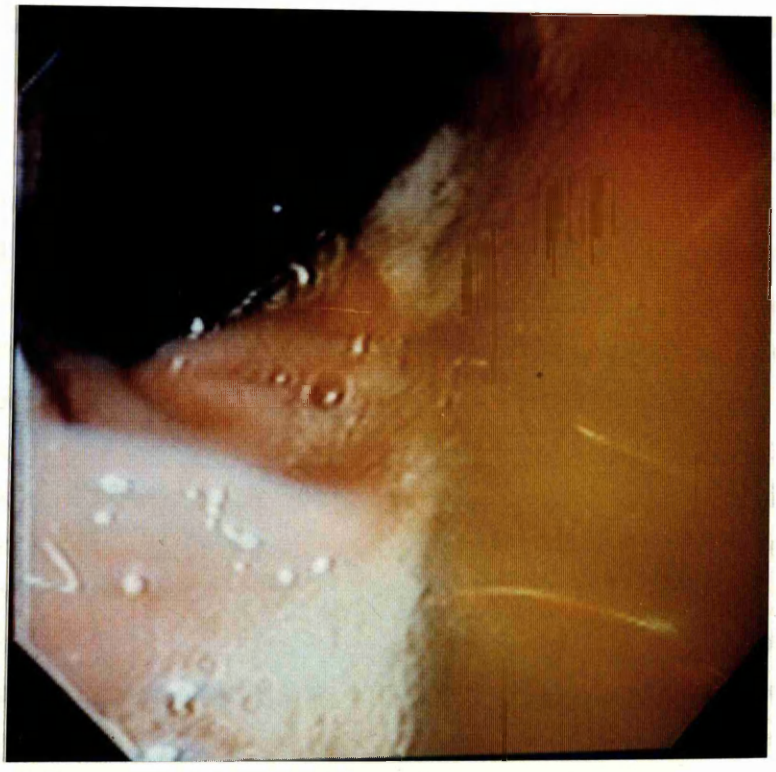


Figure 2.15. Retroflexed view of the cardia with a large yellow bile pool filling the stomach.

Condition	no	Endoscopic Findings: inflamed mucosa	follicular speckling	mucosal hypertrophy	erosion <0.5cm	ulcer	size (cm)	distribution: S	F	G
None Diagnosed	18	0	0	0	0	0	-	-	-	-
Chronic gastritis	9	9	0	0	0	0	-	-	-	9
Hypertrophic gastritis	9	5	0	9	1	0	-	-	9	-
Peptic ulcer	9	0	0	0	0	9	2	8	1	-
Gastroenteropathy	9	4	1	0	0	0	-	-	-	4
Erosive gastritis	6	6	3	0	6	0	-	-	-	6
Duodenogastric reflux	5	5	0	0	0	0	-	-	5	-
Follicular gastritis	3	1	3	0	0	0	-	-	-	3
Atrophic gastritis	2	2	0	2	0	0	-	-	2	-
Foreign body	2	2	0	0	0	0	-	2	-	-
Pyloric stenosis	2	0	0	0	0	0	-	-	-	-
Gastric dilation	2	0	0	0	0	0	-	-	-	-
Parasitic ulceration	1	0	0	0	0	1	1.5	-	1	-
Piroxicam toxicity	1	0	0	0	0	0	-	-	-	1
Gastric carcinoma	17	13	0	0	0	14	>2	13	-	4
Lymphosarcoma	3	3	0	0	0	3	1-5	1	-	2
Leiomyosarcoma	1	0	0	0	0	1	>2	1	-	-
APUDoma	1	0	0	0	0	1	>2	1	-	-

S = single lesion, F = focal lesions, G = generalised, no = number

Table 2.9. Mucosal features as determined by endoscopy in 100 vomiting dogs.

In those cases where no diagnosis was reached the endoscopic examination was normal (Table 2.9, Figures 2.16-2.21). In the neoplastic group there were two sets of findings. In those dogs with lymphosarcoma there were multiple small shallow ulcers (1-2cm) dotted throughout the mucosal surface, with only one dog having a large 5cm fundic ulcer (Figures 2.22-2.24). In those animals with gastric carcinoma, there was either an ulcer on the lesser curvature (14) or a blanched and irregular mucosal appearance due to submucosal spread (3). The ulcer was usually centred on the incisure angularis, but was present at the cardia in one and at the pylorus in another. These ulcers were large (4-5cm), deep, excavating with irregular edges, and bases filled with necrotic debris and blood. The inner walls of the ulcer were ragged with the rim occasionally overhanging the inner wall. Fixed and thickened rugae ran up to the outer walls of the ulcers, which were raised well above the surrounding mucosa (Figures 2.25 and 2.26). In three cases there was evidence of retrograde oesophageal spread. Here the oesophagogastric sphincter was open with the Z-line visible accompanied by a loss of the normal plicae of the oesophagus.

In the non-neoplastic group the animals with peptic ulcers were those that most required differentiation from gastric neoplasia. The peptic ulcers were always single and found in the antral area, with the incisure angularis being the predilection site. The ulcers, in contrast to tumour ulcers, were smaller (never more than 1-2cm in diameter) and shallower. Six of the nine cases had stigmata of haemorrhage with staining of the ulcer base (Figure 2.27). Three had an adherent clot, but none were actively bleeding at the time of examination. The bases had a cleaner appearance and the inner walls were more upright. The rim of the ulcer rarely projected much above the surrounding mucosal surface (Figure 2.28). Although rugae ran into the walls of the ulcers these were small and did not appear particularly fixed.

The dog with parasitic infestation had three ulcers 1.5cm in diameter running in a semicircle from the dorsal part of the incisure angularis across dorsal surface. These ulcers closely resembled peptic ulcers in size, location and appearance.

The dogs with pyloric stenosis had folds that failed to disappear on insufflation due to underlying muscle hypertrophy (Figure 2.29).

Those animals with hypertrophic gastropathy had either large folds of mucosa present in the pyloric canal (Figure 2.30), or large areas of irregular "morocco leather"-like mucosa that did not disappear once the stomach had been fully inflated with air (Figures 2.31-2.32).

The animals with chronic gastritis had an inflamed mucosa surface with a glistening appearance of oedema in some cases. In some classified as follicular gastritis the changes gave the impression of "measles" (Figure 2.33). Those animals with duodenogastric reflux had the above findings but also excessive luminal bile and bile reflux (Figure 2.34). In those animals with erosive gastritis similar changes were present, but in addition there were innumerable small erosions less than 5mm in diameter pitting the surface. Few of these bled and where there were large numbers they gave a "paintbrush effect" (Figures 2.35-2.37).

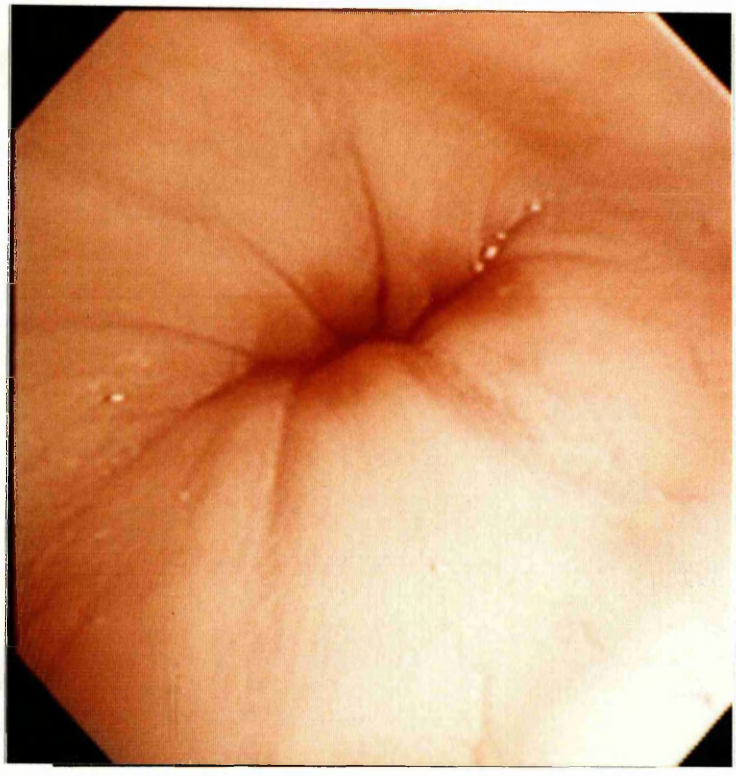


Figure 2.16. Normal oesophago-gastric junction seen from the oesophagus.

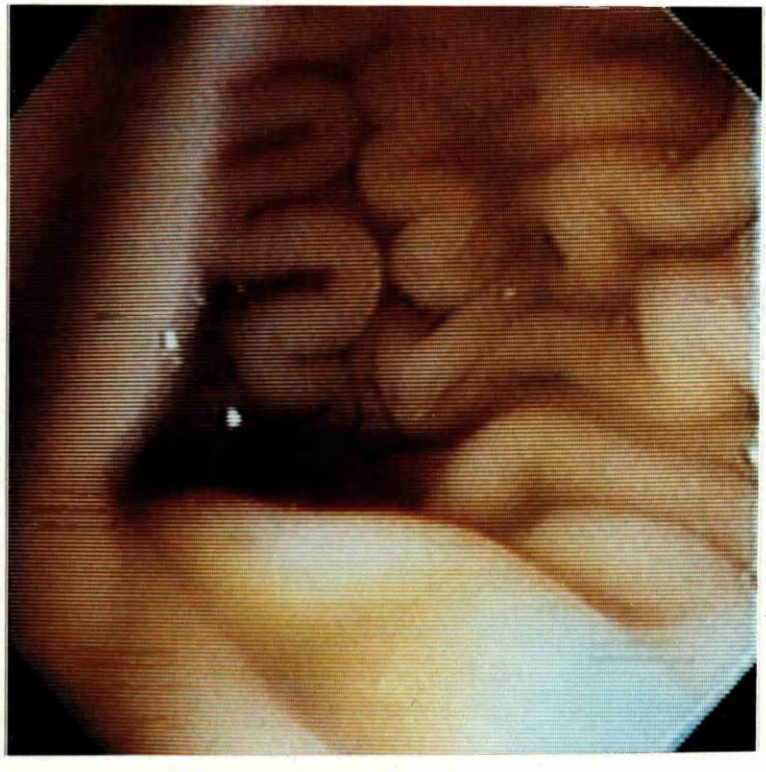


Figure 2.17. Normal view of the greater curvature of the body on entry to the stomach.

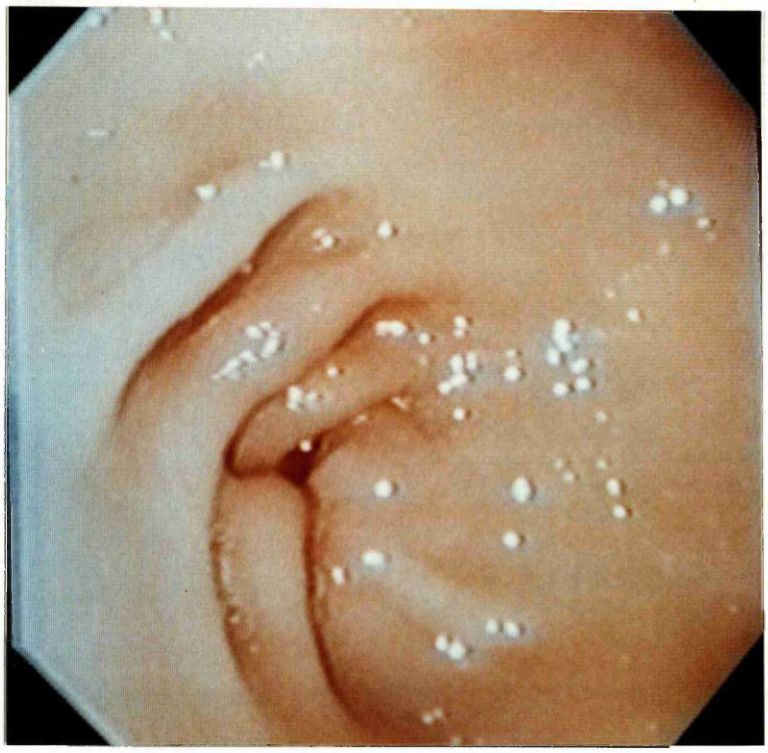


Figure 2.18. The pylorus of a partially insufflated stomach.

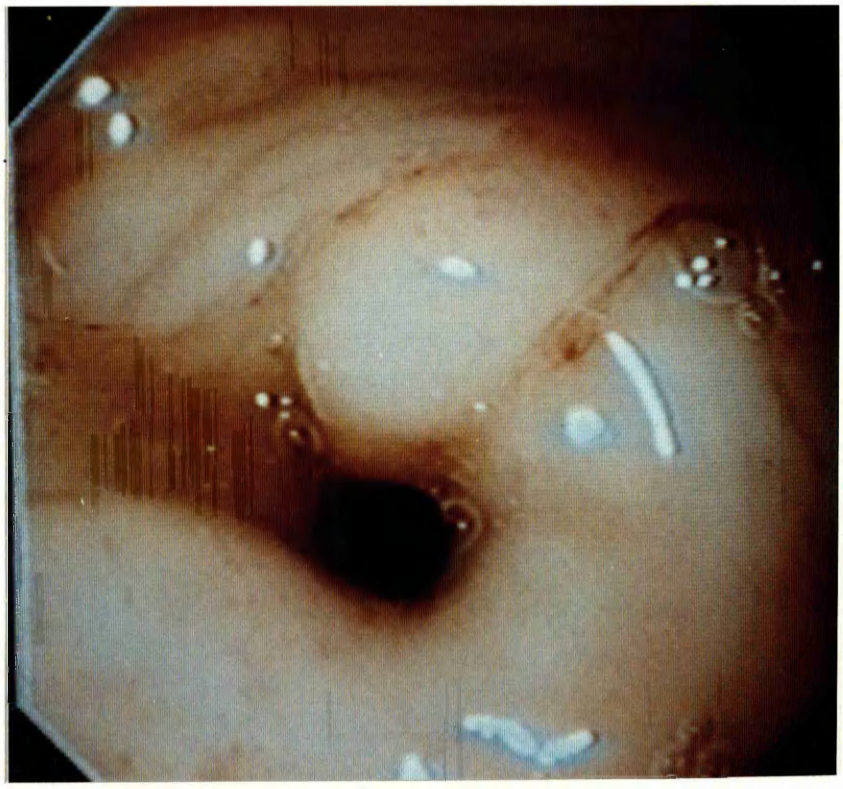


Figure 2.19. Open gastroduodenal junction following duodenoscopy. The blood streaked mucus is iatrogenic.

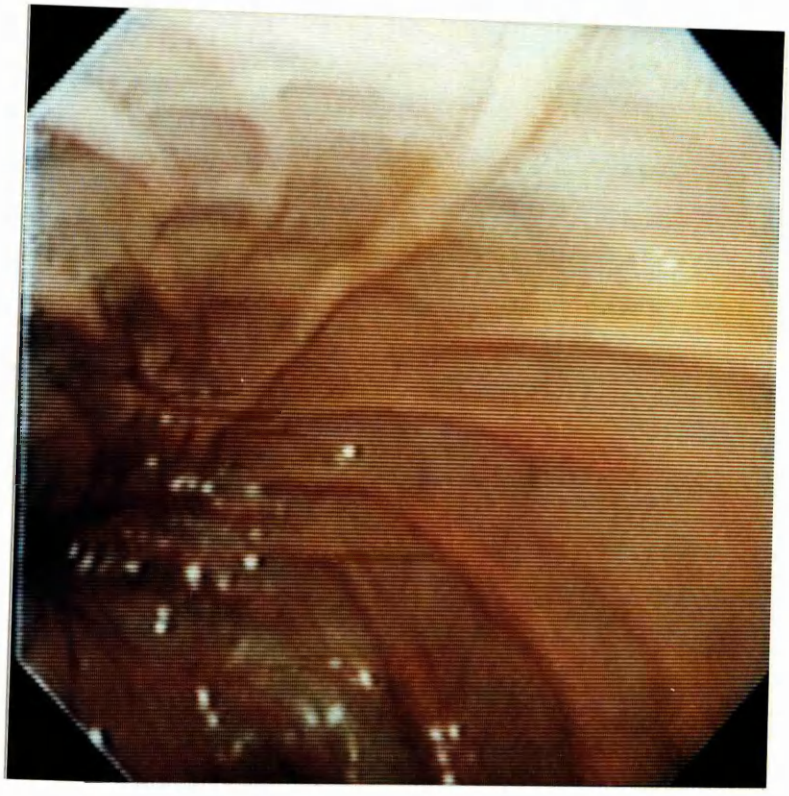


Figure 2.20. The bulk of the rugal folds are ablated following insufflation.

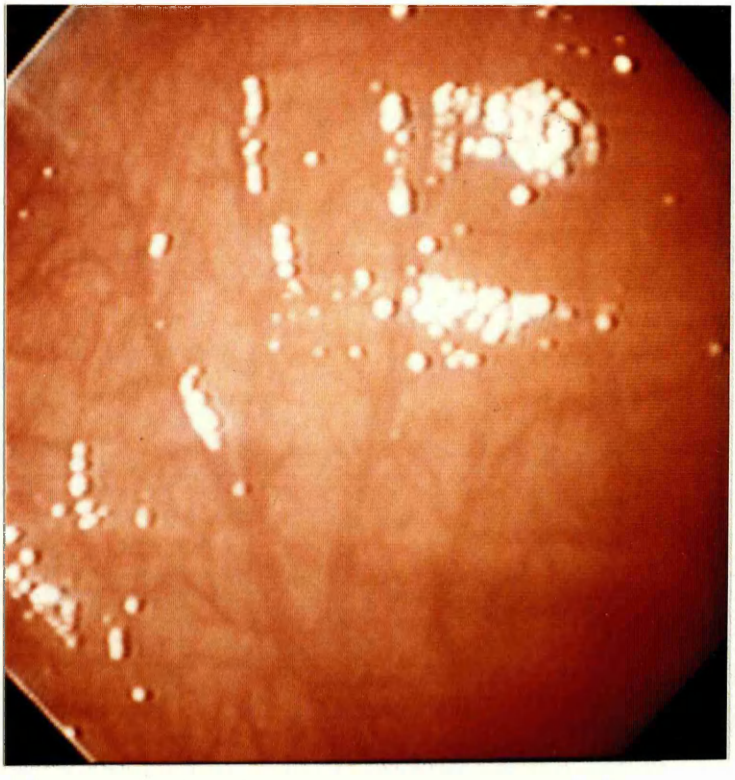


Figure 2.21. The normal submucosal vascular pattern is demonstrated when the stomach is fully insufflated.

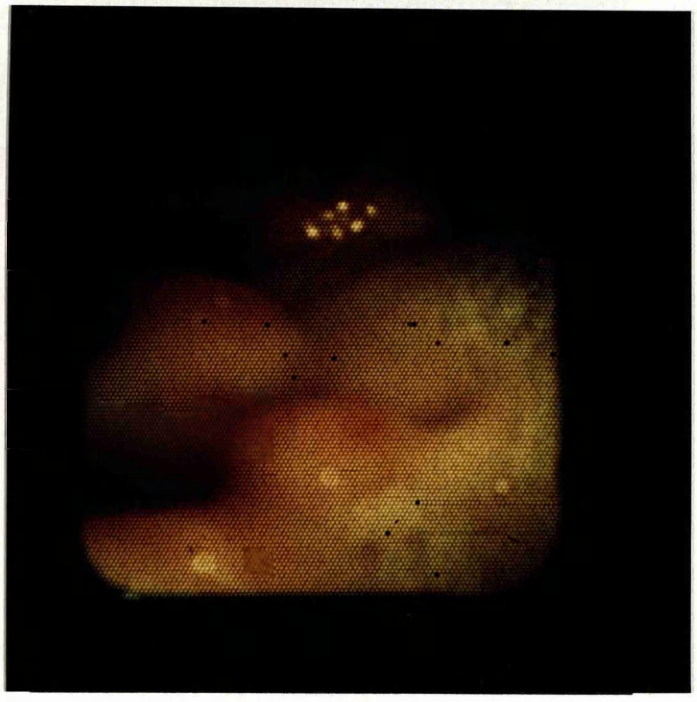


Figure 2.22. Endoscopic view- Lymphosarcoma. A small shallow ulcer is present on the mucosal surface without extensive depression or elevation from the surrounding mucosa.

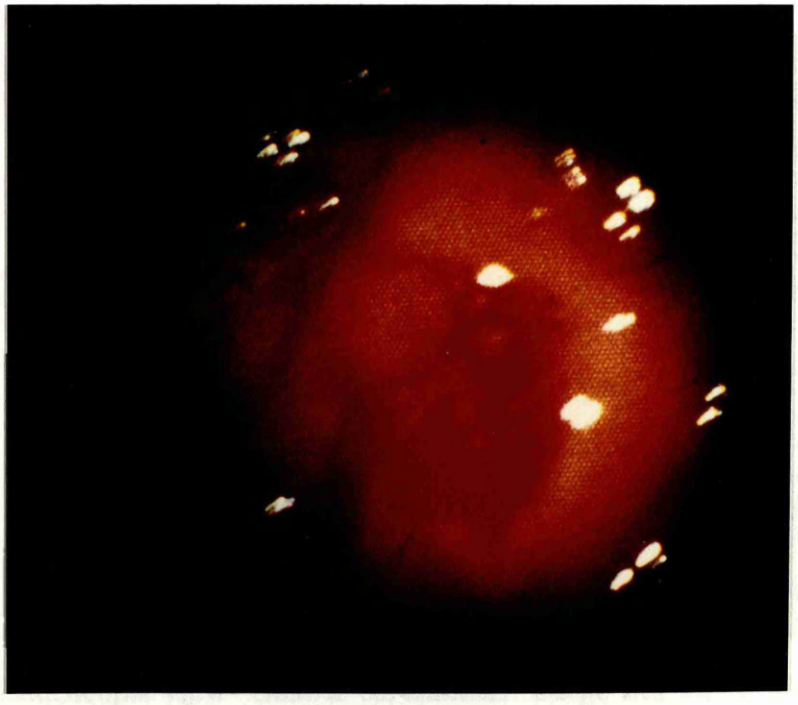


Figure 2.23. Endoscopic view- Lymphosarcoma. One of a large number of active bleeding ulcers on the mucosal surface.

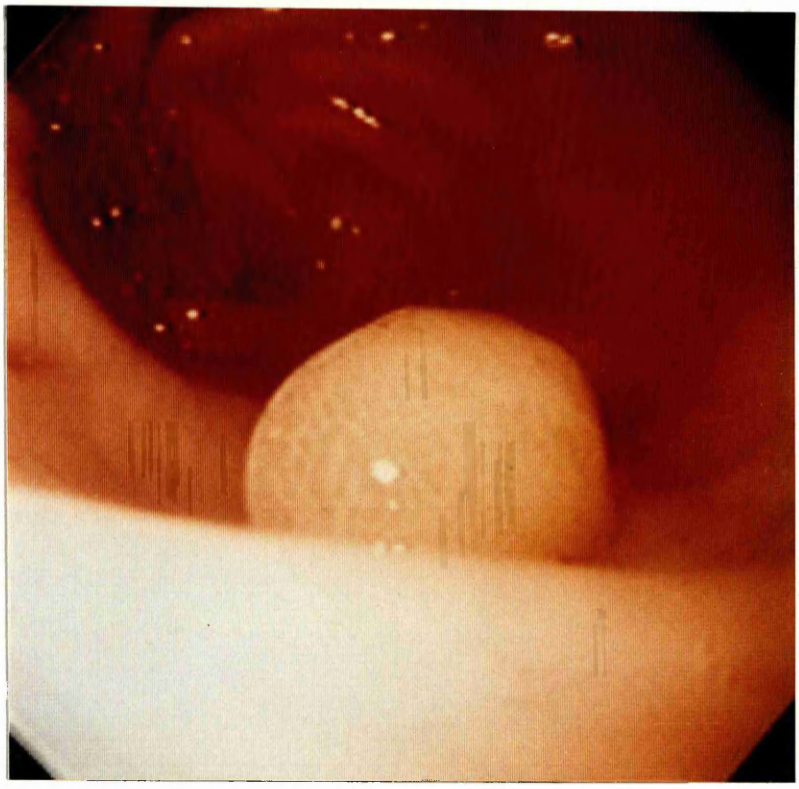


Figure 2.24. Endoscopic view- Lymphosarcoma. Small lymphoid nodule in the antrum of the stomach of a dog with generalised alimentary lymphosarcoma.

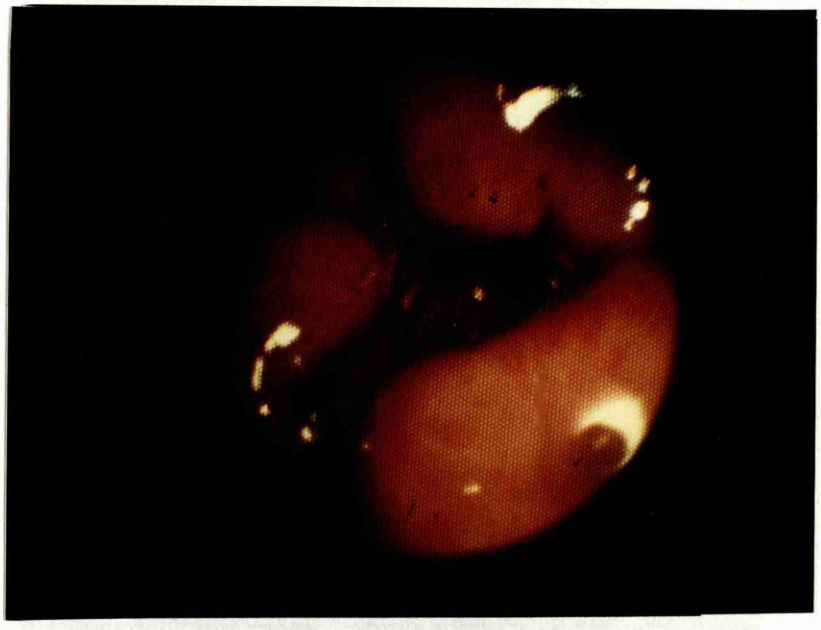


Figure 2.25. Endoscopic view- Gastric carcinoma. Large flat based ulcerated lesion at the junction between the body and the antrum. The walls have a chewed out appearance and a large thickened rugal fold is visible.

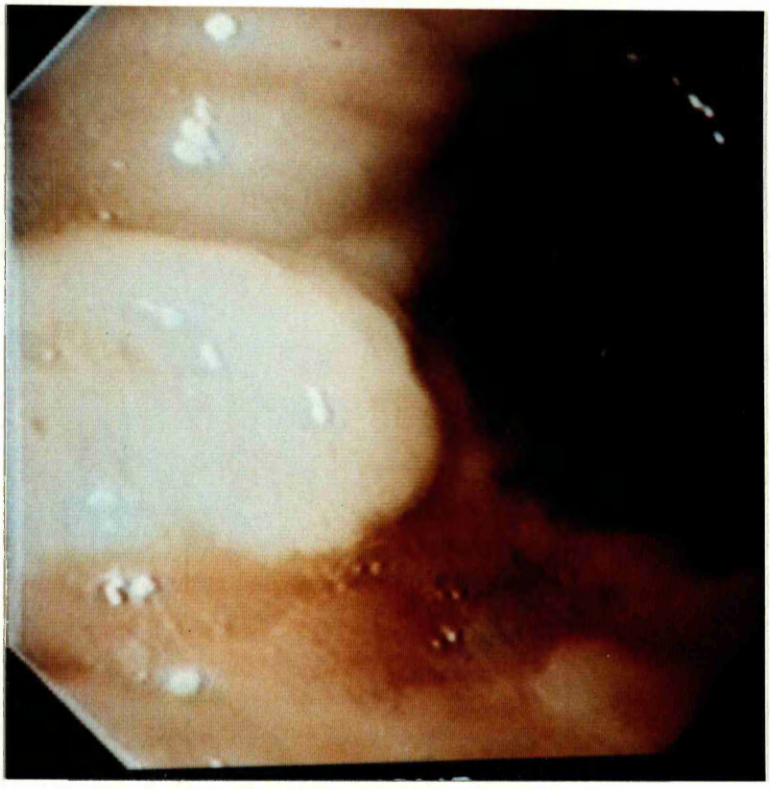


Figure 2.26. Endoscopic view- Gastric carcinoma. Large deep ulcer with overhanging irregular thick rim and blood-stained base.

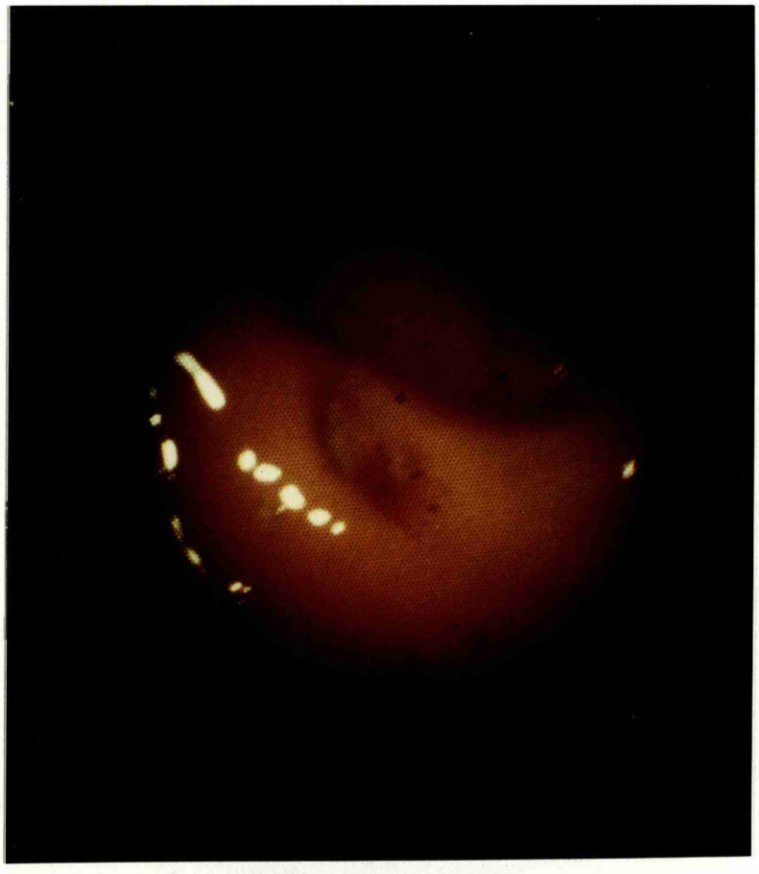


Figure 2.27. Endoscopic view- Peptic ulcer. A small ulcer on the incisure angularis. The base is largely white with an active bleeding point at one end.

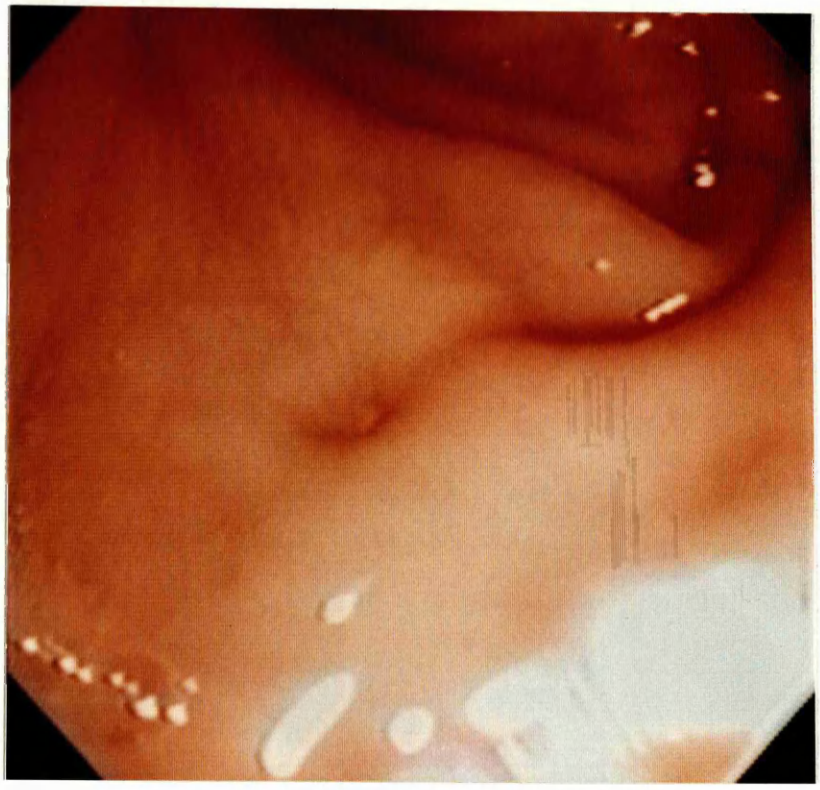


Figure 2.28. Endoscopic view- Peptic ulcer. A small ulcer on the visceral surface. The ulcer is not elevated above the surrounding mucosa.

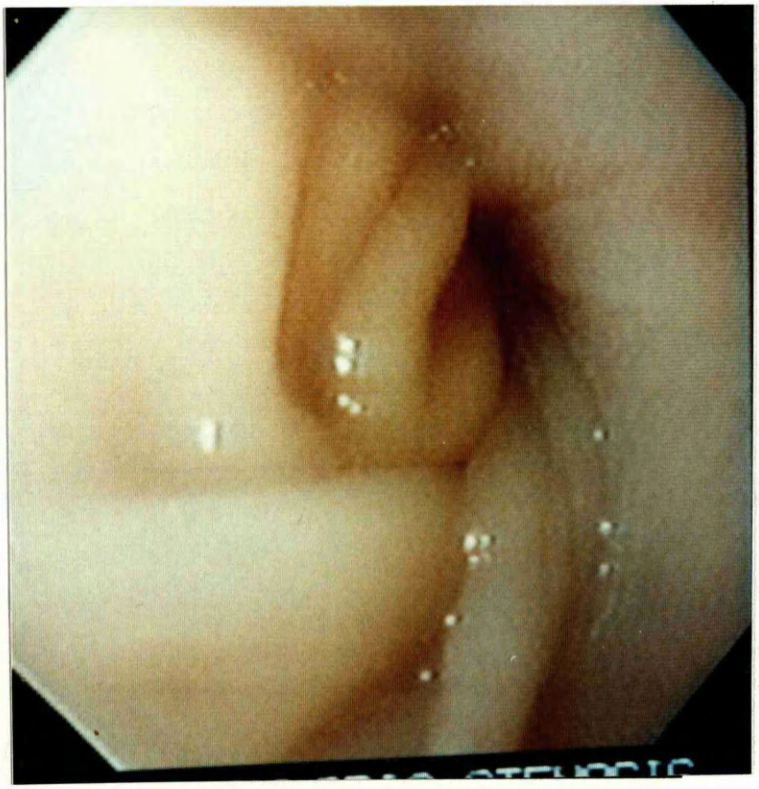


Figure 2.29. Endoscopic view- Pyloric stenosis. The rugal folds are normal but are unable to disappear on insufflation because of underlying mucosal hypertrophy.

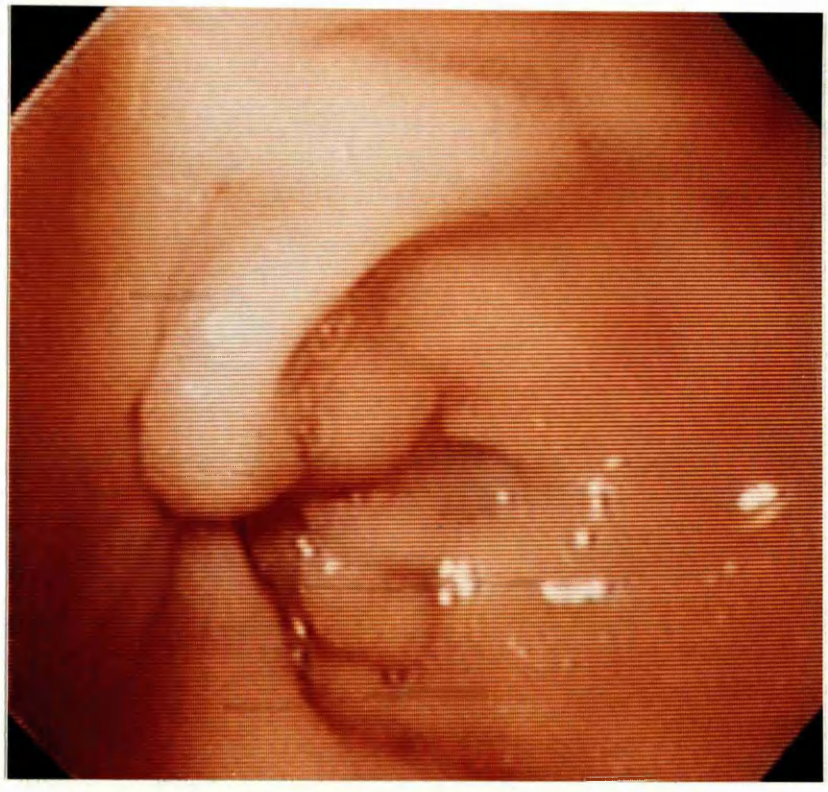


Figure 2.30. Endoscopic view- Hypertrophic gastritis. A series of thickened rugal folds are present around the sphincter.

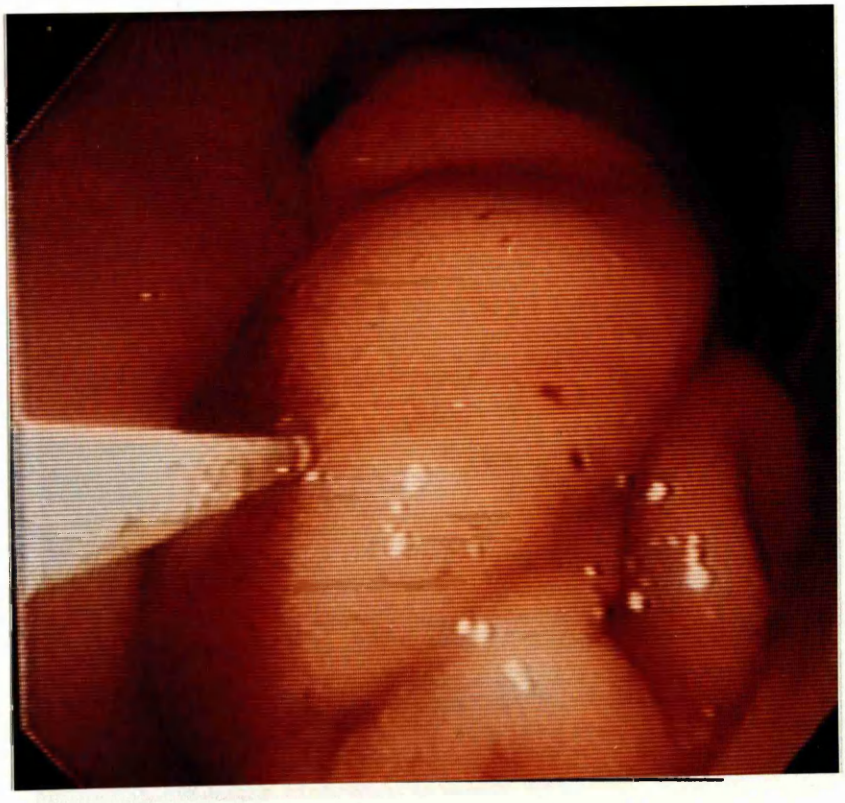


Figure 2.31. Endoscopic view- Hypertrophic gastritis. Large folding of hypertrophied mucosa appearing as an intraluminal mass.

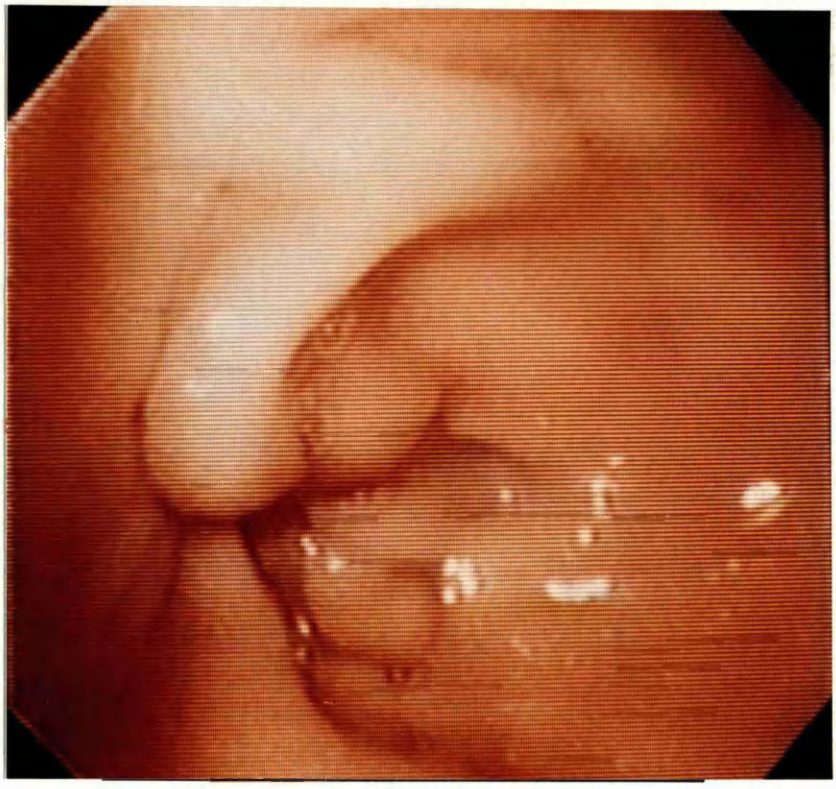


Figure 2.30. Endoscopic view- Hypertrophic gastritis. A series of thickened rugal folds are present around the sphincter.

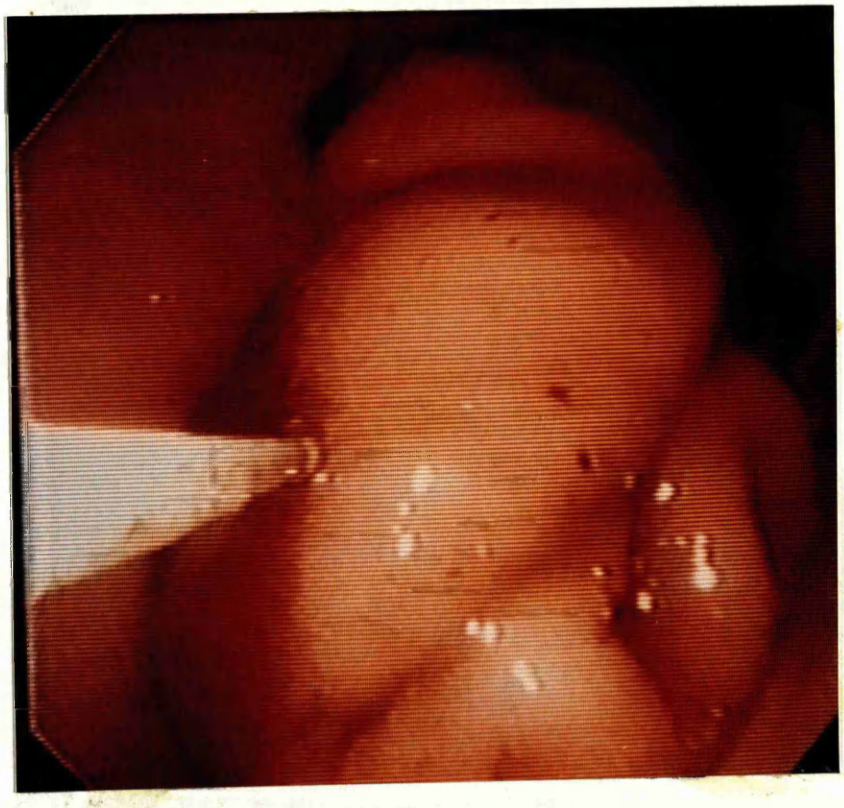


Figure 2.31. Endoscopic view- Hypertrophic gastritis. Large folding of hypertrophied mucosa appearing as an intraluminal mass.

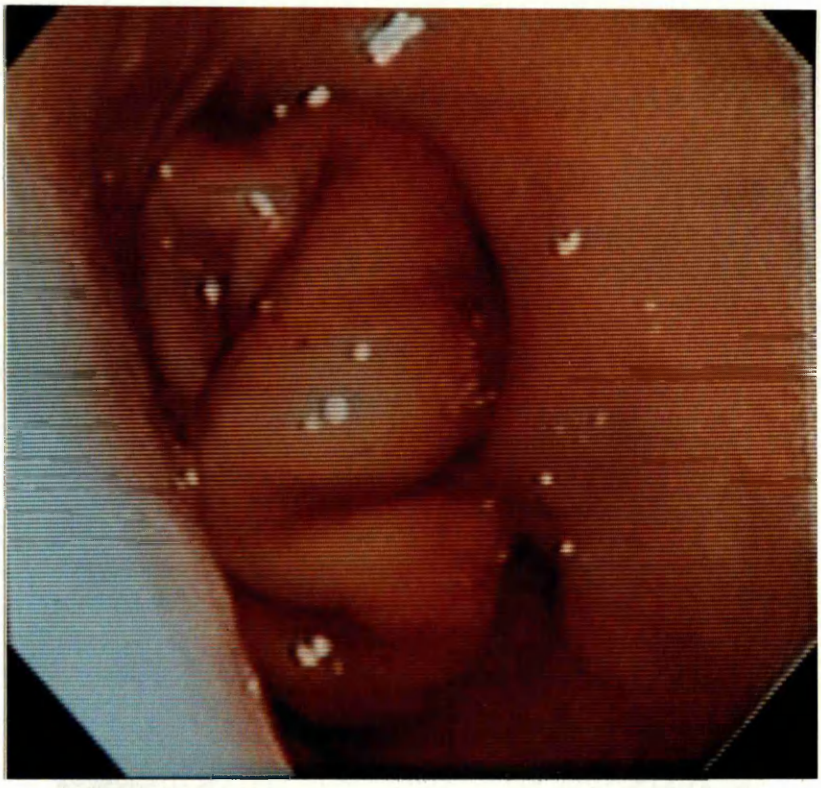


Figure 2.32. Endoscopic view- Atrophic gastritis. There is a surfeit of folded mucosa appearing as an intraluminal mass.

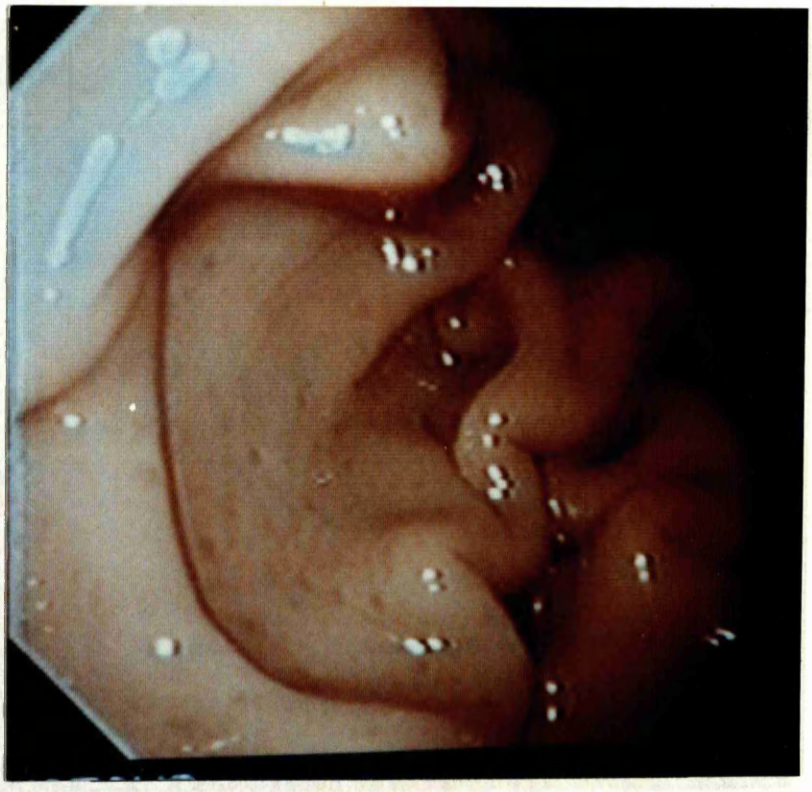


Figure 2.33. Endoscopic view- Follicular gastritis. The *measles* like pattern is dotted throughout the stomach.

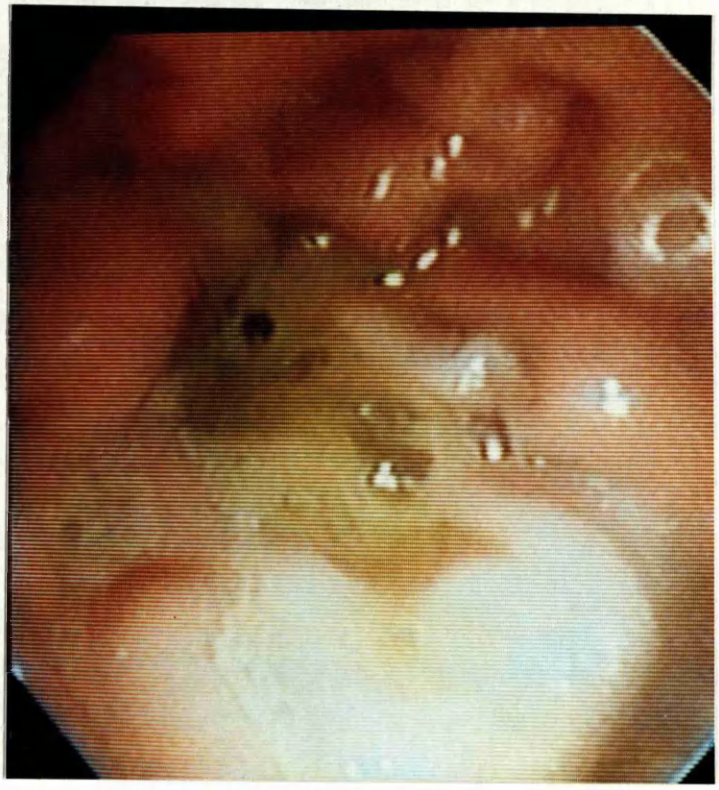


Figure 2.34. Endoscopic view- Duodenogastric reflux. A volume of bile is present amongst some swollen rugae.

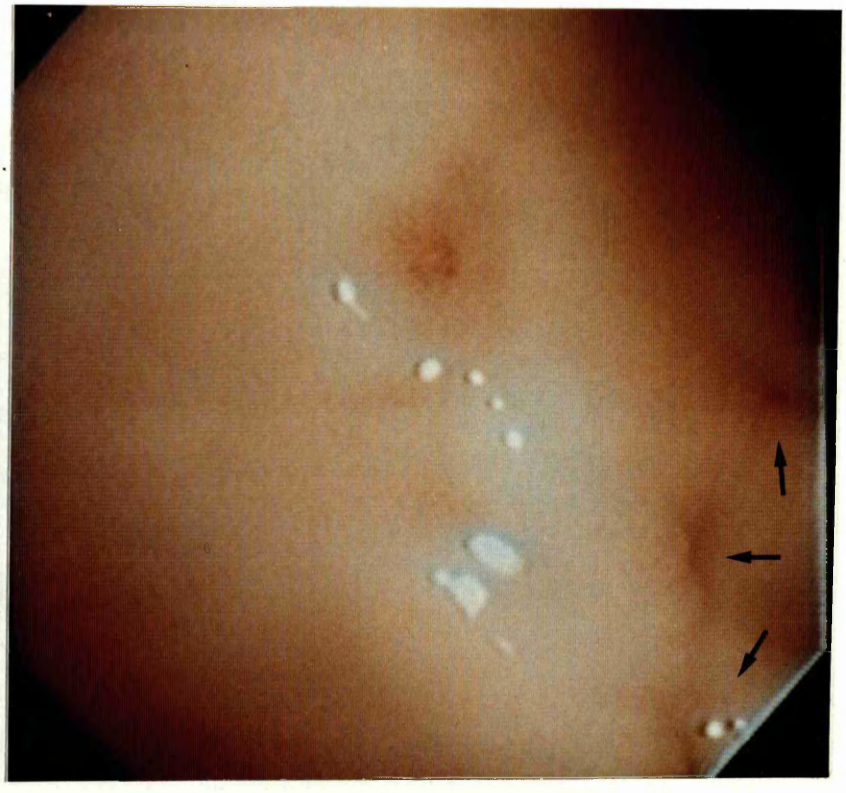


Figure 2.35. Endoscopic view- Erosive gastritis. One active erosion is visible and a number of healing stellate scars are visible (*arrows*).

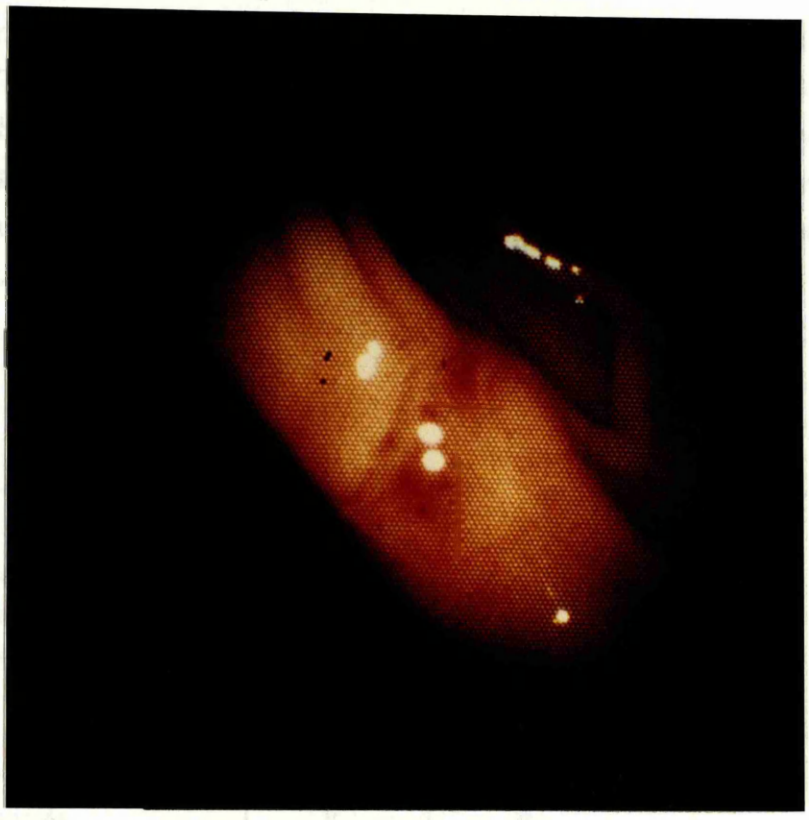


Figure 2.36. Endoscopic view- Erosive gastritis. An area of erosion on the pillar that represents the incisure angularis in an insufflated stomach.

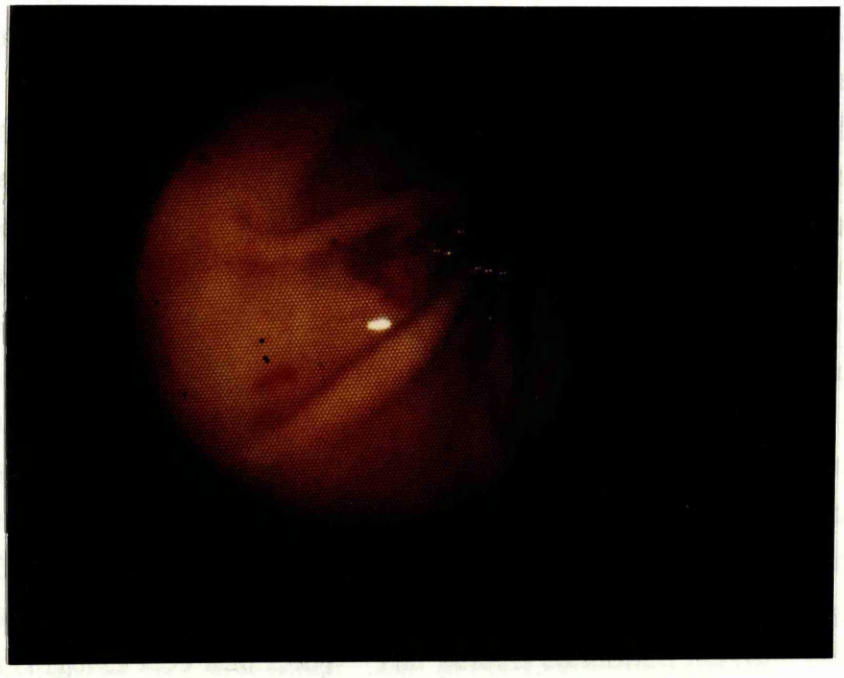


Figure 2.37. Endoscopic view- Erosive gastritis. Multiple areas of erosion in the antrum giving a *paintbrush* effect.

A comparison of the radiological and endoscopic features of selected diseases is shown in Table 2.10. Inflamed mucosa was not detected by radiology and mucosal hypertrophy was more sensitively picked up by endoscopy. Similarly, endoscopy was more sensitive in the detection of peptic ulcers. However, in animals with gastric carcinoma radiology was almost as accurate in the recognition of neoplastic ulceration.

Condition Number	Chronic gastritis 9	Hypertrophic gastritis 9	Peptic ulcer 9	Gastric carcinoma 17
Radiology				
intraluminal filling defect	0	5	0	10
mucosal irregularity	0	0	3	10
contrast retention	0	4	3	3
ulcer	0	0	6	13
Endoscopy				
inflamed mucosa	9	0	0	13
mucosal hypertrophy	0	9	0	0
erosion	0	1	0	0
ulcer	0	0	9	14

Table 2.10. Comparison of selected diseases - radiology versus endoscopy.

2.3.9 Pathology

Biopsies from the "no diagnosis" group were all normal or inconclusive (Figures 2.42 and 2.43). Biopsies from those cases where a diagnosis was reached were sometimes unhelpful (Figure 2.47). The animals with chronic gastritis had thickening of the lamina propria with increased numbers of lymphoid and plasma cells scattered diffusely or forming small aggregates. The gastric pits showed some degree of dilation and distortion (Figures 2.44 and 2.48). Those cases of eosinophilic gastroenteropathy had oedema and thickening of the lamina propria with marked infiltration of eosinophils into the lamina propria. Hypertrophic gastropathy was characterised by follicular hyperplasia with an increase in the amount of connective tissue present in the lamina propria (Figure 2.46). There were also scattered mononuclear cells with a plasma cell component present between the mucous glands (Figure 2.38). In those animals with peptic ulcer that had biopsies taken the ulcerated areas showed complete loss of the mucosa and adjacent congestion of the submucosa (Figures 2.39 and 2.45). The defects contained necrotic debris and were bounded by active granulation tissue.

Cases of lymphosarcoma had areas of ulceration with raised and thickened areas of white tissue centred ulcers. The sheets of tumour cells formed solid nodules in the submucosa with infiltration of the surrounding mucosa. Gastric carcinomas had large single ulcers (Figure 2.41). There was extensive invasion by cords and sheets of large epithelial cells containing varying amounts of mucin. In most cases there was extensive permeation of the lymphatics. The sole case of APUDoma also had a large ulcerated lesion, which contained sheets and clumps of spindly cells with oval nuclei and indistinct eosinophilic granular cytoplasm (Figure 2.40). The nuclei had moderate mitotic rates with abnormal mitotic activity. The cells were separated by fine connective tissue septae. In areas the cells appeared to be lined up in a "dendocrine pattern" with small islands of similar cells in the lamina propria.

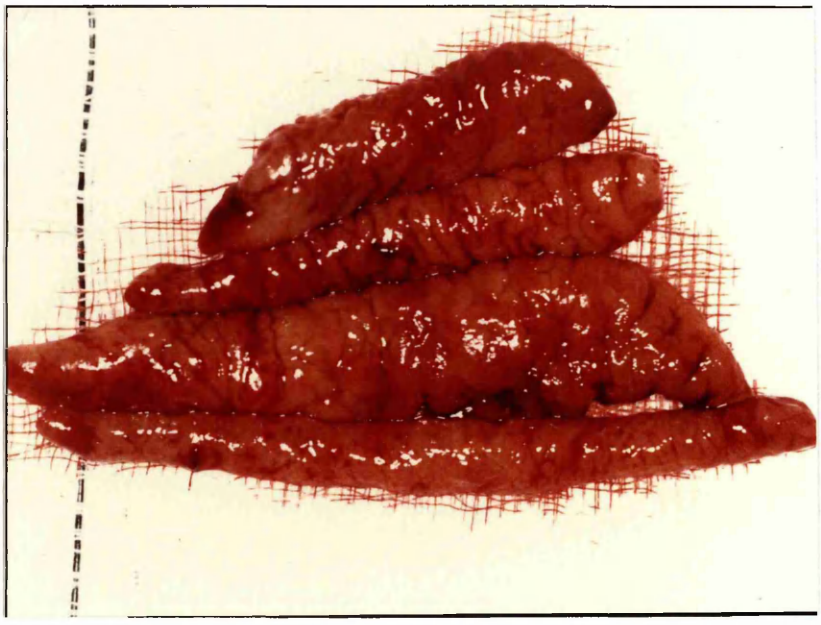


Figure 2.38. Surgical specimen of hypertrophic gastritis. Strips of gastric mucosa were removed from the body and antrum to alleviate pyloric outflow obstruction.

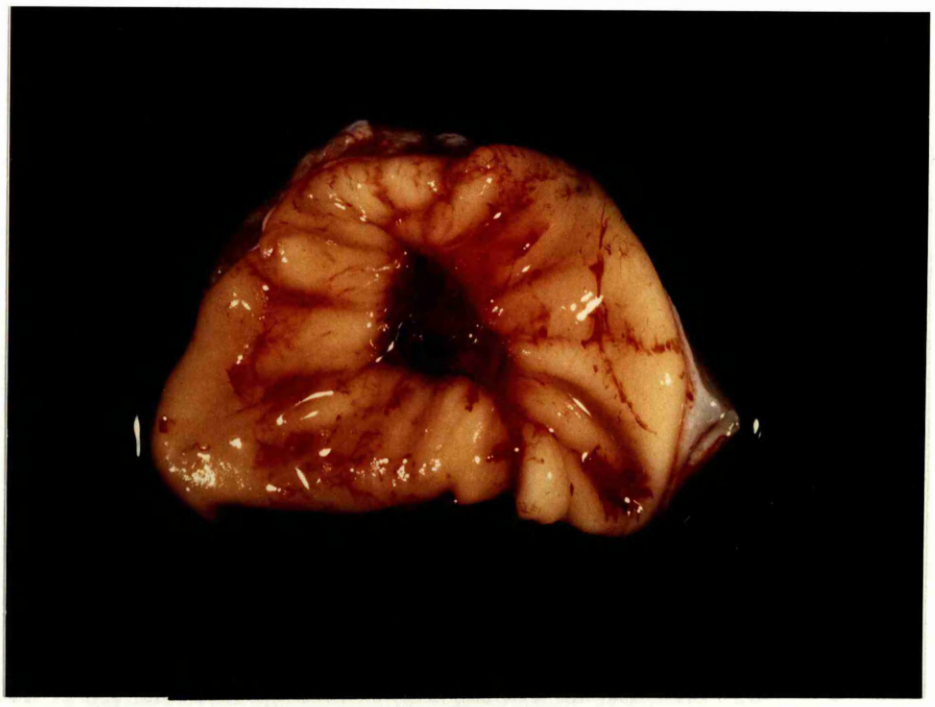


Figure 2.39. Surgical specimen of a peptic ulcer. The peptic ulcer has fresh blood filling the crater and on the surrounding gastric mucosa indicating active bleeding.

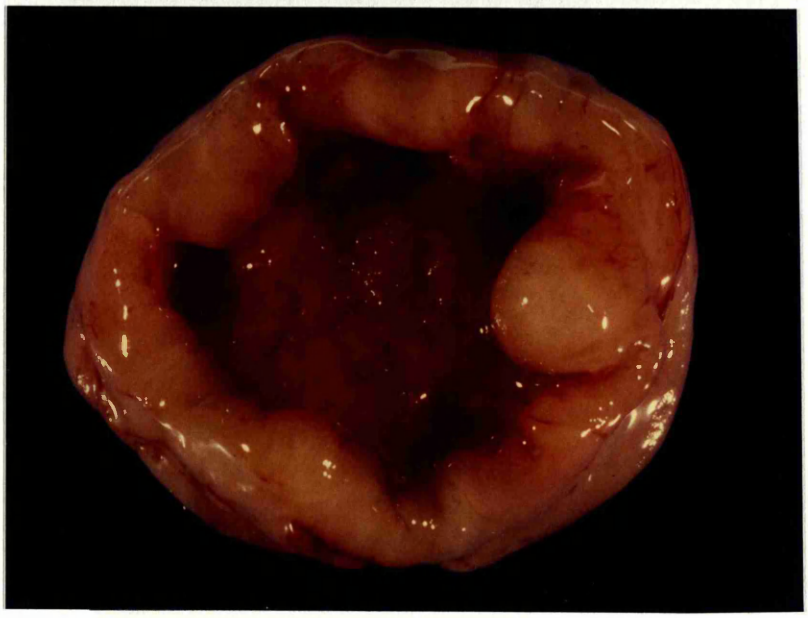


Figure 2.40. Surgical specimen of an APUDoma. The large ulcerated mass was found to be somewhat pedunculated at surgery.

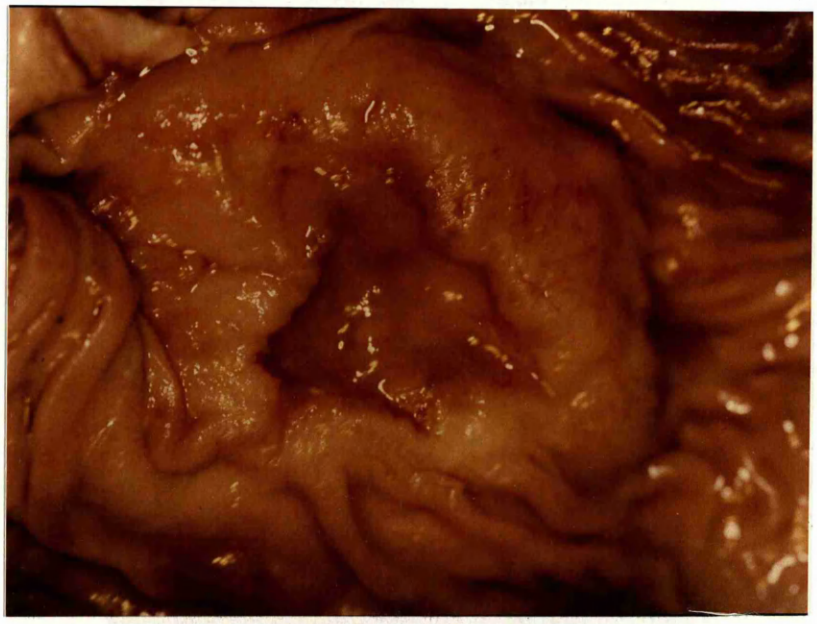
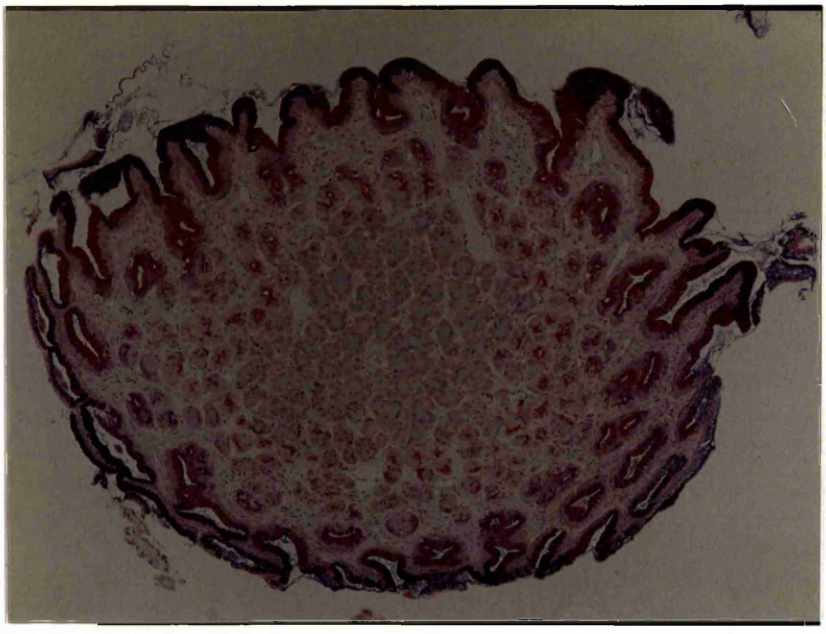


Figure 2.41. Post-mortem specimen of a gastric carcinoma. This is a large ulcerated mass with thick irregular rim. Several rugae can be seen running into the outer face of the tumour wall.



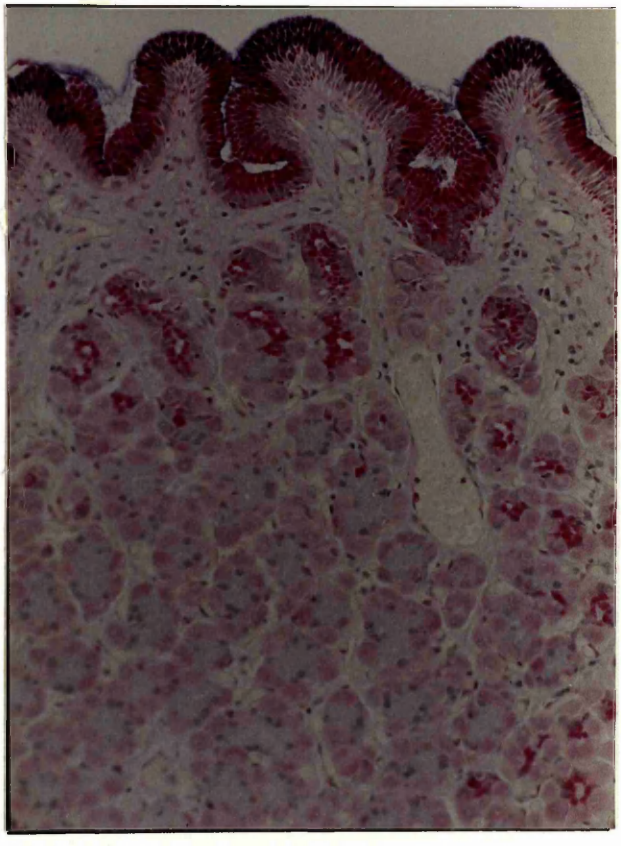
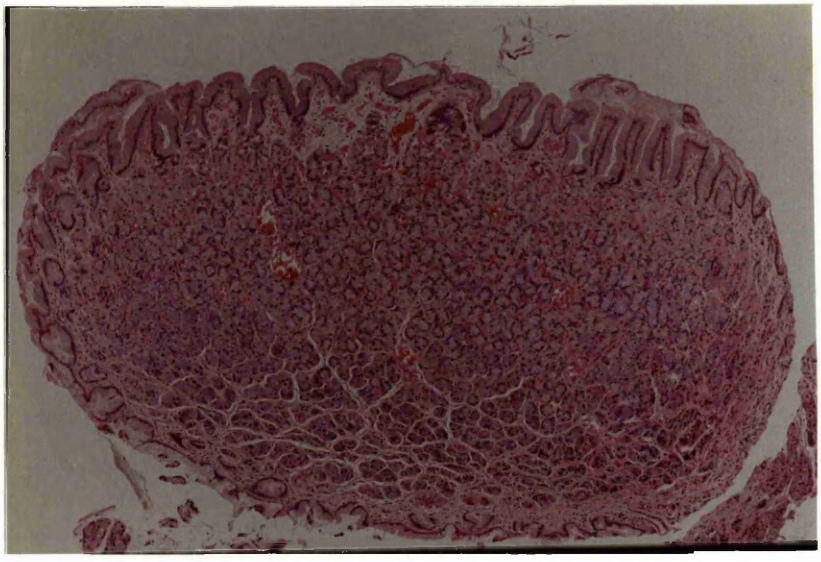


Figure 2.42a & 2.42b. Photomicrographs of an endobiopsy. The tissue is distorted with no base included in the biopsy. Inconclusive. (PAS/Alcian Blue x60, x300)



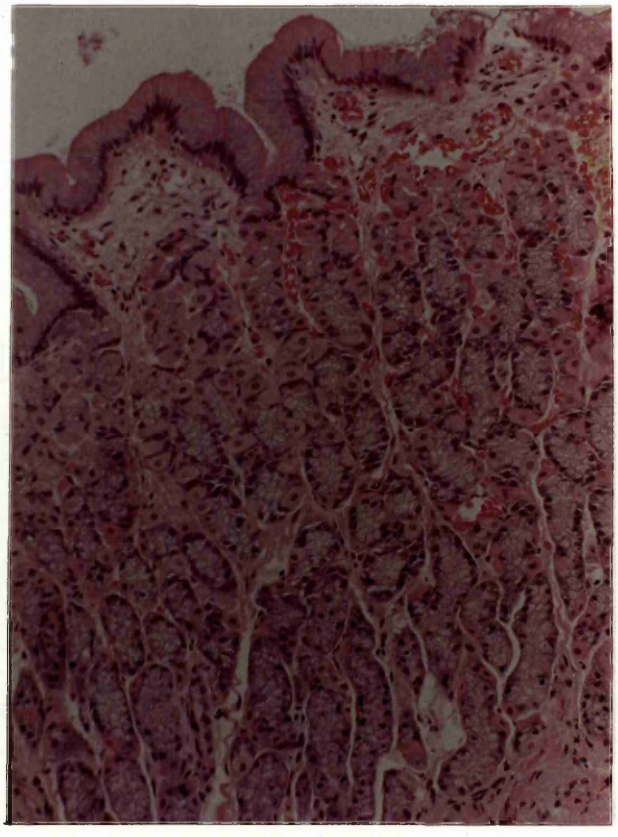
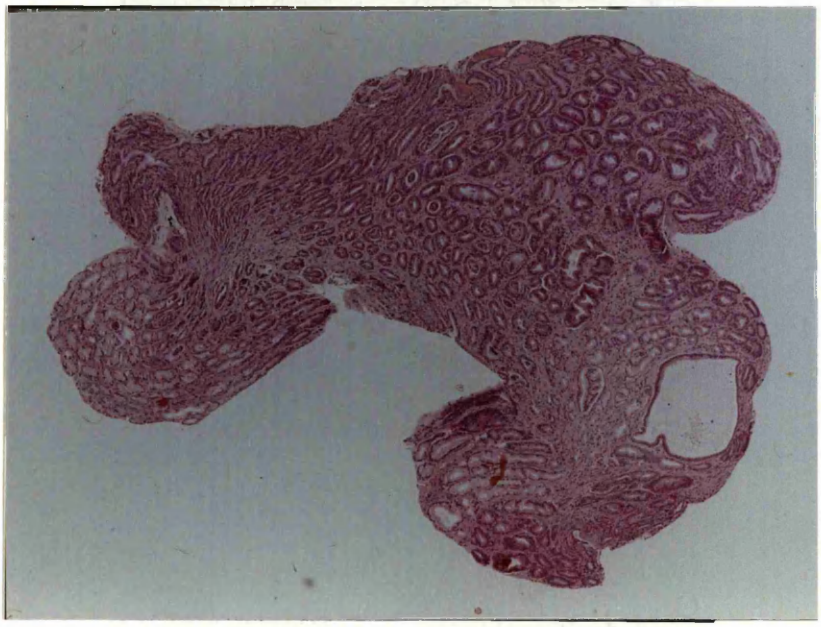


Figure 2.43a & 2.43b. Photomicrographs of an endobiopsy. The tissue is distorted and is not full thickness. There is some congestion. Inconclusive. (H&E x60, x300)



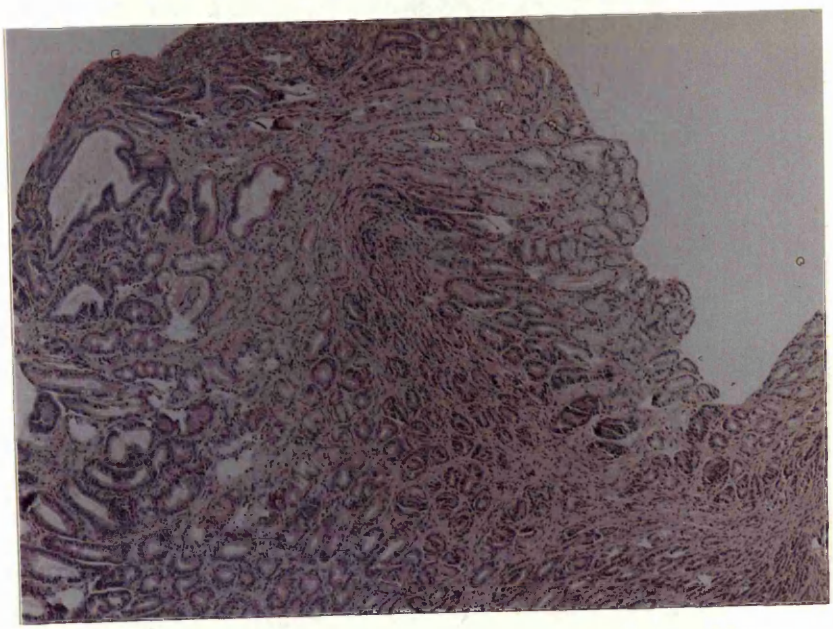
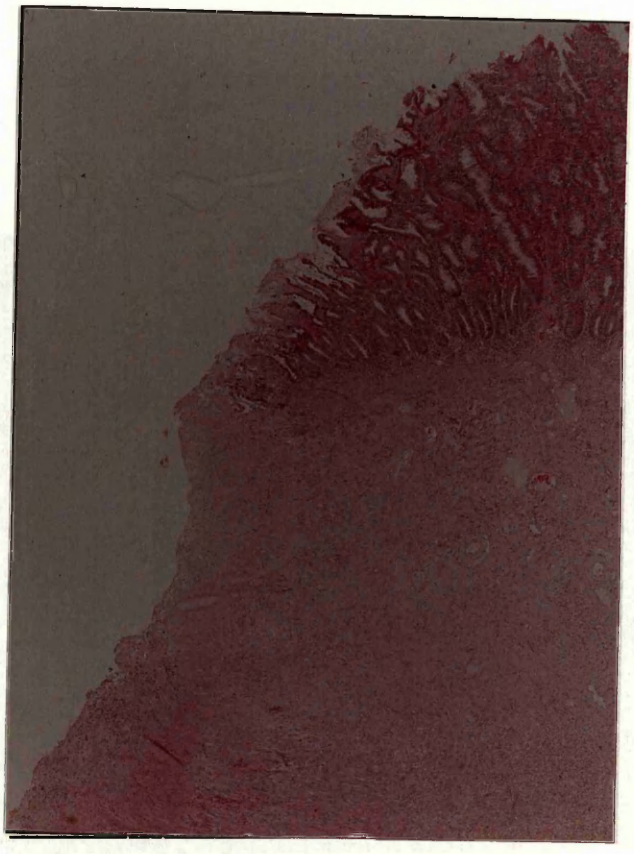


Figure 2.44a & 2.44b. Photomicrographs of an endobiopsy. There is crush artefact which has caused distortion of the mucosal architecture. Follicular gastritis. (H&E x40, Southgate x60)



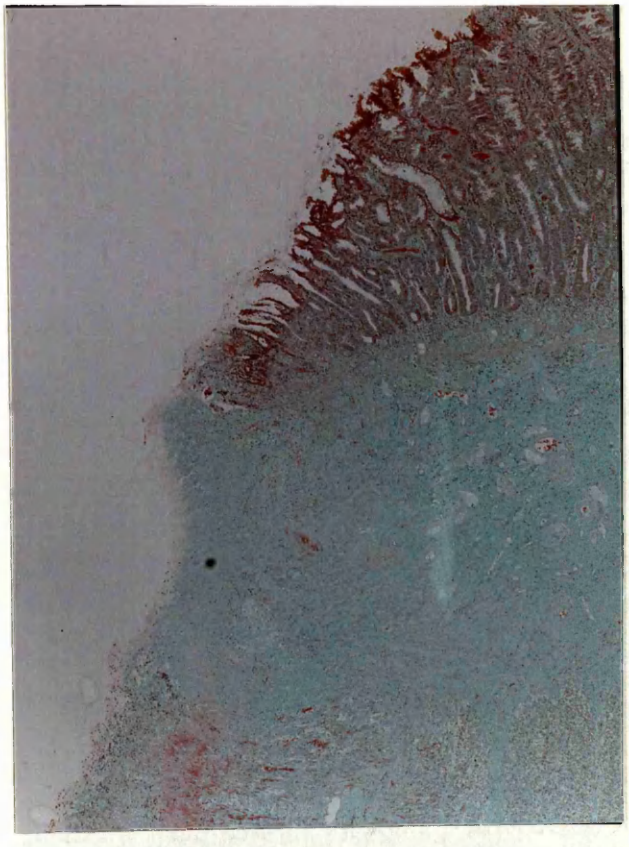
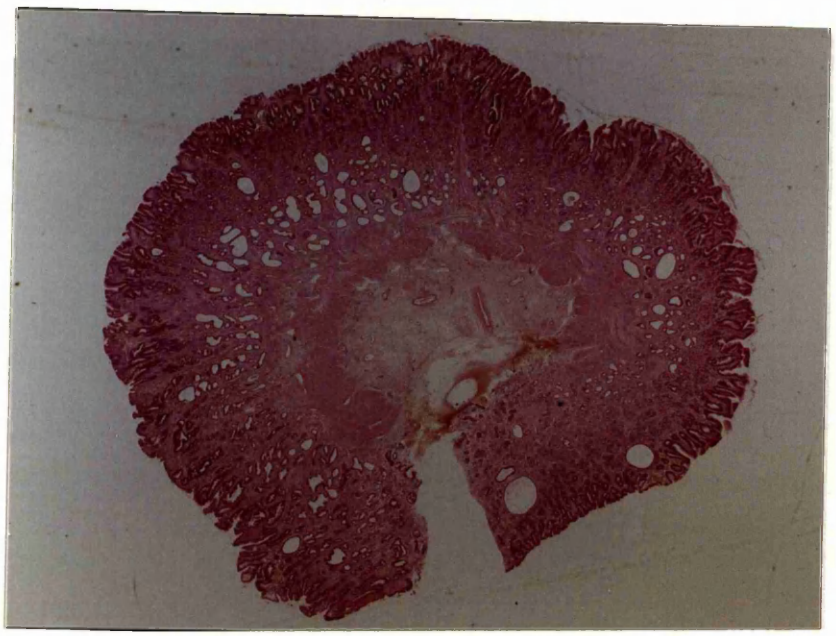


Figure 2.45a & 2.45b. Photomicrographs of a peptic ulcer The lateral wall of the ulcer is delineated. The granulation tissue at the base is highlighted by the Masson stain. (H&E x30, Masson's x30)



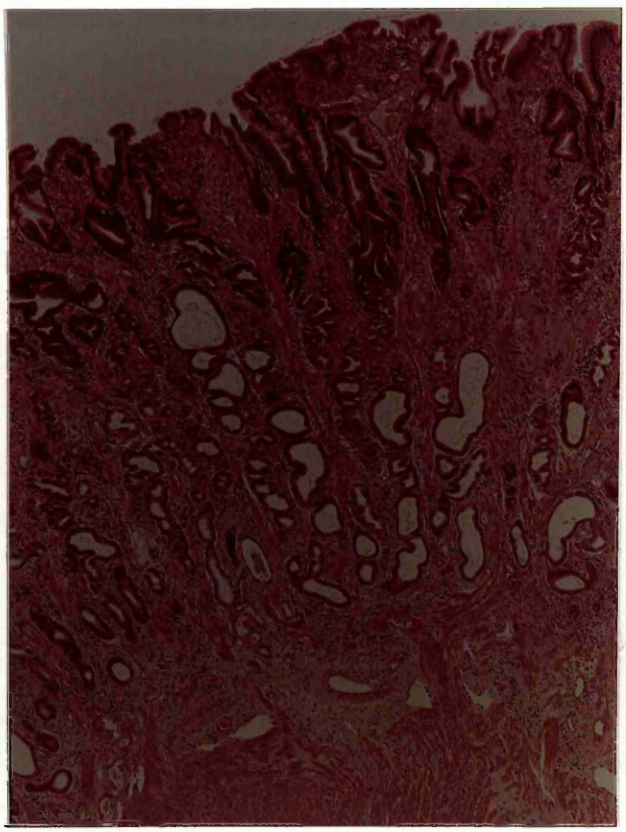


Figure 2.46a & 2.46b. Photomicrographs of hypertrophic gastritis. There is cystic dilation of the glands and intestinal metaplasia. (H&E x10, x40)

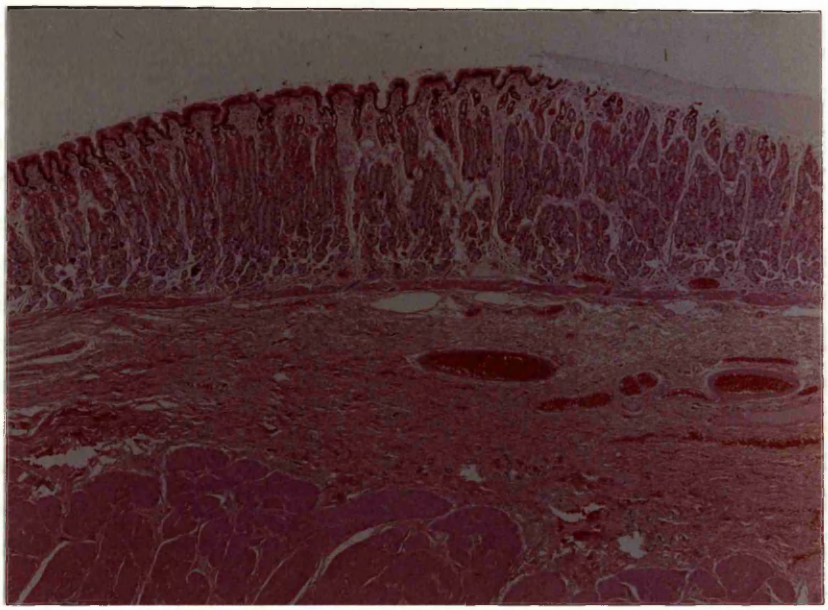


Figure 2.47. Photomicrograph of a gastric biopsy. Part of the epithelium has been sliced off during the biopsy procedure. No diagnosis. (H&E x30)

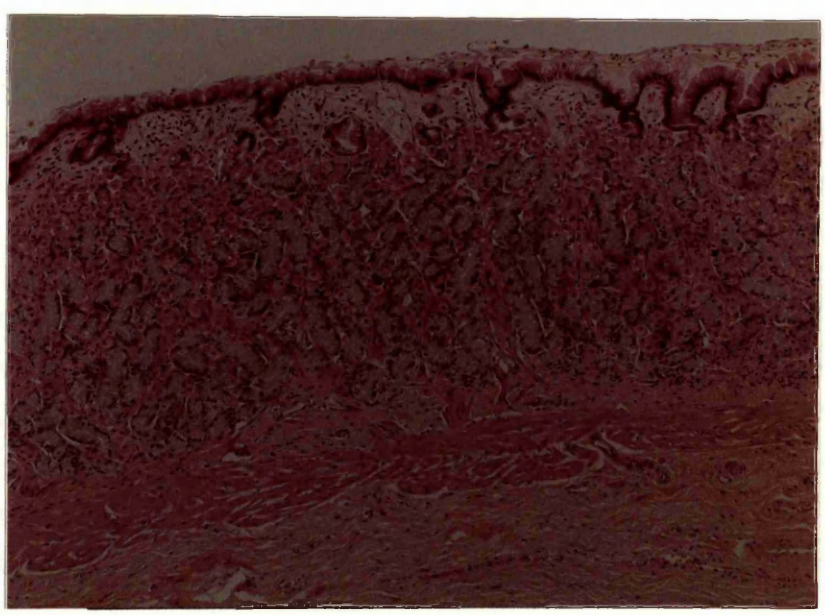


Figure 2.48. Photomicrograph of a gastric biopsy. There is subepithelial oedema and some lymphoid cellular infiltrate. Chronic gastritis. (H&E x75)

2.3.10 Treatment and Outcome

A summary of the treatments used and the outcome is presented in Table 2.11.

Diagnosis	no	Treatment	Outcome
None Diagnosed	18	none	C17:S1
Chronic gastritis	9	antibiotics (5), H_2 blocker (4)	C9
Hypertrophic gastritis	9	FR(3), HM(3), Y-U(3)	S7:C2
Peptic ulcer	9	H_2 blocker (7), GJ (2)	C9
Gastroenteropathy	9	corticosteroids	C9
Erosive gastritis	6	H_2 blocker	N1:S5
Duodenogastric reflux	5	metoclopramide/fatty meal	S2:C3
Follicular gastritis	3	H_2 blocker	C3
Atrophic gastritis	2	none	N1:E1
Foreign body	2	gastrotomy and retrieval	C2
Pyloric stenosis	2	FR pyloromyotomy	S2
Gastric dilation	2	metoclopramide and gastropexy	S2
Parasitic ulceration	1	fenbendazole	C1
Piroxicam toxicity	1	NSAID withdrawal and H_2 blocker	C1
Gastric carcinoma	17	none	E
Lymphosarcoma	3	none	E
Leiomyosarcoma	1	none	E
APUDoma	1	resection	E

C = complete resolution, E = euthanasia, N = no improvement, S = satisfactory. FR = Fredet-Ramstedt pyloromyotomy, HM = Heineke-Mikulicz pyloroplasty, Y-U = Y-U pyloroplasty, GJ = gastrojejunostomy.

Table 2.11. Treatment and outcome of 100 vomiting dogs.

Those animals in the neoplastic group were ultimately euthanased following endoscopic diagnosis, exploratory laparotomy, or once the histopathological confirmation had been made. The dog with the APUDoma stopped vomiting for 2 months after resection, but relapsed following regrowth and the owners opted for euthanasia at this point.

Those animals where "no diagnosis" was reached were, in general, given no treatment. Where scavenging was implicated or suspected the owners were advised to attempt to curtail this activity by restricting the animals freedom to roam, particularly on those days when refuse collection was anticipated. Follow-up on these animals found that all (with one exception) had ceased to vomit within three months of discharge from the hospital.

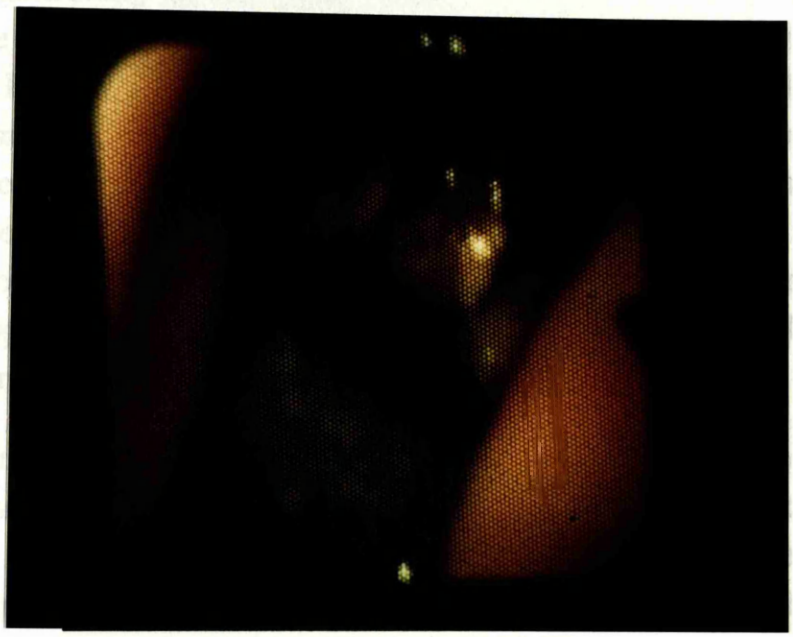


Figure 2.49. Endoscopic appearance of a foreign body lying between rugal folds.

Figure 2.50. The foreign body, a piece of chewed furniture upholstery, after removal by gastrostomy.

The animals from the non-neoplastic group were treated in a variety of ways. Those animals with foreign body, hypertrophic gastropathy or pyloric stenosis were treated by surgery to improve the outflow from the stomach. Removal of the foreign body was done via a gastrotomy in the dorsal surface of the stomach (Figures 2.38 and 2.39). Those animals with outflow problems were treated by, either a pyloromyotomy, Heineke-Mikulicz or Y-U pyloroplasty. These animals showed a marked improvement, but 50% continued to vomit occasionally despite surgery. The animals with duodenogastric reflux were managed by either metoclopramide or by increasing frequency of feeding with the introduction of a fatty meal in the late evening. Those given metoclopramide were reported to have made some unsatisfactory improvement, with frequency of vomiting little reduced. Those given a changed dietary regime made satisfactory improvement.

The animals with gastroenteropathies were treated with prednisolone starting at $1mgkg^{-1}$ twice daily. This dose was gradually reduced over four weeks to $0.5mgkg^{-1}$ every other day. Once this course had been completed the owners were directed to try and find a maintenance dose less than $0.5mgkg^{-1}$ every other day that kept the signs of vomiting under control. All owners reported that a significant improvement took place and was maintained as long as the steroid therapy was continued. Some owners had been unable to find a maintenance dose below $0.5mgkg^{-1}$ every other day. Those animals with chronic and follicular gastritis were treated speculatively with cimetidine for 30 days or a 21 day course of potentiated sulphonamide, and the owners of those with a history of scavenging were given the same advice as those in the "no diagnosis" group (see above). All this group improved within the first month of discharge from hospital and none had recommenced vomiting three months later. Those with erosive gastritis were also treated speculatively with cimetidine at $5mgkg^{-1}$ four times daily. After this most owners reported some improvement. No treatment was given to the two dogs with atrophic gastritis.

Those dogs with peptic ulcers were treated in two ways. Four dogs had surgery to remove the peptic ulcer. In one dog this was for biopsy purposes, in two it was because of the massive haemorrhage that the dogs were sustaining if cimetidine therapy was stopped. In these dogs the antrum and pylorus were removed and a gastrojejunostomy was done. In the fourth dog that had multiple peptic ulcers one was removed for histopathology and the dog was treated with cimetidine. However, this dog subsequently proved to have a parasitic infestation of the stomach thought to be due to *Ollulanus tricuspis*, which was treated successfully with a one month course of fenbendazole.

Six of the seven dogs with peptic ulcers that were treated with H_2 antagonists alone

had a history of NSAID ingestion (Table 2.12). This prior treatment included ibuprofen, piroxicam, phenylbutazone and aspirin, and was stopped immediately on presentation. Three dogs had been receiving two NSAIDs prior to presentation. The first drug in each case was phenylbutazone and shortly before presentation this drug had been substituted by a different NSAID. In one further dog with piroxicam toxicity it was not possible to identify a gastric lesion because of the volume of blood in the stomach, and it was treated successfully with cimetidine. Only one dog was treated with ranitidine. These animals all responded to anti-ulcer therapy and had healed ulcers at the end of 1-2 months treatment. In two dogs the treatment was continued for a further month as a precaution because the ulcer scar did not look sufficiently quiescent on repeat endoscopy. In nearly all cases the treatment was then discontinued with no resumption of clinical signs. In one Bernese Mountain dog it was necessary to continue the H_2 antagonist therapy because the severity of degenerative joint disease made continued NSAID therapy essential.

breed *age, sex*	*NSAIDs ingested*	*length of* *>ingestion* *>signs*	*X-ray*	*Endoscopy*	*Treatment* *>length of*
JRT 8years female	PBZ ASP	12months 7days	no ulcer	ulcer 1.5cm bloody base	cimetidine 2months
Bernese 3years male	PBZ IBF	6months 10days	ulcer	ulcer 1.75cm bloody base	cimetidine continuous
WHW 4.5years female	PBZ IBF	3months 10days	ulcer	ulcer 1cm clean base	ranitidine 1month
GSD 12years male	PBZ	uncertain 90days	ulcer	ulcer 1.5cm clean base	cimetidine 1month
Doberman 3years male	IBF	11days 5days	no ulcer	ulcer 2cm bloody base	cimetidine 1month
Cross 13years male	ASP	10days 5days	focal mucosal irregularity	ulcer 1.5cm clean base	cimetidine 2months
JRT 10years male	PIR	4days	soft tissue opacity in stomach	bloody fluid	cimetidine 1month

JRT=Jack Russell terrier, WHW=West Highland White terrier, GSD=German shepherd dog, Bernese=Bernese Mountain dog, PBZ=phenylbutazone, ASP=aspirin, IBF=ibuprofen, PIR=piroxicam, 1m=1 month.

Table 2.12. Details of dogs with gastric damage arising from non-steroidal anti-inflammatory ingestion: drugs ingested, investigation, treatment and outcome.

2.4 Discussion

The population of dogs that are referred for second opinion consultations are a selected group of animals and the mean ages of those animals in the "no diagnosis" group and the non-neoplastic group thus probably reflect the general hospital population, and are possibly of little significance with regard to the age of occurrence of gastric problems in dogs. However, one explanation for the low mean age range in these two groups could be that younger animals are more prone to gastrointestinal upset because they are more inclined to scavenge. The material that they scavenge is very different from their normal diet. The introduction of, for example, a decaying chicken carcase is likely to upset the normal digestive processes and cause the animal to vomit in an attempt to remove the *"foreign"* material. However, this contact with strange food would need to occur on a regular basis to account for the duration of the vomiting in some of these dogs. The mean age of those dogs with gastric neoplasia was approximately twice that of the "no diagnosis" and non-neoplastic groups. This is not unexpected as, in general, neoplasia affects the older animal, and tumours of the alimentary tract are no exception (Church, Melhaff and Patnaik 1987, Kosovsky, Matthieson and Patnaik 1988). The results presented here agree with other studies of gastric neoplasia in the dog (Lingeman, Garner and Taylor 1971, Sautter and Hanlon 1975, Patnaik, Hurvitz and Johnson 1977, Krauser 1985). In man, and as yet with no evidence to suggest that a similar situation is not the case in the dog, gastric carcinoma is thought to be present in the stomach for 20-30 years before the person is thoroughly investigated for the complaint. The evidence for this long occult period comes from two sources. First, endoscopic examination has revealed that the mean age of patients with early gastric cancer in man is 10 years less than when patients are more commonly seen with the advanced stage (Demling and others 1982). Second, using radiology Kawai, Miyaoka and Kohli (1974) found that the time taken for a tumour to increase twofold is relatively long, implying that development of the condition is fairly slow. The most likely explanation for not seeing cases of gastric neoplasia in the early stages is that the nascent tumour is small and produces few symptoms until it ulcerates. Initially, the small area of ulceration heals quickly because of the normal rapid repair processes that occur in the stomach. Thus this cycle of ulcer/repair may be repeated many times in the years before the tumour reaches such a size that it causes either significant or persistent symptoms such that the patient undergoes thorough investigation.

Considering the effect of breed on the different types of gastric abnormalities in the dogs investigated in these studies, in the neoplastic group the Rough collie was the breed in which gastric carcinoma occurred most frequently. However, this apparent breed predilection commented on previously by Sullivan and others (1987) could not

be substantiated in this series as the numbers were not large enough to allow statistical confirmation. In that series the terrier type, as well as the Rough collie, was thought to show a breed predisposition. Breed predisposition has been identified in other reports of gastric carcinoma in dogs. Scanziani and others (1991) reported that the Belgian shepherd dog suffered from the condition more frequently than others. The variation in the affected breeds identified in different parts of the world could be because of a difference in diet and/or environmental influence rather than a specific genetic predisposition (Lingeman, Garner and Taylor 1971, Murray and others 1972a, Sautter and Hanlon 1975, Patnaik, Hurvitz and Johnson 1977).

Support for this comes from man where there has been a general decline in mortality (Howson, Hiyama and Wynder 1986), and where it has been shown that a reduced incidence of gastric carcinoma occurs in second generation Japanese people living in the United States of America compared to those suffering from the complaint in Japan. Indeed, the incidence in the United States approached the incidence of gastric carcinoma in native born Americans (Haenszel, Kurihara and Segi 1972). The explanation proposed by these authors was that the change in diet resulted in the drop in incidence. As dog food comes from the same food chain as humans, diet may also be related to the occurrence of some gastric cancers in the dog. Environmental influences are also thought to be of importance with excessive ingestion of salt and nitrates implicated in the occurrence of gastric carcinoma in humans and similar findings have been reported from experiments in the rat (Correa, Cuello and Duque 1970, Hill, Hawksworth and Tattersall 1973, Correa 1984, Fontham and others 1985, Takahashi and Hasegawa 1986, Takahashi and others 1986). In the dog, dimethylamine, a precursor of the implicated carcinogen nitrosodimethylamine, has been found to concentrate in the gastric juices with little absorption into the blood (Zeisel and others 1986).

Genetic influence cannot be ruled out as the genetic pool of pedigree dogs in one particular area is often quite small with marked in-breeding used to establish particular lines. It is possible that this may explain the discrepancy in the breeds reported to be susceptible to gastric carcinoma from across the globe. Evidence to support this assertion comes from the fact that crossbred dogs are almost always under-represented in published series (Lingeman, Garner and Taylor 1971, Sullivan and others 1987). There is also some evidence in man of a genetic factor in that relations of those who have had gastric carcinoma have a higher incidence of atrophic gastritis and therefore are more at risk of developing gastric cancer (Kekki and others 1991).

In the non-neoplastic group there was no specific breed identified with any one

condition. This is most probably because, although the group contained the largest number of animals, it consisted of a wide and disparate range of conditions such that meaningful conclusions as to breed disposition were difficult to draw from the results recorded. In the subgroup of dogs with hypertrophic gastropathy there was no breed predilection as has been reported previously by Matthiesen and Walter (1986) in the largest study (45 dogs) published to date. This may be because the number presented here is small. However, the results from two other publications where the numbers of animals investigated were also small, indicated that toy breeds, such as the Lhaso Apso and miniature poodle showed a predilection for hypertrophic gastropathy (Walter and others 1985, Sikes and others 1986).

In the "no diagnosis" group small breeds and Labradors predominated. In considering the numbers of toy breeds in this situation, owners of such dogs are inclined to be more involved with their animals than those who own larger breeds and thus be more aware and concerned when the animal vomits. Thus there may be an owner effect in the frequency of presentation in these cases. Furthermore, small breeds by being more temperamental in their eating habits are frequently tempted by and fed on the same diet as their owners. This diet may be more inconstant and less acceptable to the canine digestive system than food normally fed to pets and so cause the gastric upset. These explanations do not apply to Labradors. Here a more likely explanation is that this breed, apart from being very common, are notorious scavengers and their frequent appearance in this group may represent the effects of refuse eating and the problems it presents, as already discussed.

There was no apparent sex predilection for those animals in the "no diagnosis" group. Where there were reasonable numbers of a specific condition in the non-neoplastic group only those with peptic ulcer showed a male preponderance. In man, this relationship was recognised by Menguy 1970, but not by Murray and others (1972b) in the dog. The most likely explanation for the disparity between the animals in this study and those reported by Murray and others (1972b) is that both were comprised of small numbers and therefore contained the element of chance, which is not the case in man where large cohorts with peptic ulcers have been studied. The male was more prone to gastric carcinoma in this series of dogs as has been reported by other workers (Lingeman, Garner and Taylor 1971, Murray and others 1972a, Sautter and Hanlon 1975, Patnaik, Hurvitz and Johnson 1977, Krauser 1985, Sullivan and others 1987). In man, the male predisposition is probably influenced by co-factors such as smoking and excessive alcohol intake (Domellöf 1979, Fontham and others 1985). Other dietary factors such as charred or overheated fats and oils in the diet have been suggested (Ivy 1955). This would not be the case in the dog fed proprietary dog food suggesting that there is a genuine male predilection.

The variation in duration of clinical signs prior to referral possibly includes a human factor in that referral depends to some extent on the owner recognising the severity of the vomiting and also on the primary veterinary surgeon referring an animal promptly. Obviously, the nature of the vomiting has great bearing on the owners' response. Animals that are intermittent vomiters producing foam or small volumes of bilious fluid from an empty stomach are likely to be dismissed initially by the owners as not requiring investigation. However, the spectacular production of blood explains why such animals tend to be referred quickly usually within three days after the onset of haematemesis. The same situation occurs in man where acute upper gastrointestinal haemorrhage is a serious problem (Dawson and Cockel 1982).

The mean and maximum duration of symptoms in those animals with neoplasia was approximately one third less than the other two groups. Animals with neoplasia often have other signs that aggravate the problem in the owners eyes, namely that these dogs develop polydipsia and significant weight loss which encourages owners to seek advice (Murray and others 1972a, Sullivan and others 1987).

With regard to the haematological investigations carried out, the eosinophil count in two dogs from the "no diagnosis" group and one with erosive gastritis was elevated. Eosinophilic gastroenteropathy may have been missed in these two animals. On the other hand the eosinophilia may merely have been the result of injury to the gastrointestinal tract with the release of histamine. In only 3/6 dogs with eosinophilic gastroenteropathy was there a circulating eosinophilia, which was not particularly marked in any of the three animals. This is not surprising as other forms of eosinophilic disease such as eosinophilic bronchitis or pulmonary infiltrate with eosinophils do not always have a corresponding circulating eosinophilia (Lord, Schaer and Tilley 1975, Calvert and others 1988, Brownlie 1990, Moon 1991). Coracoran and others (1991) found that circulating eosinophil counts of 6/14 cases of eosinophilic bronchitis were normal. This apparent lack of haematological evidence in haematologically related disease also occurs in man. Talley and others (1990) found that 23% of their patients with eosinophilic gastroenteritis had normal peripheral eosinophil counts. This may be because eosinophils are rapidly attracted to tissue with increased concentrations of histamine, which can arise as a result of "*injury*" (Archer 1970, Honsinger, Silverstein and van Arsdel 1972), or because the sample may have been taken during that part of the diurnal variation where the circulating numbers are lower and so any response is less exaggerated and appears to fall within normal range. Few authors have offered much of an explanation for this apparent anomaly. Parasitic infections have been implicated as being one of the causes of this eosinophilic syndrome (Hayden and Van Kruinigen 1973, Moon 1991), but could also be due to diet (Narama and others 1990).

In those animals with neoplastic ulceration accompanied by blood loss into the alimentary tract only the dog with an APUDoma demonstrated anaemia. This situation was reported previously (Sullivan and others 1987), and contradicts the findings of Murray and others (1972a). In contrast 3/9 cases of dogs with peptic ulcers, which had been vomiting for more than a few days, had some evidence of anaemia. This is in agreement with the findings of Murray and others (1972b) and Stanton and Bright (1989). The discrepancy between the occurrence of anaemia in two groups of animals may be because the peptic ulcer, although smaller than the neoplastic lesions, is provided with a richer profusion of blood vessels supplying the granulation bed of the ulcer. Indeed in humans it is the rupture of larger blood vessels in the base of the peptic ulcer that is responsible for the acute upper gastrointestinal haemorrhage, which results in emergency hospitalisation (Brearley and others 1985).

Overall the haematological investigation undertaken in all three groups of dogs was not particularly rewarding as most of the animals had normal values in the parameters investigated. There is often a delay in the receipt of the results of haematological investigations and treatment or further investigations have already taken place before their return. This tends to discourage more intensive use of haematological investigations in either the diagnosis or treatment of gastric abnormalities in the canine. A faster return of information and an adoption of the latest haematological procedures now able to be undertaken needs to be encouraged as advances in haematology are providing very useful ancillary aids to diagnosis in so many other previously under-diagnosed conditions in both animals and man (Norburn and Tvedten 1992, Klag, Giger & Shofer 1993).

The biochemical screening did not reveal any dramatic findings in the groups of animals investigated. However, animals investigated did not include dogs with associated renal disease where biochemistry can be of particular benefit in reaching a diagnosis. In a number of the animals there was a small and inconsistent rise in the non-specific enzymes serum alkaline phosphatase and AST, which may merely indicate an ongoing increase in metabolism arising from cell necrosis in the gut and increased catabolism in the liver as a result of vomiting (Kramer 1980).

Elevation of ALT was the most commonly encountered biochemical change in all three groups. In the "no diagnosis" group it was elevated in 6/18 cases. ALT activity is elevated in plasma under circumstances where there is an increase in hepatocyte membrane permeability. There may therefore be a number of possible explanations for the rise in these cases.

One possible explanation for the increase in this specific hepatic enzyme in the group

where no diagnosis was reached is that these animals were vomiting not because of gastrointestinal disease, but because of some non-specific mild hepatic disease. This then implies that 10 animals from the non-neoplastic group also had evidence of liver damage. However, the increase in permeability could also have arisen from ascending infection or inflammation, or where there may have been some period of anoxia, so that the cause of the increase in ALT need not be strictly hepatic in origin. Indeed many dogs with alimentary signs have mild elevation of enzyme activities (Sherding 1989). None of the animals with peptic ulceration had elevated ALT interpreted as hepatic dysfunction, in contrast to the hepatic pathology reported by Murray and others (1972b) in their series of peptic ulcers. Hypoproteinaemia was rare in this study and was found in only three dogs. Certainly one might have expected hypoproteineamia in those gastroenteropathies that had a inflammatory cell infiltrate. However, there was little change in total protein in any of these animals suggesting that the disease was not severe enough for the animal's compensatory mechanisms to be overcome. Thus although serum biochemistry was not pursued to any extent in the animals referred to in this thesis there were certain biochemical findings in some of the cases of gastric abnormalities that warrant further investigation.

The use of radiology both as a straightforward approach and in combination with barium, in the investigation of gastric complaints in the dog has been the mainstay of diagnostic aids for many years (Douglas and Williamson 1970, Gibbs and Pearson 1973). The recent necessity for sedation to minimise handling of the animal, has not reduced the usefulness of this technique as it does not affect the emptying of barium from the stomach (Zontine 1973, Leib, Wingfield and Twedt 1985). However, the results of radiographic examination in some of the gastric abnormalities in the dogs investigated were disappointing. This was mainly associated with the detection of small lesions and in differentiating between pathological lesions and artefacts. Radiology is especially effective in detecting forms of outflow obstruction (Gibbs and Pearson 1973, Couto and others 1989, Brawer and Bartels 1990), and whilst this certainly was the situation with the cases of pyloric stenosis, atrophic and hypertrophic gastropathy in this study, there were the two cases of foreign bodies that escaped detection. This was most probably due to the shape and size of the foreign bodies. These were flat and composed of materials that project as radiolucent or faint soft tissue opacities on radiographs. Such objects may not be sufficiently radiolucent or radiopaque to be demonstrable against the inherent or added contrast that barium supplies. Furthermore because of their size and shape they do not show up well even in the presence of barium and are easily overwhelmed by the volume of barium given and become masked. An anticipated delay in emptying may not occur because liquid barium is used. The lack of impairment of barium emptying arises because fluid is

preferentially and more rapidly emptied since the emptying rate is determined largely by particle size (Carlson, Code and Nelson 1966, Kelly 1980, Miyabayashi and Morgan 1984, Leib and others 1986, Burns and Fox 1990). Unfortunately, if food is added to the barium to demonstrate a delay in emptying of particulate material, then the mottled appearance of this medium camouflages the small flat foreign bodies that escaped detection in this series (Burns and Fox 1990).

Radiographic examination failed to detect all of the peptic ulcers in this series of dogs. Such ulcers are small and in addition they are often located on that part of the gastric wall that is most difficult to image, namely the junction of incisure angularis and dorsal wall (Wallace, Zawie and Garvey 1991). This is because on lateral views the pylorus and duodenum curve cranially across this area, and on the dorso-ventral or ventro-dorsal views the area is overlain by the incisure angularis (O'Brien 1978).

In man this problem is overcome by the extensive use of double contrast studies (Ichikawa 1973), which have not been found to be very helpful in the dog (Jakovljevic 1988). The success of the technique in man is in no small measure due to the radiologist being able to instruct his human patients to contort their bodies into specific positions to encourage specific movement of the contrast within the gastric lumen (Ichikawa 1971).

In the normal stomach some areas on radiographic examination can project in such a way as to mimic quite severe ulceration, and so differentiating normal mucosa from ulcerated mucosa can be difficult. This can be overcome, to a certain extent, with the use of image intensification that allows continuous examination of the barium-filled stomach, but results in a poorer quality image. Image intensification allows peristalsis to be examined and thus the appearance of an area of interest on a spot film that may or may not be artefactual can be viewed repeatedly and a diagnosis reached. However, even the use of image intensification does not always permit definitive diagnosis of neoplasia on the basis of radiological examination alone (Sautter and Hanlon 1975, Sullivan and others 1987). In one series of 31 cases of gastric carcinoma in which 17 had image intensification used, only 10/17 could be reliably interpreted as having a neoplastic lesion present (Sullivan and others 1987). Thus the results of the radiological examinations carried out in this study, while providing useful information, did not always provide sufficient information to permit a definitive diagnosis to be reached.

Endoscopy is being used with increasing frequency in diagnosing gastric conditions in both humans and animals (Happé and van der Gaag 1983, McCloy 1985). Endoscopy already has overcome many of the problems of radiology, especially those related to the examination of abnormalities of the gastric mucosal surface as it allows

direct detailed examination of this aspect of the stomach (Happé and van der Gaag 1983, Happé 1986, Sullivan and Miller 1985). However, a combination of radiology and endoscopy should be used as a means of diagnosis as they compliment each other (Wittenberg and Kantrowitz 1970). The limiting factors in endoscopy are that it does not allow examination of peristalsis, or the subsurface tissues and is severely limited if there is excessive gastric content present. However, the recent introduction of more sophisticated equipment that combines the endoscope with an ultrasonographic unit has permitted the subsurface tissue to be investigated (Kimmey and others 1989, Silverstein and others 1989, Lightdale 1992).

When the dogs in the control group underwent endoscopic examination many had bile, fluid and foam present in the gastric lumen, which limited thorough evaluation of the luminal surface in a few animals. Excessive fluid can be removed by aspiration if suitable suction pumps are available. The foam can be reduced by the use of anti-foaming agents given prior to endoscopy (Gasster, Westwater and Molle 1954). In man the administration of pre-procedural simethicone mixed with 100ml of water has been found to clear the stomach and duodenum of foam and bubbles; this must be given less than 10 minutes before examination (Bertoni and others 1992). In the initial examination carried out none of these measures were taken in the dogs examined, but would certainly be well worthwhile considering for future endoscopic examinations in the dog.

As the "no diagnosis" group could be described as being most similar to the control group in most of the parameters examined by endoscopy, it is difficult to explain why these animals had an emptier stomach at the time of examination. There are two possible explanations, either the vomiting "no diagnosis" group were eating less because of a temporary gastric upset and therefore had less to empty from their stomachs, or because the clinical cases were fasted for two successive nights to allow examination of a barium meal after the first night of fasting and an endoscopic examination after the second night of fasting.

The assumption that the finding of duodenogastric reflux of bile on endoscopic examination indicates the cause of the gastritis in some of the clinical cases must be re-evaluated in the light of the frequency with which pools of bile were seen in the control dogs. However, in man the presence of bile was found in 28% of normal stomachs in contrast to 68% of abnormal stomachs and there is some association between dyspepsia and endoscopic evidence of bile reflux (Eyre-Brock, Holroyd and Johnson 1984). Normal dogs with duodenogastric reflux may or may not have abnormal histological changes in the gastric mucosa (Happé, van den Brom and van der Gaag 1983). Experimentally in the dog it has been shown that pyloric sphincter

incompetence with duodenal reflux will lead to chronic gastritis (Adair and Wlodek 1968). In man the occurrence of duodenal regurgitation has been found to vary between individuals and between examinations. Siurala and Tawast (1956) found duodenal contents in the gastric contents of many fasting patients and some of these patients had evidence of atrophic gastritis. That duodenogastric reflux occurs in the dog following feeding is accepted (Sonnenberg and others 1982).

The duodenogastric reflux found in normal dogs and in the clinical cases may have been due to fasting. Nevertheless, the inflammatory effect of bile has been confirmed experimentally by Lawson 1964, and reflux of bile through the pylorus will induce a gastric mucosal hyperaemia (Keighley, Asquith and Alexander-Williams 1975). Furthermore evidence has been presented to show that duodenogastric reflux can produce clinical signs in human beings (Kilby 1970, Keighley, Asquith and Alexander-Williams 1975, Brough, Taylor and Torrance 1984). In man reflux of bile can be demonstrated not only after diversional surgery of the antrum and pylorus (Capper, Butler and Buckler 1966, Kilby 1970, Graffner, Florén and Nilsson 1984), but also in the patient with normal antrum and pylorus (Johnson 1972). In one study in man (Glikmanas and others 1984) patients were divided into two groups on the basis of the endoscopic identification of a bile pool and a telemetry device was placed in the stomach to measure subsequent pH. Those patients who had a clear mucus were found to have few and short lasting rises in pH. In contrast those patients with a bile pool had frequent and prolonged rises in pH suggesting that duodenogastric reflux causes the bile pool and that it is not necessarily induced by endoscopy. Despite much work, just what the importance reflux of bile in the stomach of man and animals is not fully understood and requires further investigation. In the control dogs used in this study a bile pool was found in 36.67% of animals. However, in those animals from the non-neoplastic group a similar sized bile pool was found in 23%, and in a further 18% of dogs a large pool was found. Although bile reflux was observed in both control and non-neoplastic groups, there was a difference in severity. None from the control group had a bile reflux scored 2 or 3 in contrast 30% from the non-neoplastic group. Bile reflux occurs during normal alimentation but the bile is not very concentrated and is rapidly swept from the stomach, but bile will injure the mucosa of an empty stomach where it lies in contact with the mucosa without being swept away. The time that this situation is most likely to occur is during the night when the animal is asleep. Borg (1959) has shown in man that the bile reflux occurs during the night and rises to a peak between 1-2am in the morning. His explanation for this was that there was a nocturnal relaxation of the pylorus possibly induced by a preponderance of parasympathetic stimulation during sleep and by the recumbent pose adopted by sleeping people.

If the classification of Karvonen and others (1983) of erosive gastritis in man is applied to the findings in the animals in this study with the same condition, then the incomplete flat erosions seen were of an acute nature. However, Sauerbruch, Schreiber and Schüssler (1984) and Gad (1986) found that there was some discrepancy between the endoscopic diagnosis of erosion and confirmation by histology of endobiopsies in the human. Gad (1986) suggested that this was most probably due to inaccurate translation of the sections produced from the biopsy material because the samples obtained by endobiopsy were unsatisfactory in amount and content. Therefore one might ask just what is the benefit of taking endoscopic biopsies (Karvonen and others 1983, Franzin and others 1984). However, published reports suggest that endoscopic examination is one of the most successful methods used routinely in the diagnosis of erosive gastritis. Taor and others (1975) found that the endoscopic findings in the majority of cases of gastritis agreed with the subsequent histological changes in sections prepared from endobiopsies. They also found that a third of the samples taken from endoscopically normal mucosa had histological changes indicative of gastritis. This tends to suggest that some of the "no diagnosis" group that gave negative results on endoscopy may have had gastritis. However, as Whitehead, Truelove and Gear (1972) pointed out finding gastritis on one or two samples does not necessarily reflect the state of the mucosa as a whole. In one of the few studies published relating endoscopic and histological findings in the dog, Roth and others (1990) found that none with normal endoscopic findings had abnormal histology. In contrast they also reported that a few animals were seen to have endoscopic change that was not substantiated histologically. Nevertheless it does make the examination of biopsies from the gastric mucosa a possible means whereby diagnosis of gastric abnormalities can be made, which currently might go undiagnosed.

The histological samples obtained by exploratory laparotomy or *post-mortem* examination presented little difficulty as far as interpretation was concerned in the animals in this review. This was because a suitable amount of tissue was obtained by these methods. However, despite samples being taken during endoscopic examination the results from the sections prepared were inconclusive and a significant number of cases were diagnosed on grounds other than endoscopic biopsy. The handling and interpretation of endobiopsies has been a problem since endoscopes became a major diagnostic aid. Morrissey (1972) in reviewing gastrointestinal endoscopy felt that perhaps several different types of endoscopes, such as side-viewing as opposed to panendoscopes, might be needed to get adequate biopsies. Danesh and others (1985) examined different sizes and types of biopsy forceps as well as the technique of actually grabbing the mucosa in an attempt to determine some of the factors that might determine the quality of the resultant quality of the endobiopsy.

They concluded that the endobiopsies obtained using paediatric gastroscopes with round cupped forceps gave the most inadequate sample volume and the poorest samples since they are small, tend not to penetrate particularly deep into the mucosal layer, fragment easily and are difficult to orientate. Unfortunately, this set of circumstances prevailed for a large part of this study and the forceps used with the video-endoscope were also round rather than ellipsoid. Despite this, it may be argued that endoscopy is fairly accurate as there has been good correlation between abnormal appearance and histological confirmation, and that the main flaw lies in those stomachs that appear to have normal gastric mucosa endoscopically (Dekker and Tytgat 1977), which could perhaps be resolved by widespread sampling of the mucosa.

Endoscopy is very effective in the identification of ulceration of the gastric lumen and on the basis of this series is a much more sensitive investigative aid than radiological investigation. Gastric carcinoma ulcers seen in this series were large and presented with a "*chewed out*" appearance in similar fashion to those reported previously (Sullivan and others 1987, Scanziani and others 1991). Two of the lymphosarcoma dogs had small multiple marginally raised ulcers with a sharp border between normal and abnormal so that there was no surrounding rugal change. This type of appearance is similar to that reported in man where a comparison between the endoscopic appearance of carcinoma and lymphoma showed the same disparate appearance of the two tumour types (Fork and others 1985). The third case was atypical in that it was a large ulcerated mass located in the fundus on the greater curvature. The location would have been uncharacteristic for gastric carcinoma, but does raise the possibility of endoscopic confusion because the large ulcer is typical of gastric carcinoma, which is easily the commonest gastric tumour (Howson, Hiyama and Wynder 1986).

Typically, the benign ulcer is found at the junction between antrum and pylorus (Ruding 1967), less than 2cm in diameter and lacks the "chewed out" inner wall with overhanging lip and thickened, fixed rugae that are seen in neoplastic ulcers. Six of the animals with peptic ulcers had had previous treatment with NSAIDs. Interestingly, three of these had been ingesting a particular drug over a fairly long period of time before an abrupt switch to another of these preparations was made. The endoscopic appearance of several of these ulcers suggested that they had been present for longer than the clinical signs, or the length of time that NSAIDs were reported to have been ingested by the dog.

In man the use of NSAID drugs has been implicated in peptic ulcer disease, where the association seems to be more positive for the person over 60 years of age (Collier and

Pain 1985, Sommerville, Faulkner and Langman 1986). However, peptic ulcer may be silent in many people until the ingestion of NSAIDs cause the peptic ulcer to become clinically active (Glise 1990). Certainly 3/6 cases presenting with peptic ulcer in this series after ingesting NSAIDs, and the dog with piroxicam toxicity could be considered aged, that is over 10 years of age, suggesting that the sequence of silent peptic ulcer exacerbated by NSAID administration also pertains in the dog. The NSAIDs produce this effect by interfering with the normal defense mechanisms of the stomach. The main route of action is by suppressing prostaglandin synthesis (Ferreira, Moncada and Vane 1971, Vane 1972, Vane and Botting 1978, Kauffman and others 1979). The role played by prostaglandins in the defense of the gastric mucosa is well recognised and operates by stimulating blood flow (Cheung 1980, Cloud and Ritchie 1982, Konturek and Robert 1982, Pihan and others 1986), by playing an integral part in the secretion of bicarbonate into the lumen, the production and release of glycoproteins (Johansson and Bergström 1982, LaMont and Szabo 1984), by maintaining the hydrophobic nature of the adsorbed layer of surface-active phospholipids (Lichtenberger, Richards and Hills 1985), and cellular restitution (Morris 1986). Thus in a situation where the stomach has a pre-existing lesion that is attacking the integrity of the stomach, but which is being held in check, the administration of a drug that removes the defensive prop will lead to this lesion overwhelming the mucosa and the patient experiences obvious symptoms. This appears to be what happens to these silent peptic ulcers in the presence of prostaglandin suppressing NSAIDs. The NSAID drugs including aspirin, indomethacin and phenylbutazone are also capable of producing acute erosions and ulcers in a variety of species (Varró, Csernay and Jávor 1959, Watt and Wilson 1959, Davenport 1969, Nicoloff 1968, Bugat and others 1976, Miller and Jacobson 1979).

Thus in the initial investigation of vomiting dogs it is essential to ascertain any previous administration either by the owner or by the referring veterinary surgeon of this class of drugs, as they are clearly provocative to the gastric mucosa, and their potential role in the induction of gastritis, by breaking the mucosal barrier, as opposed to exacerbating cases of peptic ulcers needs to be investigated further.

One of the most interesting findings of this study was the identification of a dog with ulcers caused by parasites. The parasitic ulcers resembled both endoscopically and grossly the other forms of peptic ulcers. The parasite thought to be responsible *Ollulanus tricuspis* is seen most commonly in cats, particularly big cats, and has not been described in the dog before (Hargis, Prieur and Westcott 1981, Guy 1984, Hasslinger 1984, Wilson and Presnell 1990). Stanton and Bright (1989) reported that peptic ulceration is almost always related to a single lesion. They reviewed 43 cases of gastroduodenal ulceration and found only one of 21 NSAID-induced cases with

multiple ulcers in the stomach. All the dogs with peptic ulcers in this study presented as having a single lesion. Therefore the multiple lesions found in the dog with the parasite helps to differentiate this condition from other cases of peptic ulcers. Unfortunately, attempts to demonstrate the extremities of the parasite by scanning electron microscopy and thus confirm absolutely the presence of this species of parasite were unsuccessful. Therefore although this case of parasitism in the dog may be an aberration, this condition must be considered in the future in the differential diagnosis of peptic ulcers.

In 1984, Dooley and others published a comparative study of 100 hundred dyspeptic patients, evaluating endoscopy versus double contrast radiography as diagnostic aids and found that endoscopy was superior. The evidence presented in this series of cases tends to concur and suggests that endoscopy is a more sensitive and specific diagnostic tool than radiology. However, it would be arrogant to dismiss radiology. The two procedures should be considered complimentary in view of the limitations inherent in each technique.

The lack of treatment for those where "no diagnosis" was reached did not appear to be detrimental to those animals since all had ceased to vomit within three months of discharge from the hospital. This may be because those that were scavenging were successfully stopped in this behaviour, or in other cases because the underlying cause of the vomiting, which had not been determined, disappeared or resolved. The last possibility is that these animals had not been followed up for long enough to ensure that the problem had truly resolved, and that if relapse occurred the owners did not push for re-investigation as the condition was not serious, accepting that the dog was going to have bouts of vomiting.

Of those animals that underwent surgery, those that had foreign bodies completely recovered, as one might expect. Those animals with hypertrophic gastropathy and pyloric stenosis all improved, though some not completely. The possible explanations for this are that the degree of gastric distension that existed prior to surgery failed to resolve after surgery so that the stomach persisted as a rather large ineffective sac leading to excessive retention of food. Alternatively, the pyloric relief surgery undertaken in these cases of pyloric outflow obstruction failed to adequately widen the outflow tract, resulting in continuing outflow obstruction. This hypothesis is difficult to prove in the clinical case as the underlying pathological change and not the surgery may be responsible. Certainly experimental work in the dog where the three main techniques used to relieve these conditions have been compared has shown that all were perfectly capable of allowing adequate emptying of the stomach, though all showing a tendency towards slowing of gastric emptying (Fox and Burns 1986,

Papageorges, Breton and Bonneau 1987, Stanton and others 1987), and produce an increase in duodenogastric reflux (Müller-Lissner and others 1983, Müller-Lissner and Blum 1984. One last explanation is that neither persistent dilation or surgery are responsible for the complete resolution of signs, but the procedures permit excessive reflux of bile when the stomach is empty and induce an alkaline gastritis. Alkaline reflux gastritis has been identified in man and also in the experimental dog after diversional surgery (Lawson 1964, Capper, Butler and Buckler 1966, Keighley, Asquith and Alexander-Williams 1975, Müller-Lissner and Blum 1984, Kellosalo, Alavaikko and Laitinen 1991), and the fact that bile can cause cell necrosis of the surface epithelial cell has been known for some time (Grant and others 1951).

The rationale for treating dogs with chronic, follicular and erosive gastritis with potentiated sulphonamide or cimetidine was mainly speculative, but also offered a placebo effect. However, there is some evidence to support the use of both these type of drugs in the above conditions. One approach to counteract the deleterious effect of injury to the stomach is to suppress the acid secretion, since it plays such a significant role as an aggravator of mucosal damage (Guth and Code 1978, Guth, Aures and Paulsen 1979). Histamine is a mediator in the stimulation of gastric acid and its release occurs during the early phases of gastric secretion. Acetylcholine and gastrin are also involved in the release of acid. However, the H_2 receptor would appear to be the major controller of the parietal cell hydrogen ion production line (Code 1977). Cimetidine was the first of the commercially successful H_2 antagonists. It attaches to the H_2 receptor and blocks the activation of the parietal cell (Brimblecome and others 1975, Burland and others 1975). There are now recognised side-effects of cimetidine in man such as pruritus and gynaecomastia (McGuigan 1981, Gailbraith and Michnovicz 1989) Many of the side-effects and drug interactions of cimetidine can be traced to the inhibition of cytochrome P_{450} (Peura and Freston 1987). However, from a patient point of view, one of the drawbacks of cimetidine is the requirement for it to be taken four times daily. Ranitidine has overtaken cimetidine in terms of market share because it lacks many of the side-effects, but importantly needs only be taken twice daily (Bohman, Myren and Larsen 1980, Woodings and others 1980). Both these drugs will suppress basal and stimulated acid secretion (Ostro 1987).

Konturek and others (1981a) noted that at sub-suppressive doses, which did not affect prostaglandin levels, ranitidine would protect the stomach of the rat against the injurious challenge. This same effect was not noted with cimetidine where the healing or protective effect, rather than acid inhibition was due to endogenous prostaglandin release (Konturek 1985). Thus the class of drugs to which cimetidine belong, because they suppresses acid secretion and as consequence prevent

pepsinogen becoming active, will in fact allow healing of the stomach in less invidious conditions than normally prevail (Gurll and Damianos 1981). Further the reduction in acid secretion will limit the amount available for back-diffusion that has been shown to stimulate pepsin output in dogs (Johnson 1971). The use of potentiated sulphonamide is rather harder to justify. One might argue that there was a possibility that these animals may have had a form of *Heliobacter pyloris* (*Campylobacter pyloridis*) specific to the canine. In humans, this bacteria has been established as a major cause of gastritis in adults and, perhaps more importantly, in children, and has been implicated in the pathophysiology of peptic ulceration (Buck and others 1986, Hazell and Lee 1986, Hazell and others 1986, Marshall 1986, Glise 1990, Maaroos and others 1991, Wallace 1991). Although a *Heliobacter* species has been identified in the laboratory ferret there is still no convincing evidence of its occurrence and pathogenicity in the antrum of the dog (Fox and others 1990), though the presence of spiral bacteria on the surface of the gastric mucosa has been recognised for nearly 100 years (Bensley 1899).

The relationship between previous treatment and the occurrence of peptic ulcers was one of the important areas that was investigated. All but three of the dogs with peptic ulcers had a history of NSAID ingestion prior to the onset of clinical signs. For most of these dogs the delay from the commencement of NSAID ingestion and appearance of clinical signs was of the order of 7-10 days, yet on endoscopic examination the peptic ulcers looked quite mature. This raises the possibility that some of these ulcers may have been clinically quiescent with the animal living in balance with the ulcer, and the ingestion of NSAID provoked the ulcer to change from a relatively quiescent state to an aggressively active state, and to become clinically manifest. In man, superficial severely haemorrhagic 1cm erosions not ulcers were present three days after ingestion of aspirin tablets (Roth and others 1963), and other NSAIDs have been implicated, such as ibuprofen, indomethacin and sulindac (Loiudice, Saleem and Lang 1981). Certainly, three of the dogs with NSAID induced peptic ulceration had been on prolonged intermittent therapy with phenylbutazone that had not appeared to induce signs of illness that the owner was able to detect. One cannot discount the possibility that a silent ulcer had been induced by the phenylbutazone. Indeed 8/22 cases described by Murray and others (1972b) were occult. No single NSAID was common to the cases. The variety used indicates the damage inducing capability all NSAIDs appear to have. Reports in the literature implicate aspirin, flunixin meglumine, ibuprofen, meclofenamic acid, naproxen and phenylbutazone as being associated clinically with peptic ulceration in the dog (Ewing 1972, Roudebush and Morse 1981, Cosenza 1984, Walter and others 1985, Daehler 1986, Thomas 1987). However, Stanton and Bright (1989) in a review of 43 cases found that only three dogs had no identifiable underlying disease or drug

ingestion that might be implicated suggesting spontaneous clinical peptic ulcers do occur, but are rarely themselves clinically manifest.

For all but two of the animals with peptic ulcers treatment with H_2 receptor blockers resolved the problem and allowed the ulcer to heal. The fact that it has not proved necessary to treat some of these animals with continued medication adds weight to the suggestion that the peptic ulcers in these cases were pre-existing and silent, a feature recognised in man (Glise 1990).

For one dog cessation of H_2 blocker therapy led to relapses that were accompanied by massive haemorrhage - it was necessary to remove the pylorus and antrum of this animal's stomach. Acute upper gastrointestinal haemorrhage is a problem in man that can lead to death. In man it has been found that treatment with H_2 blockers during these episodes makes little substantial difference (La Brooy and others 1979, Dawson and Cockel 1982). In the one large published series of peptic ulcers in the dog there was a mortality rate of 10/43, however, not all the ulcers were due to NSAID therapy (Stanton and Bright 1989).

In a second case the cessation of NSAID ingestion was contraindicated as the severity of degenerative joint disease meant that the animal could not lead a satisfactory pain-free life without the use of NSAIDs, yet the animal had an active peptic ulcer. Here it was found necessary to institute a prophylactic regime of H_2 blocker therapy to prevent further ulceration, and then to maintain a NSAID regime to control the pain associated with the degenerative joint disease. This has permitted the animal to follow a relatively pain free and mobile life, without the recurrence of clinical or, more importantly, endoscopic evidence peptic ulcer.

It is recognised in both man and experimentally in the dog and rat that ingestion of NSAIDs, even if they do not induce or exacerbate peptic ulceration, will produce erosions and inflammation in the stomach (Muir and Cossar 1955, Hurley and Crandall 1964, Lev, Siegel and Glass 1972, Lanza 1984, Lanza and others 1986, Laine and Weinstein 1988). However, it has been shown that the presence of acid in the lumen is an essential prerequisite for the full effect of NSAIDs to be exerted (Brodie and Chase 1967). This is because the absorption of the NSAID is reduced unless an acid pH exists in the lumen allowing the NSAID drug to be undissociated, thus lipid soluble in the gastric juice, thus move through the cell membrane, then into ionic solution where the damage is done (Brodie and Chase 1969). The damage caused is probably accentuated by increased duodenogastric reflux that has been shown to occur sometimes following the administration of NSAID. Admittedly the effect was most marked for those dogs given aspirin as opposed to indomethacin and phenylbutazone (Pantoja and others 1979). The additive effect of bile and NSAID in

the induction of mucosal damage has been demonstrated using indomethacin in the dog and other species (Davenport 1964, Ivy 1971, Djahanquiri, Abtahi and Hemmati 1973, Semple and Russell 1975, Whittle 1977, Moody and others 1978). Whilst aspirin has long been recognised as being toxic to the gastric mucosa, Lanza and others (1979), Lanza (1984) and Lanza and others (1986) have presented work that shows that endoscopically in man the newer NSAIDs including flurbiprofen, ibuprofen, indomethacin, naproxen all produce less damage to the stomach mucosa than aspirin.

Histamine H_2 receptor blockers have been shown to afford protection to the gastric mucosa when attacked by barrier breakers experimentally, though this concept is not universally accepted (Kenyon, Ansell and Carter 1977, Levine, Sirinek and Pruitt 1979). However, H_2 blockers have been used in man in the treatment of erosive gastritis (Gurll and Damianos 1981).

Thus there is clearly need for greater information about the circumstances surrounding the use of NSAIDs in the management of the clinical patient where there will be conflict between the need to protect the gastric mucosa in the face of drugs required by the patient for other conditions. The mechanisms which increase the toxicity to the mucosa of these drugs and the action of which can be accentuated by the presence of physiological fluids refluxed into the stomach needs further investigation.

Thus the overall conclusions drawn from the results produced from these 100 vomiting dogs clearly indicated that further investigation in almost all aspects of diagnosis are required. Whilst endoscopy provided most useful information there is obvious room for improvement in the methods used for obtaining suitable biopsy material and the ability to translate the histological findings from this source. The role played by bile in the production of the variety of different gastric complaints known to occur in the canine is of special importance. Finally the gastric problems that may be being produced or exacerbated by some of the everyday treatment given to dogs for some of the other common complaints in small animal practice requires clarification. In an attempt to increase our knowledge in some of these important areas it was decided to study changes in the rat under varying experimental conditions, including the administration of acid, bile and NSAID drugs. The results were investigated by examining the stomach and its contents grossly, and by the evaluation of the histological changes by light and scanning electron microscopy.

3. General Material and Methods

3.1 Experimental Animals

3.1.1.1 Rats

Sprague-Dawley rats bred in-house were employed throughout this study. The rats were young adults weighing between 200-225g. The rats were used as batches, no attempt was made to distinguish them on basis of sex, and were allocated to groups at random. All experiments were carried out between 10.00 and 11.00 GMT.

3.1.1.2 Maintenance

The rats were housed in RC1 cages (North Keat Plastics) and fed on a commercial diet (*Rat & Mouse No1*, Special Diet Services).

3.1.1.3 Anaesthesia

The rats were deprived of food for 36 hours prior to induction of anaesthesia. Water was not withheld but given *ad libitum*. The rats were anaesthetised with a combination of fentanyl-fluanisone (*Hypnorm* - Janssen Animal Health) at a dose of $0.2mgkg^{-1}$ given as an intramuscular injection into the biceps femoris, followed immediately by diazepam $2.5mgkg^{-1}$ (*Valium* - Roche Products Ltd) given via the intraperitoneal route. The rats were placed in dorsal recumbency on a bed of cotton wool, which was covered in thin plastic and overlaid with a single layer of paper towel to circumvent hypothermia. Respiratory rate, depth and palpebral reflexes were monitored to assess depth of anaesthesia. Rectal temperature and mucous membranes were checked regularly to monitor anaesthesia for adverse effects.

3.1.1.4 Surgical Approach

The stomach was approached via a small cranio-ventral midline laparotomy incision. Using the xiphoid as a landmark, a 2cm incision was made through the skin, subcutaneous tissue and linea alba commencing 2cm caudal to the xiphoid. The hepatic lobes of the liver were easily identified. By pushing the greater omentum laterally and cranially, the edges of the left hepatic lobes could be identified. The free edge of the medial hepatic lobe was visible as it emerged from beneath the lateral hepatic lobe. Using this as a landmark, the proximal duodenum was picked up and exteriorised by passing forceps underneath and dorsal to the lobe. The constriction and colour difference between the duodenum and pyloric canal made demarcation straightforward.

The pyloro-duodenal junction was ligated with 1.5 or 2 metric silk. Care was taken to avoid damage to the blood vessels (right gastric and gastroepiploic arteries and veins) and accompanying branches of the vagus nerve. Intragastric infusion of agents was

done by injecting the solutions into the non-glandular region near the greater curvature with a 2ml syringe and fine 25 gauge needle. The skin was closed with a single 3.5 metric silk suture in those rats where the exposure times were to be greater than five minutes. This was to reduce heat loss through fluid evaporation from exposed moist tissues.

3.1.1.5 Euthanasia

The rats were sacrificed by injecting sodium pentobarbitone (*Euthatal* - RMB Animal Health Ltd) into the cardiac chambers to cause respiratory and cardiac arrest, and is an accepted and humane way to kill animals.

3.2 Harvesting of Tissue

3.2.1 Fixatives used are described in Appendix 9.1

3.2.2 Gross Examination

The anatomical terminology applied to the stomach for descriptive purposes follows that proposed by (Robert 1971). The forestomach refers to that part of the stomach which lies proximal to the limiting ridge, the corpus to the area from the limiting ridge to the antrum, which is clearly delimited from the corpus by its lighter yellow colour and thinner wall. The pylorus is that part of the stomach which separates the stomach from the duodenum.

The stomachs were removed from the rats immediately following cardio-respiratory arrest. The duodenum was opened along the mesenteric border and the incision extended through the pylorus cutting the silk ligature and then along the greater curvature of the stomach to the cardia. The contents were gently flushed with isotonic saline followed by 0.1M sodium cacodylate buffer to remove any food and other lightly adherent particles. The surface of the mucosa was then examined grossly. Since the infusions and the opening of the stomachs were made through and along the greater curvature, any alterations seen along these margins were ignored because of the possibility of iatrogenically induced change.

3.2.3 Sample Areas

The stomachs were quickly placed mucosal side up on a small cork board. They were fastened to the board with mapping pins placed along the greater curvature at the non-glandular/corpus, corpus/antral and pyloro/duodenal junctions. The cork board and stomach were then placed in a Petri dish filled with Karnovsky's fixative to prevent autolysis and permit more leisurely harvesting of tissue for microscopic examination. All the samples were cut from the stomach with a single-edged razor

blade. From one half of the stomach large samples (approximately 0.5x1cm) of corpus and antrum were removed and pinned at one end to small pieces of cork to aid orientation. These were then placed in fresh Karnovsky's fixative. From the other half of the stomach small pieces of corpus and antrum were removed for light and transmission microscopy.

3.2.4 Light Microscopy

Tissue samples were removed from the Petri dish containing Karnovsky's and were immersion fixed in 4% BNF for a minimum of 48 hours. The samples were removed from the BNF and processed in a Shandon-Elliot automatic tissue processor on a 24 hour cycle (Appendix 9.2). Sections were cut at 3-5μm at right angles to the surface and mounted on glass slides. The sections were stained with haematoxylin and eosin and periodic acid Schiff & Alcian blue (PAS/Alcian blue). The preparation of stains is described in Appendix 9.2.

3.2.5 Scanning Electron Microscopy

The samples were stored in Karnovsky's fixative for a minimum of 24 hours and dehydrated in a series of acetone baths, critical point dried and mounted on aluminium stubs (Appendix 9.2). The samples were examined with a Philips 501B scanning electron microscope operating at 15 kilovolts. Photographic records were obtained with a Steinheil Optronic camera using Ilford FP4 film.

3.2.6 Transmission Electron Microscopy

Small pieces of tissue approximately 2mm wide and 4mm long were placed in 2.5% gluteraldehdye in isotonic sodium cacodylate buffer (0.075M) for more than 48 hours. The samples were processed and on the basis of semi-thin sections areas were prepared for examination by transmission electron microscopy by ultra-thin sectioning (Appendix 9.2). The resulting grids were examined with a transmission electron microscope.

3.3 Assessment of Samples

3.3.1 Gross Examination

The stomachs were examined on removal and after they had been pinned out. Evidence of oedema, erythema and erosions were looked for in the defined anatomical regions of the stomach. The findings were recorded on an annotated gastric map (Figure 3.1).

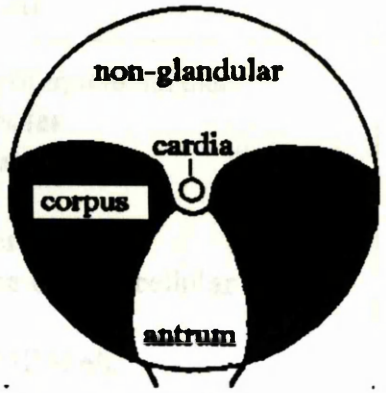


Figure 3.1. Gastric map.

3.3.2 Light Microscopy

The surface epithelial cells, mucus layer and luminal contents were examined. Evidence of polymorph accumulation at the lamina propria was evaluated by examining 4 fields per section at x400 and counting the number of polymorphs present to give an average. The submucosal blood vessels were examined for evidence of congestion. The degree of congestion was assessed subjectively (scored 0-2) on the basis of the volume of blood cells in the veins between the mucosa and the muscularis.

Migration of WBCs to the wall of the blood vessels was noted, if more than two WBC were found migrating, and if this was present in more than two blood vessels. Emigration of white blood cells was also looked for.

The presence of oedema was assessed by measuring the thickness of the submucosal layer against the thickness of the epithelial and submucosal layers. The measurements were made at three sites and averaged to produce a representative ratio. This was done using a 19mm graticule on a 6.3x eyepiece.

The apical epithelial cells and luminal contents were examined. Damage to the apical cells was qualitatively graded 0-3 to describe the severity of involvement. The thickness of the overlying mucus was noted either as a trace, otherwise it was considered to be thick. The cell content of the mucus was evaluated as none/few or many.

3.3.3 Scanning Electron Microscopy

Initial assessment concerned the mucus layer thickness and extent of covering. The features then looked for were extrusion of surface epithelial cells, rosette formation, enlargement of the gastric pits, loss of the normal cobblestone appearance of the surface epithelial apical surface, excessive convexity of the individual cells and changes in the intercellular junctions (Table 3.1).

General	Individual
mucus covering	ruffling of apical surface
mucus thickness	apical holes
extrusion of cells	apical concavity
rosette formation	
enlargement of gastric pits	apical erosions
loss of cobblestoning	exposure of intracellular
contents	
prominence of intercellular junctions	empty cell shells
cell convexity	

Table 3.1. Scanning electron microscopy features.

The stubs were examined at a variety of magnifications, and subjective analysis of the surface epithelial cell layer was quantified by assigning the observations to a scale from 1-5 (Table 3.2).

Further features noted were absence of microvilli, ruffled apical surface, presence of a hole in the luminal surface, concavity of the apical membrane, visible intracellular contents and the presence of empty cell shells. Each features was looked for was also assessed subjectively and ascribed a score from 1-5. The scores from the general features and the individual cell features were summed to give an overall score for each grouping.

3.3.4 Transmission Electron Microscopy

The surface epithelial cells were examined with reference to the general integrity of the mucosal surface, integrity of the apical membrane, the distribution of the subsurface cluster of mucous granules, nuclear chromatin clumping, alterations in mitochondria and assessment of tight junctions.

3.4 Data Analysis

Excel v3.0 (Microsoft) was chosen as the method of entry because of an excellent user interface and powerful but simple editing tools. The data from the spreadsheets were then exported to the following statistical software packages for further analysis, *Minitab* v7, *BMDP* and *GLIM*. The statistical tests used were the *t*-test, Polychotomous Logistic Regression and Generalised Linear Models respectively. Stastistical significance was set at the 95% level ($p < 0.05$).

	Qualitative score of features on scanning electron microscopy				
	1	2	3	4	5
General					
mucus covering	none	sparse	few islands	profuse	complete
mucus thickness	no cover	thin	distinct outline	bare outline	not visible
cell extrusion	occasional	few	moderate	pronounced	marked
rosette formation	occasional	few	moderate	pronounced	marked
enlargement of gastric pits	none	few	moderate	pronounced	marked
loss of cobblestoning	none	few	moderate	pronounced	marked
prominent i/c junctions	none	few	moderate	pronounced	marked
cell convexity	none	few	moderate	pronounced	marked
[pooled score 1]					
Individual					
apical ruffling	none	few	moderate	pronounced	marked
apical holes	none	few	moderate	pronounced	marked
apical concavity	none	few	moderate	pronounced	marked
apical erosions	none	few	moderate	pronounced	marked
exposed i/c contents	none	few	moderate	pronounced	marked
empty cells	none	few	moderate	pronounced	marked
[pooled score 2]					

i/c = intracellular

Table 3.2. Definition of scanning electron microscopy scores.

4. Normal Rats

4.1 Introduction

Grossly, the rat gastric mucosa can be divided into three regions. The corpus is separated from the nonglandular portion by a limiting ridge, and the antrum can be distinguished from the corpus, being thinner with a yellow hue, unlike the red colouration of the corpus (Berg 1942, Robert 1971). Histologically, these two regions are different. The fundus has short foveolae and relatively long straight glands, and apart from mucous neck cells are lined by substantial numbers of chief and parietal cells. In the antrum, the glands have long foveolae and short coiled glands, which make it difficult to see a perpendicular section of the gland itself (Harvey 1906, Berger 1934, Grossman 1958, Grossman and Marks 1960).

Although the initial work on scanning electron microscopy of the stomach was done by Pfeiffer (1970a) in the man and the ferret, work on the rat has shown that his descriptions can also be applied to the rat, as there is relatively little difference between species (Parisio and Clementi 1976, Harding and Morris 1977). The surface epithelial cells present as a sheet of cells covered by mucus (Pfeiffer 1970a), from which the mucus can be cleared Wood and Dubois (1981). Even if the mucus is not cleared, there are windows that allow inspection of the surface epithelial cells, albeit covered by strands of mucus or debris (Harding and Morris 1977). The sheet of cells does not have a flat surface because of the intrinsic convexity of individual cells. In normal gastric mucosa, two features are noted as the cells age and are discarded. The pattern of change appears to be the development of a small hole or defect in the apical membrane followed by some collapse of the surrounding membrane producing a degree of concavity. The defect enlarges to become an erosion. Extrusion of the cell takes place and the surrounding cells encroach to close the gap so created. In doing so, the cells adopt a rosette formation (Pfeiffer 1970b, Harding and Morris 1977).

Transmission electron microscopy shows the surface epithelial cells to be intimately linked by desmosomes and tight junctions. The nucleus is located at the base of the cell, and apically the mucous granules are clustered beneath the luminal membrane. The apical membrane has numerous short microvilli (Lillibridge 1964, Toner and Carr 1971, Parisio and Clementi 1976, Kelly and others 1979).

The rat is well established as an experimental animal for use in gastrointestinal modelling. However, it seemed wise to establish the normal appearance, grossly histologically and ultrastructurally for tissues harvested and processed in our

laboratories.

4.2 Material and Methods

Fourteen Sprague-Dawley rats were starved, anaesthetised and the pylorus ligated as described in section 3.1. Two rats were euthanased immediately. In the remaining 12, 2ml of isotonic saline were injected into each stomach, to mimic the volume of fluid to be used when infusing the stomach with the injurious agents. Of the 12 rats, groups of four rats were killed at 2, 5 and 15 minutes post-infusion. The method of gross examination and description of the harvesting of tissues is detailed in section 3.2.

4.3 Results

4.3.1 Gross

All but two of the stomachs removed from the rats appeared normal. The corporal mucosa was a glistening pink colour, in contrast to the more translucent and yellowed appearance found in the antrum. In one rat killed immediately after anaesthesia, a small peptic ulcer/erosion was found. It was located in the body of the stomach near the corpus/antral border, just off the lesser curvature. The erosion was 2mm in diameter, with a crater lined by fresh and changed blood extending onto the surrounding mucosa. In a second rat, there was a small 4mm area of reddening on the dorsal aspect of the corpus. Six of the rats contained small quantities of what seemed to be ingesta.

4.3.2 Light Microscopy

The surface epithelial cells presented as columnar cells covered by occasional thin strands of lumenal mucus. On H&E sections the cytoplasm was poorly stained but the nuclei were visible at the base. At the tips some of the cells were rather triangular in shape with a pinched base. Little or no mucus was evident on the H&E sections. A thin coating of mucus was visible, as was periapical intracellular accumulation of mucus, when sections were stained with PAS/Alcian Blue. None of the sections examined had evidence of areas with apical damage or disruption with sloughing of the cells into the lumen (Figures 4.2-4.5).

No time effect was detectable statistically ($p < 0.05$), therefore the results from all the rats for oedema, and numbers of WBCs in the lamina propria were pooled. The

mean scores obtained for oedema in the corpus (0.2) and antrum (0.21) were compared and no statistical difference was detectable ($p<0.05$). A similar lack of region effect for WBCs was also confirmed ($p<0.05$).

In the corpus the mean number of neutrophils was 3.9 (range 1-6), and in the antrum 4.38 (range 1.5-8). The submucosal vessels showed no evidence of congestion in any of the sections examined, and thus were scored zero. Consequently, no WBCs were found to be migrating or emigrating through the blood vessels.

A specific section was obtained from the rat with the erosion and this showed destruction of the surface epithelium and the proximal part of the glands. The wall was fairly sharp, and there was an obvious intense inflammatory infiltrate into the base and lamina propria.

Figure 4.1. Gross appearance of normal rat stomach. The mucosa is pinned out and the red mark (*arrow*) is iatrogenic.

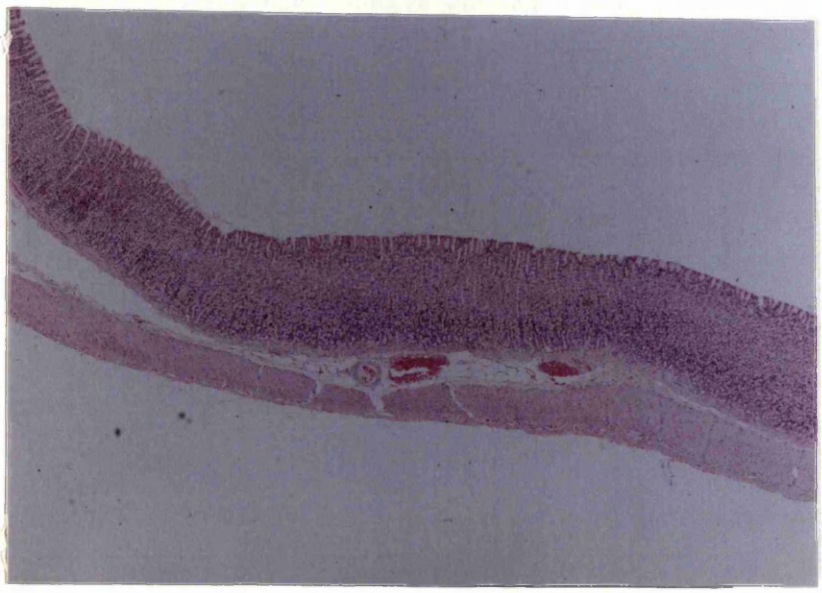


Figure 4.2. Photomicrograph of corpus of normal rat. (H&E x30).

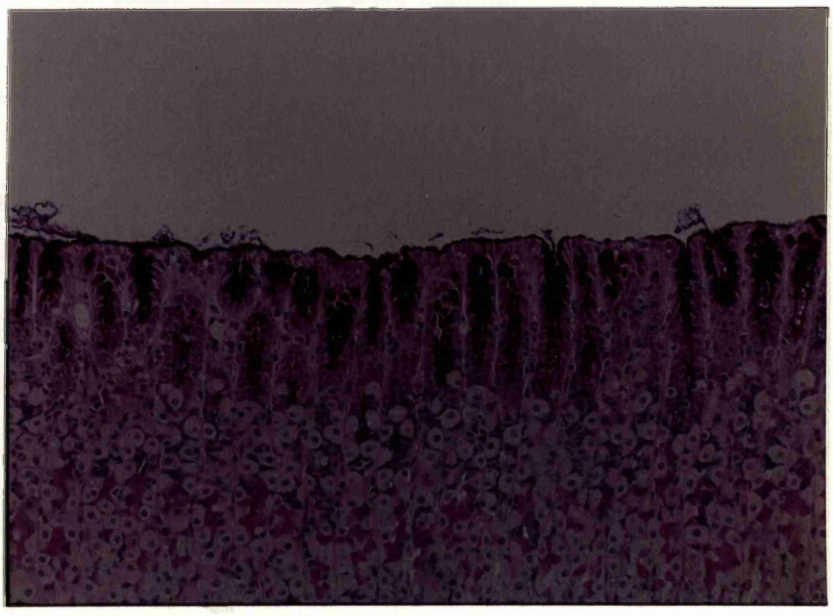


Figure 4.3. Photomicrograph of corpus of normal rat. (PAS/Alcian Blue x150).

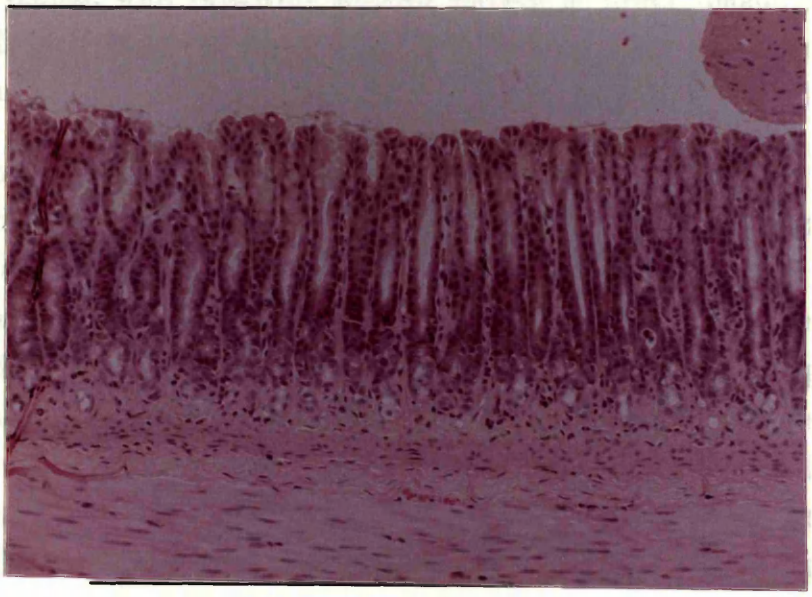


Figure 4.4. Photomicrograph of antrum of normal rat. (H&E x150).

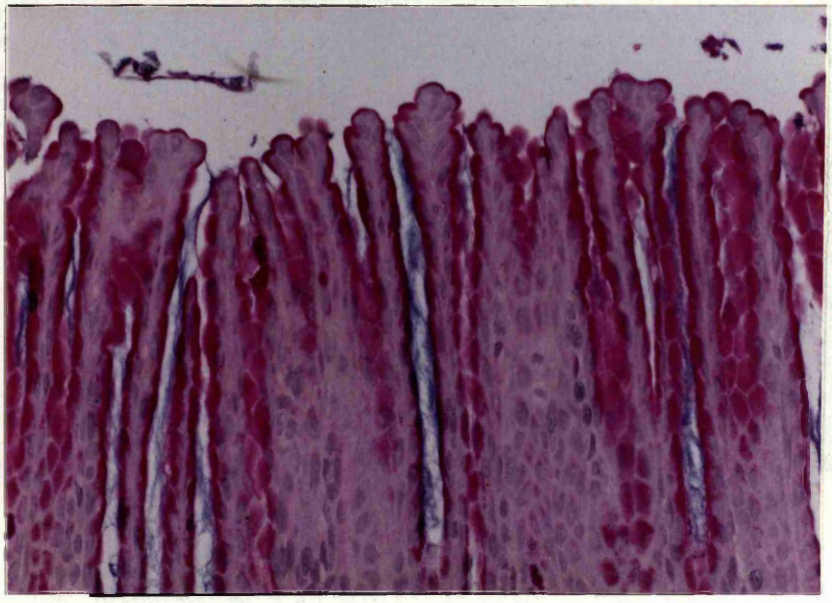


Figure 4.5. Photomicrograph of antrum of normal rat. (PAS/Alcian Blue x300).

4.3.3 Scanning Electron Microscopy

The surface of the stomach was seen as a sheet with the cell contour giving a cobblestone appearance. On closer examination, the entrances to the *foveolae* were either seen clearly or as indentations in the mucus layer (Figures 4.6-4.8). Individual cells were polygonal, with relatively smooth surface at lower magnifications. At higher magnifications the surface had a gentle undulating appearance. The intercellular junctions were visible as raised ridges surrounding the apical membrane.

There was no time effect or difference between scores for corpus and antrum ($p < 0.05$). The surface epithelial cells were covered by a layer of mucus which did not appear in all rats as a complete carpet over the cells (Table 4.1). In eight rats the covering was complete or substantially so. However, even in these rats there were always windows of exposed sheets of cells. In the rest, the covering was seen as scattered islands surrounded by extensive windows (Figure 4.9). These areas had little mucus, or only strands covering the cells (Figure 4.10). The thickness was assessed by determining how easily the underlay of cells was visible. The thickness in each individual rat showed little variation over the area of the stub. However, there was some variation between individuals, but all, except three rats, scored less than 4.

Figure 4.6. Scanning electron micrograph (SEM) of normal luminal surface of the rat antral mucosa The entrance to a gastric pit is evident with epithelial cells partially covered by a lacy mucous overlay. (x2800)

Features	Corpus	Antrum
General		
mucus covering	3.57	3.14
mucus thickness	2.87	2.57
cell extrusion	1.71	1.36
rosette formation	1.57	1.43
enlargement of gastric pits	1.07	1.00
loss of cobblestoning	1.07	1.00
intracellular junctions	1.71	1.86
cell convexity	1.43	1.50
[pooled score 1]	**15.00** ±0.61	**13.86** ±0.51
Individual		
apical ruffling	1.14	1.50
apical holes	1.36	1.37
apical concavity	1.07	1.21
apical erosions	1.21	1.07
intracellular contents	1.07	1.13
empty cells	1.29	1.29
[pooled score 2]	**7.14** ±0.72	**7.36** ±0.8

Table 4.1. Scanning electron microscopy scores obtained from normal rats.

In general, extruding cells were not, or only occasionally, seen in nearly all the rats (12/14). Scores for rosettes of cells seen in the interfoveolar areas essentially matched those given for extruding cells (13/14 scoring 1 and 2) There was no evidence of enlargement of the gastric pits. The cobblestone appearance of the whole surface was obviously affected by the convexity of the individual cells and the scores for both these were small. Excessive convexity of the cells was found in three rats, which also had prominent intracellular junctions. However, this appearance was focal rather than generalised. In a number of stubs (6/14) small clumps of debris were found lying above the general contour of the mucus layer. Degeneration of cells *in situ* was seen occasionally in most of the rats. The individual cellular change observed most frequently was apical holes.

Since there was no statistical evidence of a time effect the results for all the rats in this group were pooled. The pooled mean scores and standard deviations are given in Table 4.1.

Figure 4.7. SEM of normal corpus. The surface cells are outlined beneath the mucous layer. (x720)

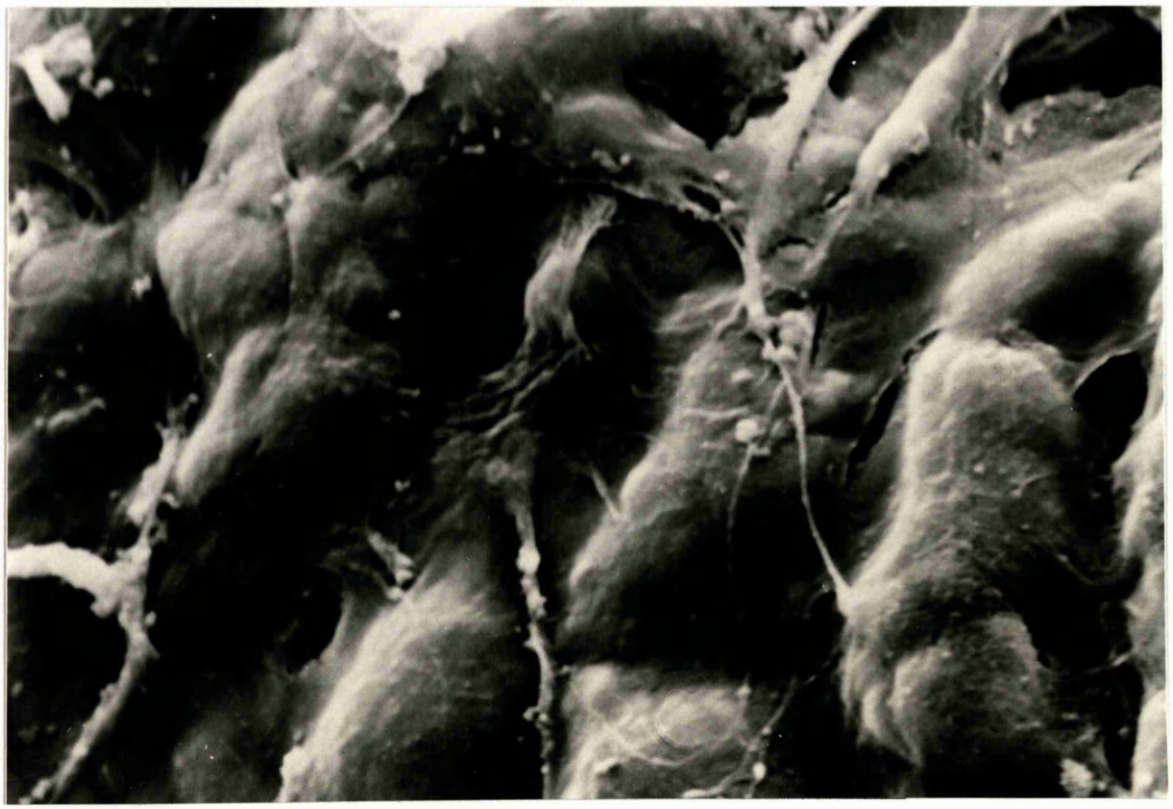


Figure 4.8. SEM of normal corpus On the mucus layer strands of mucus can be seen. (x1440)

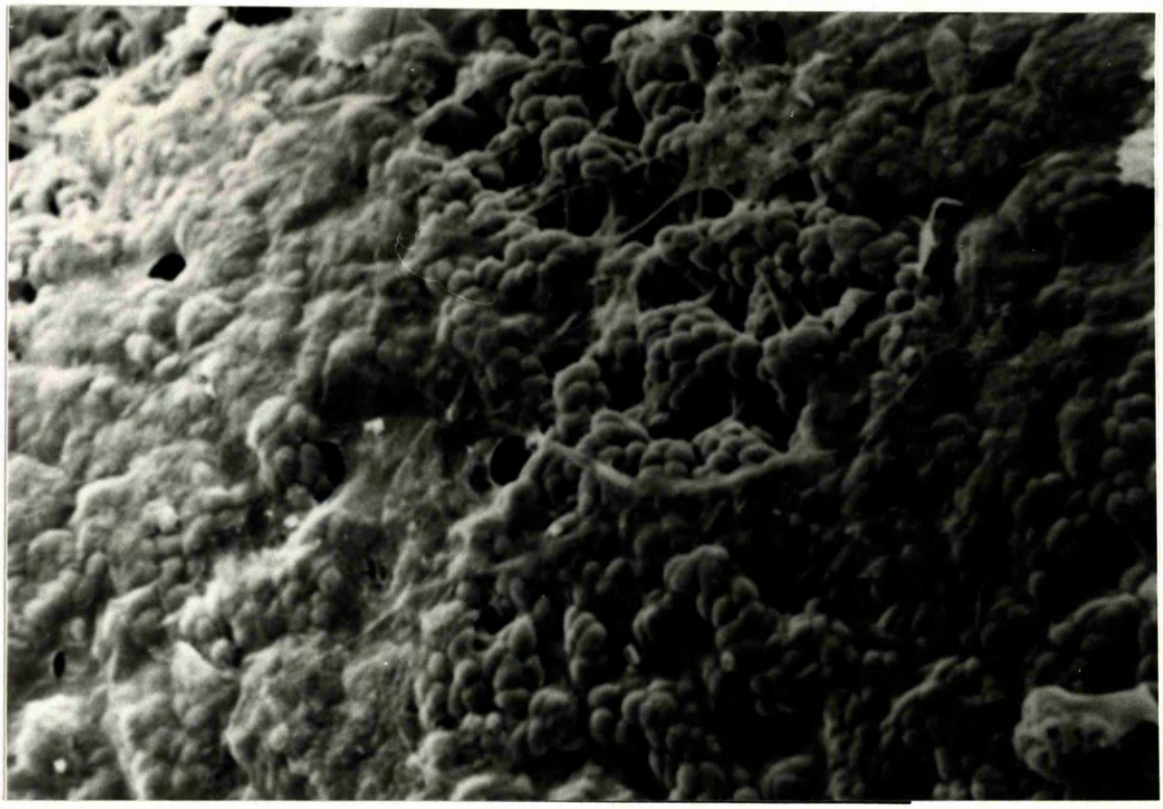


Figure 4.9. SEM of a mucus window. (x720)

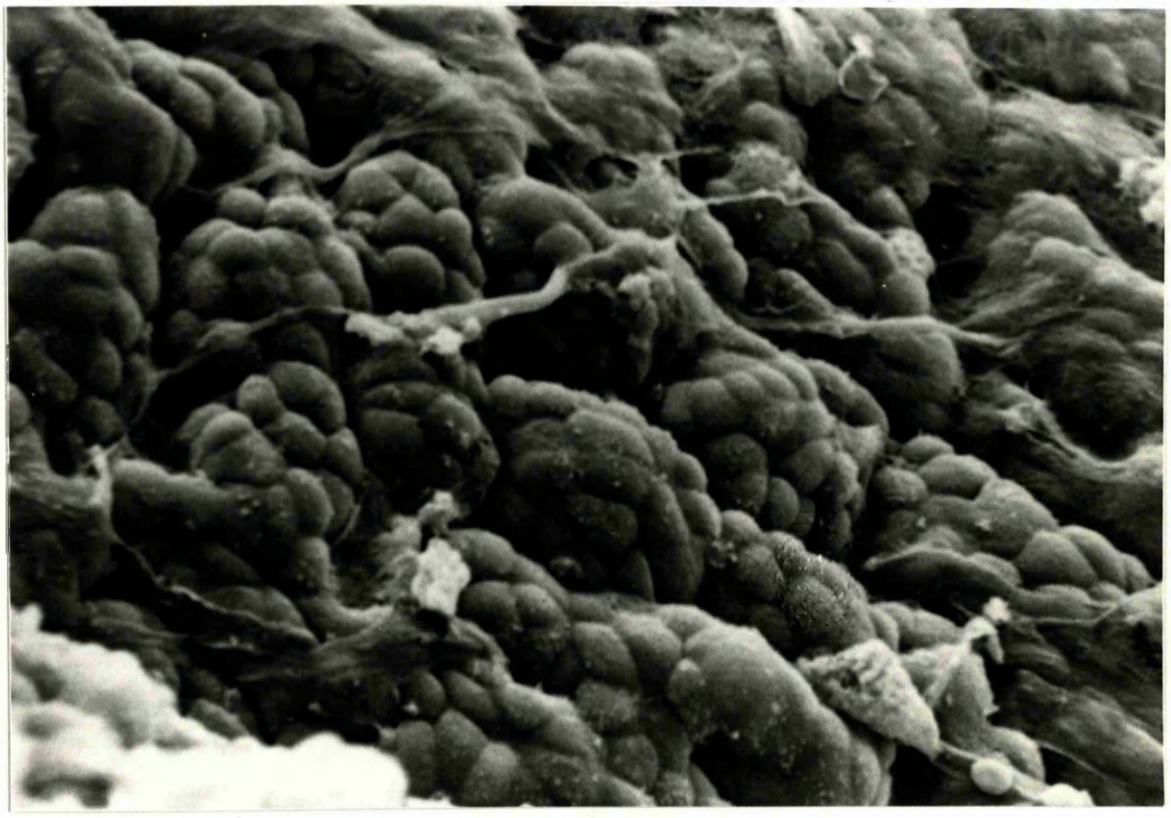


Figure 4.10. SEM of the surface epithelium. Strands of mucus overlie the epithelial cells, an intercellular junction is visible (*arrow*). (x1440)

4.3.4 Transmission Electron Microscopy

The nuclei of the cells were located at the base of the cell. The various organelles were distributed throughout the cytoplasm between the nucleus and the apical area. The apical membrane in many cells had small intraluminal projections or microvilli, and beneath this membrane were aggregations of mucous secretion granules. The tight junctions and desmosomes were all intact (Figures 4.11-4.12).

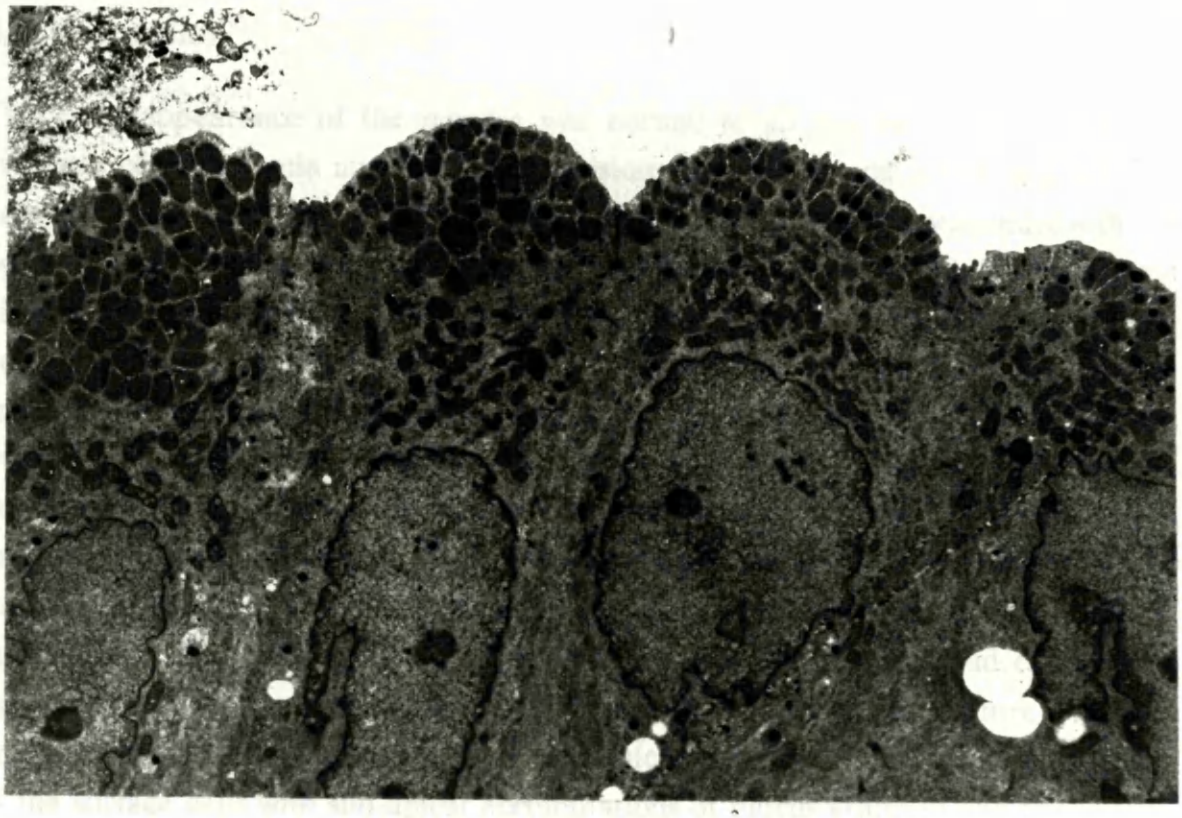


Figure 4.11. Transmission electron micrograph (TEM) of the surface epithelium of the normal rat mucosa (x5400).

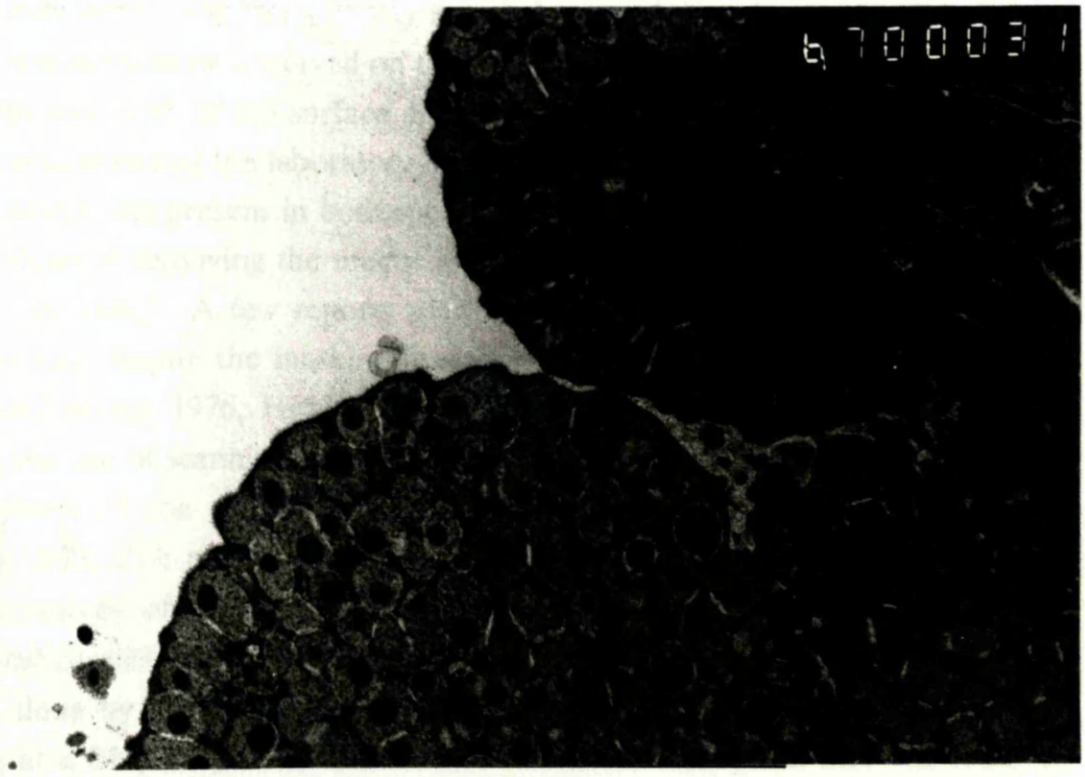


Figure 4.12. TEM showing the normal dense aggregations of discoid mucous granules found at the apex of surface epithelial cells (x10000).

4.4 Discussion

The gross appearance of the mucosa was normal in all but two rats, lacking any evidence of hyperaemia and with clear divisions between the darker fundus and the pale antrum (Robert 1971). Histologically, the surface epithelium was intact with the occasional cell with a pinched base at the tip of a foveola, presumably in the process of being ejected, and a few leucocytes in the submucosa (Kammeraad 1942). Scanning electron microscopy showed the surface epithelium as a sheet of cells with an undulating surface caused by the slightly bulging apical membranes that gave the overall surface a distinct cobblestone appearance (Ogata and Murata 1969, Harding and Morris 1977). The mucus covering was more or less complete and relatively thin in most of the rats. Surprisingly very few extruding cells were seen, considering that the surface cell layer is a dynamic covering, with cell turnover every 3 days (Leblond and Walker 1956, Messier 1960, Baker 1964). Where a few rats had exaggerated bulging apical membranes causing some noticeable convexity, this feature was focal and not generalised. Transmission electron microscopy confirmed the healthy nature of the surface cells with sub-apical accumulations of mucus granules and the nucleus with a relatively even density.

No attempt was made to remove the mucus covering on the surface of the stomach other than to flush away surface debris. This is worthy of explanation. In one of the earliest descriptions of the surface topography of gastric mucosa using scanning electron microscopy, Pfeiffer (1970a) noted that precipitated strands, globules and flakes of mucus could be observed on the gastric surface, and that in areas, mucus did obscure the microvilli of the surface epithelial cell. He also found that the surface topography in man and the laboratory ferret were essentially similar and this masking effect of mucus was present in both species. Consequently, there is a question mark over the value of removing the mucus as it forms part of the normal appearance of the gastric mucosa. A few reports specifically state that no attempt was made to remove mucus, despite the masking feature of mucus in parts of the scanned stubs (Baskin and others 1976, Harding and Morris 1976). But in most publications reporting the use of scanning microscopy of the luminal surface, no mention is made of the subject of the mucus layer as far as specimen preparation is concerned (Frenning 1973, Zoli and others 1986). Takagi and others (1974) in attempting a systematic survey of the normal foetus and adult stomachs, coupled with some pathological conditions in the adult, decided to remove the mucus layer after fixation. This was done by twice washing the specimens in fixative with an ultrasonic unit operating at a frequency of 28kHz for 30-60 seconds. They felt that this removed more of the mucus and was safer than the spray method. Parisio and Clementi (1976) and Trabucchi and others (1986) adopted a different technique for mucus

removal. First they washed the surface with saline to allow gross examination, then continued washing for a further 15 minutes in saline with *N*-acetyl-L-cysteine. However, Fallah and others (1976) compared various washing solutions used before fixation and found that they produced surface alterations in the canine stomach. Continuing in the same vein, Wood and Dubois (1981) compared, brushing - which left abundant coating material and caused denuding and rupture of the SEC, acetylcysteine - which removed little material, and glycosidase coupled with mechanical agitation - which seemed to achieve the best results. In contrast, Al-Tikriti and others (1986), using tissues from cats, compared flushing with saline alone, brushing with a cotton bud and washing and rubbing the mucosa with a surgically gloved hand. Their findings suggested that washing and rubbing with a gloved hand to remove mucus was successful, and did not damage the underlying cells. Jet action with fixative was used by Fringes, Lorenz and Oehlert (1979), though no comment was made of the efficacy of this course of action. Ohno, Ohtsuki, Okabe (1985) found that vigorous spraying with phosphate buffered saline was needed to remove adherent mucus.

Thus there is no established technique for the removal of mucus that does not run the risk of damaging the underlying cells. Since the mucus layer is considered to be an essential component of the barrier (Allen and Garner 1980), it seemed prudent not to interfere with that aspect of the barrier, nor to run the risk of damaging the underlying cells. This leaves one open to the argument that by not removing the mucus layer, surface cell change may be being masked. However, it is recognised that even in the normal stomach there are discontinuities in the mucus layer through which one can gain an appreciation of the surface cells (Morris 1985). Indeed these windows may in fact be due to preparation of the specimens, rather than actual defects in the unprepared tissue and by altering the preparation of tissues it is possible to increase the mucus coverage on a section (Morris and Harding 1974). Bollard and others (1986) pointed out that mucus is a hydrated complex of glycoproteins. The usual techniques for histology, and more importantly for scanning electron microscopy, result in the dehydration of the mucus, which may result in loss or distortion of the mucus layer. It was decided not to attempt to remove the mucus layer in the study of the stomach of the rats

Further, later work was aimed at examining the effect of sucralfate on the physical barrier and to have removed the mucus would have obviated part of that evaluation.

The range of change seen in these sections with scanning electron microscopy was rather restricted. *In situ* degeneration was visible in a small number of cells with a hole in the centre of the apical surface and other groups of cells in rosettes, indicating progression. These changes have been established as normal (Pfeiffer 1970b).

Pfeiffer (1970a) and Frenning (1973) amongst others have described what they took to be microvilli on the surface at very high magnifications. Microvilli were seen on many stubs of the normal rats gastric mucosa, but it was not possible generally to bring these into sharp focus on the machine being used, and so no further attention was paid to this feature of the cell because of the difficulty of ascribing change to the machine, and/or, to the experiment. Indeed, Parisio and Clementi (1976) found that microvilli were not homogeneously distributed amongst cells.

In two rats the gross appearance was not considered normal. The erosion that was visible grossly was seen by light microscopy as a typical chronic erosion, with significant localised epithelial loss and pronounced surrounding leucocyte infiltrate. In the other rat, there was no histological evidence to support the gross finding of reddening. It is possible that either the suspected area was not accurately sampled, or that it was a visual artefact created by an anomaly in that particular rat, for example vascular.

5. Challenged Rats

5.1 Introduction

It is strange that of all the organs of the body, the stomach must defend itself against such an inimical environment, particularly as this circumstance is self-generated. The stomach produces acid that serves to destroy ingested organisms and transform pepsinogen into the proteolytic enzyme pepsin. This combination creates an environment that axiomatically must be countered by the stomach itself in order to prevent autodigestion. The stomach is able to do this by regulating, in co-operation with other parts of the body, the production of acid and enzyme precursor (Andersson, Nilsson and Uvnäs 1967, Elwin and Uvnäs 1967, Walsh and Grossman 1975, Albinus and others 1977). The main stimulants of secretion are, of course, expectation of and actual ingesta. These stimuli serve to enhance the two secretions (Feldman and Richardson 1986). The stomach is able to protect itself against these two by several methods. The stomach wall becomes more resistant to the back-diffusion of hydrogen ion by actively secreting acid (Moody and Davis 1970, Fromm, Silen and Robertson 1976, Smith and others 1977). Additionally, the stomach will be under the influence of gastrin that not only stimulates acid secretion, but is also thought to tighten the gastric mucosal barrier and minimise back-diffusion of hydrogen ions (Wlodeck and Leach 1966). The mucus, a covering mixture of glycoproteins, water and bicarbonate ions, forms a hydrophobic barrier resisting the incursion of acid, pepsin and other ingested materials, creating a gradient against which incoming hydrogen ions are buffered and a layer against which pepsin will dissipate (Hollander 1954, Allen and Garner 1980, Hills, Butler and Lichtenberger 1983). The gross function of the stomach helps in this process, serving as a transient repository for incoming ingesta. This ingesta furnishes a large volume of material, which admittedly stimulates these relatively noxious secretions in the first place, to dilute and neutralise the secreted acid and pepsin. Luminal contents are constantly being churned to ensure thorough mixing of the food, acid and pepsin, and thence forced from the stomach into the more neutral environment of the small bowel (Cannon 1898). Here secreted bile is then mixed with the outpourings from the stomach (Jensen 1980, Guyton 1981). Unfortunately, this bile can also reflux back into the stomach. In the normal animal, this situation tends to occur during alimentation and thus the bile is diluted by the contents of the stomach, and it does not remain there for long before being returned to the duodenum (Ehrlein 1981), and in the normal human, at least, is present in only small concentrations in the region of 0.13mM (Rhodes and others 1969). Reflux movement of bile may also occur "*normally*" during alimentary upset when vomiting occurs, due to retrograde peristalsis

(Alvarez 1925, Thompson and Malagelada 1982, Lee, Park and Chey 1985, Lang, Sarna and Condon 1986).

Nevertheless, not only is bile refluxed into the stomach in patients who have had diversional surgery (Keighley, Asquith, and Alexander-Williams 1975, Heading 1983), but also by normal animals when the stomach is empty. In man, proof has come from normal conscious volunteers (Rhodes and others 1969), whilst the evidence for this in animals comes from anaesthetised or sedated animals (Happé and van den Brom 1982).

Thus the stomach may be confronted by three inimical body-generated agents, namely; acid (yielding a pH of 1), pepsin and bile. Whilst the bulk of the material swallowed by animals, including man, tends to be somewhat neutral as far as acidity and chemical activity are concerned, both take or are given products that, despite their recognised therapeutic benefits, are more than capable of injuring the stomach. Against this background it has long been recognised that one of the most common drugs taken and still taken in large quantities is aspirin (Hammond 1971). Aspirin (acetylsalicylic acid) may be considered the progenitor of the class of drugs known as non-steroidal anti-inflammatory drugs. Acetylsalicylic acid, distinguished for its antipyretic properties for many years, was also recognised as being potentially harmful to the stomach (Dodd, Minot and Arena 1930). Work by Martin (1963) defined quite clearly that because of the nature of aspirin as a molecule having a pK_a value of around three, that in the stomach most of the ingested aspirin would be in an undissociated form, and as Davenport (1964), pointed out would therefore be lipid soluble and able to move through the membrane of the surface epithelial cell. There in the more neutral intracellular environment the aspirin becomes ionised and more chemically active. Garner (1978) has shown, using 20mM aspirin, that high concentrations of aspirin were found in the cells of the upper part of the mucosa within 10 minutes, though tending to dissipate after 30 minutes.

Precise evidence has emerged that whilst this influx of aspirin can take place in the stomach, the damage done by this drug is singularly enhanced by the presence of acid (Brodie and Chase 1967). The reasons for this are twofold. First, the acid generates a pH environment that is most accommodating for the formation of the lipid soluble form of the agent. Second, should aspirin damage the epithelium, it then permits the back-diffusion of hydrogen ions from the lumen that do much of the additional injury (Davenport 1967a), compounded by the increase in pepsin output engendered by the back-diffusion of acid (Johnson 1971). The main mode of action in the damage generated by aspirin is the suppression of prostaglandins produced by the stomach to moderate the defensive mechanisms of the gastric barrier (Miller and Jacobson 1979,

Miller 1983). The consequence is that aspirin will sweep aside the normal cytoprotective influences. Whilst maintaining the gastric barrier prevents deep mucosal injury, the superficial or surface epithelium suffers, as has been demonstrated ably by Hingson and Ito (1971) using electron microscopy.

There has been a deluge of new NSAIDs over the last 20 years, to compete in the very lucrative market for anti-inflammatory drugs (Simon and Mills 1980ab). Each succeeding drug is synthesized to be more powerful and hopefully with fewer side-effects, and perhaps work in those patients who appear to be unresponsive to other NSAIDs (O'Brien and Bagby 1987). Whilst this is laudable, it is unfortunate that this side-effect profile is not always the case. An example is the belated discovery of the intense and, in some cases, permanent photo-sensitizing effect that "*Opren*" (Eli Lilly) had on many users. Indomethacin, in man, is a powerful NSAID and a potent suppressor of the generation of prostaglandins in the alimentary tract (Levine and Schwartzel 1984). Though, this drug has found a niche in the human market, its untested use in the dog resulted in anecdotal reports of the induction of severe gastrointestinal bleeding in many dogs, but only one precise report (Ewing 1972). These examples serve to highlight the variation in inter-species response to these potent drugs, which is frequently underestimated.

Carprofen (pK_a4.7) is a recent non-steroidal anti-inflammatory agent with pronounced anti-inflammatory and analgesic effects. It's anti-inflammatory, analgesic and anti-pyretic activities were identified by Randall and Baruth in 1976. It has a half-life in dogs of 40 hours compared to 13-26 hours in man (Rubio and others 1980, Brogden 1986), and is rapidly absorbed following oral administration (Schmitt and Guentert 1990). The introduction of carprofen has been mooted as a NSAID that would have the required potency as far as the control of arthritis is concerned, but have minimal gastric irritancy. Strub, Aeppli and Muller (1982) have shown carprofen to have marked anti-inflammatory and analgesic effects. In experimental animals intestinal ulcers were the only recognisable gross side-effect. The relative lack of ulceration was related to the poor inhibition of prostaglandin synthesis. As far as PGE_2 is concerned, a prostaglandin associated with the gastrointestinal tract, a lack of inhibition in the stomach has been reported by Frolich and others (1984). However, carprofen will suppress another prostaglandin, $PGF_{2\alpha}$, in the stomach to the same extent as indomethacin, and also in the post-partum cow, carprofen, just like indomethacin, has been shown to reduce the levels of PGE_2 in the synovial fluid (Minuz and others 1986, Thun and others 1989, Seppala and others 1990).

Aim

Thus it was determined to examine and compare the short-term effects (minutes), rather than the long-term (hours), of acid, bile and aspirin on the gastric mucosa, and compare any effects to those obtained with aspirin. Further, the additive effect of bile with these two NSAIDs was also to be studied.

5.2 Material & Methods

Pairs of Sprague-Dawley rats were starved, anaesthetised and the pylorus ligated as described in section **3.1.** Pairs were killed at 2, 5 and 15 minutes post-infusion. The method of gross examination and description of the harvesting of tissues is detailed in section **3.2.**

The agents used were hydrochloric acid, sodium taurocholate (Sigma Chemical Company), acetylsalyclic acid (*Aspirin 1g* B.P.) and carprofen (*Imadyl 150mg* Hoffman-La Roche).

The rats were divided into three groups. Rats in Group I received hydrochloric acid or a single injurious agent. Those in Group II and Group III received a combination of injurious agents. The agents and combinations used are shown in Tables 5.1-5.4.

Agent	concentrations	
hydrochloric acid (HCl)	0.05N	0.1N
sodium taurocholate (bile)	2.5mM	5mM
aspirin	20mM	40mM
carprofen	0.23mM	0.46mM

Table 5.1 Group I. Single injurious agent concentrations given to rats.

Agent	concentrations
aspirin & bile	20mM & 2.5mM
aspirin & bile	20mM & 5.0mM

Table 5.2 Group IIa. Aspirin combinations used in rats.

Agent	concentrations	
carprofen & HCl	0.23mM & 0.05N	0.23mM & 0.1N
carprofen & HCl	0.46mM & 0.05N	0.46mM & 0.1N
carprofen & bile	0.23mM & 2.5mM	0.23mM & 5mM
carprofen & bile	0.46mM & 2.5mM	0.46mM & 5mM

Table 5.3 Group IIb. Carprofen combinations used in rats.

Agent	concentrations
carprofen & HCl & bile	0.23mM & 0.05N & 2.5mM
carprofen & HCl & bile	0.46mM & 0.1N & 5mM

Table 5.4. Group III. Triple injurious combinations given to rats.

5.3 Results

5.3.1 Gross Appearance

5.3.1.1 Single Agents

Acid

With one exception, no gross change was evident in any of the rats exposed to either 0.05N or 0.1N HCl. The exception was a rat given 0.05N HCl acid where there was a generalised erythema after 15 minutes exposure in both the corpus and antrum.

Bile

There was no alteration detectable after 2 minutes exposure to bile at either 2.5mM or 5mM. In one rat after 5 minutes exposure to 2.5mM bile, there was a generalised erythema restricted to the corpus with widespread pin-point areas of deeper reddening. This change was not observed in the second rat, nor was it repeated at the same time interval in those rats exposed to 5mM bile. This concentration (5mM) and time interval only produced a focal mild reddening of corpus. More consistently were the findings after 15 minutes exposure. Bile at 2.5mM caused patchy erythema of the mucosa of the corpus, and at 5mM the erythema was widespread, though still visually mild. In summary, the changes detected were of erythema that appeared to be more time constrained than a consequence of concentration.

Aspirin

No gross change was demonstrable at either 20mM or 40mM at any of the three exposure times (Figure 5.1).

Carprofen

With 0.23mM carprofen there was reddening of the antral mucosa in 5/6 rats. This was widespread in 3/5 rats, but localised to the corporo-antral margin in the other two. At 15 minutes, there was no change in one rat and a localised intense change in the other. When 0.46mM carprofen was used the reddening was again centred on the corporo-antral margin, but accompanied by linear streaking in 4/6 rats. Time did not seem to make any difference to the gross change (Figure 5.2).

5.3.1.2 Combination of Agents

Aspirin & Bile

All rats exposed to 2.5mM bile/20mM aspirin showed erythematous change in both antral and corpal regions. In all rats it appeared moderate, with the exception of one rat where after 15 minutes the alteration was classified as mild. In the corpus, the reddening was extensive in contrast to the antral area where the change was more

focal in nature. The corpus of one rat after 5 minutes, also had what could be described as a cobblestone glistening appearance, ascribed to mucosal oedema.

When the concentration of bile was increased to 5mM in combination with 40mM aspirin the erythema was again widespread in the corpus varying from a mild after 5 minutes to a severe reddening at 15 minutes. However, in the antrum the reddening was again somewhat localised and gave the impression of linear streaking on the rugal crests. Therefore, there appeared to be a time and concentration effect.

Carprofen & Acid

The level of reddening was much less intense in the rats given carprofen 0.23mM/0.05N HCl than was seen in those rats given carprofen alone. Furthermore, the area of involvement was more focal in nature, essentially restricted to the antrum. Oedema was apparent in one rat after 15 minute along the incisure. A linear streak was found in the antrum of one rat after 5 minutes. Increasing the concentration of acid served to extend the area of reddening into the corpus, but linear streaks were again found in only one 5 minute rat.

Doubling the concentration of carprofen to 0.46mM coupled with 0.05N HCl caused reddening of the mucosa around the corporo-antral junction, which involved progressively more of the mucosa of the corpus as the time interval was increased from 2 minutes to 15 minutes. Linear streaking was only observed in one rat after 5 minutes exposure, and in the second 5 minute rat the rugae were more prominent than in other rats: there was also oedema in this rat. Applying 2ml of fluid containing 0.46mM carprofen/0.1N acid to the gastric mucosa resulted in essentially the same changes as those detected with lesser concentrations of carprofen and acid. There was widespread reddening of the corpus and antrum centred on the corporo-antral junction, with no linear streaking in the antrum.

Carprofen & Bile

A solution of carprofen (0.23mM) and bile (2.5mM) produced a similar degree of reddening of the mucosa to that caused by carprofen and acid. Four of the six rats had generalised hyperaemia of the corpus mucosa, but in only one (15 minute) did this extend to include the antrum, where it was widespread. In two further rats there were single focal areas which resembled erosions. Oedema was a notable feature being present in 5/6 rats. Linear erosions were not however seen in any rat.

Doubling the concentration of either the bile (5mM) or carprofen (0.46mM) made no appreciable difference to the range of hyperaemia and oedema noted in the corpus. However, antral hyperaemia was seen in 2/6 rats with doubled concentration of bile. In those rats given carprofen 0.46mM there was a focal reddening in 4/6, with the

other two having widespread involvement at 5 and 15 minutes.

Further, the application of a solution containing 0.46mM carprofen and 5mM bile caused a more intense reddening of the mucosal surface when compared subjectively to that seen with other mixtures of bile and carprofen. This change affected all six rats and was accompanied by oedema in 3/6, with erosions in the corpus in two of these rats (Figure 5.3).

Carprofen & Acid & Bile

Solutions containing either 0.23mM carprofen/2.5mM bile/0.05N acid or 0.46mM carprofen/5mM bile/0.1N acid produced much more marked change. The mucosa was a deeper red and involved the complete surface of the corpus. In the antrum the presence of linear erosions was noted in 10/12 rats. The solution containing the higher concentration produced corporal pin-point erosions superimposed upon the generalised hyperaemia at 15 minutes.

In summary, the single agents produced sparse evidence of grossly visible mucosal change, with the most consistent change being produced by those rat given carprofen. The addition of acid to carprofen also produced reddening of the mucosa, particularly in the antrum, with the development of what appeared to be linear erosions in some rats. Carprofen/bile caused reddening of the corpus, which spread to involve the antrum as time and concentrations were increased. Bile and aspirin produced hyperaemia in both corpus and antrum and at the higher concentrations caused some linear streaking. The effect of using triple concentrations was to enhance the reddening induced in the mucosa and produce in nearly all the rats linear erosions and some pin-point erosions at the higher concentrations.

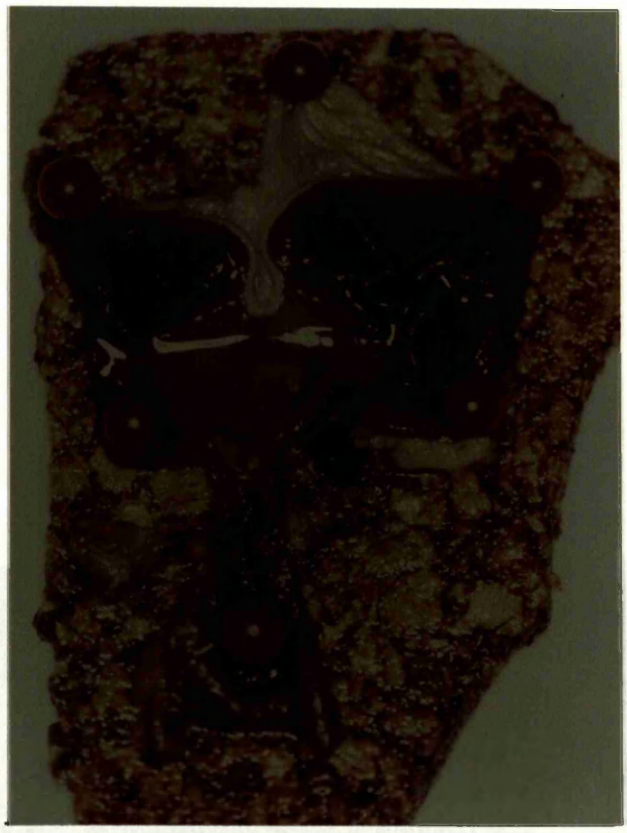


Figure 5.1. Gross appearance - aspirin.The normal colour of the corpus and antrum is unchanged.

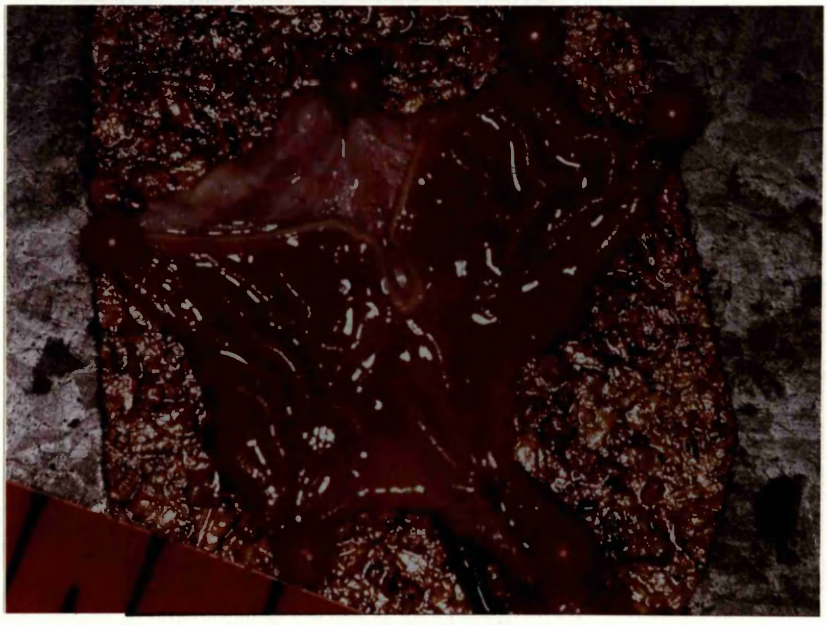


Figure 5.2. Gross mucosal appearance - 0.46mM carprofen. There is deepening of the colour of mucosa at the corporo-antral border causing loss of the normally distinct margin.

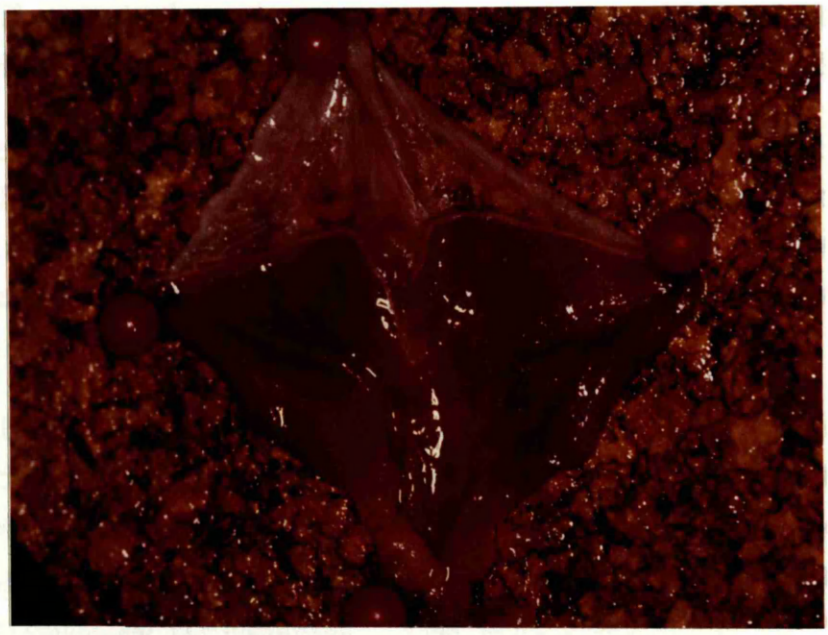


Figure 5.3. Gross mucosal appearance - 0.46mM carprofen & 5mM bile. There is marked reddening of both corpus and antrum.

5.3.2 Light Microscopy

(See Figures 5.5-50.17)

5.3.2.1 Oedema

In group I, four potentially injurious agents were injected singly into the stomachs of paired rats. No time effect was detectable with the oedema scores obtained from the corpus and antrum of the rats given single injurious agents. The scores were also compared to those obtained from the control rats (Table 5.5). There was no observable difference in tissue taken from the corpus of rats given single injurious agents. However in the antrum, there was a statistically significant ($p < 0.05$) increase in oedema score for the antrum of rats given aspirin 40mM and carprofen at 0.23mM. Comparison of the different concentrations of individual agents showed that there was no difference in the oedema score generated with acid, aspirin, bile or carprofen, but 40mM aspirin induced a higher score than 20mM aspirin ($p < 0.05$).

The scores generated by carprofen were compared to those from the other single agents. In the corpus there was no difference between agents. In the antrum, the scores were also similar for acid, bile concentrations. However, 40mM aspirin and 0.23mM carprofen produced significantly higher scores than the other agents, though there was no difference between the these two.

In group IIa combinations of aspirin and bile were placed in the stomachs of the rats (Table 5.5). No time effect was demonstrable in the corpus or antrum. When the two combinations were compared with each other there was a significant difference in the antrum only, with 40mM aspirin/5mM bile producing a higher score than the lower concentration combination. This same antral effect was evident when the higher concentration of aspirin/bile was compared with corresponding single agents, but not with the lower concentration combination.

In group IIb the effect of adding different agents and concentrations to carprofen was tested (Table 5.6). No time effect was demonstrable amongst this group of combinations, nor were there any significant differences between corpus and antrum. Almost all combinations of carprofen induced more oedema in the corpus and antrum compared with the controls. The exceptions were 0.23mM carprofen & 0.05N HCl and 0.46mM carprofen & 2.5mM bile.

Group	Agent	mean corpus	±sd	mean antrum	±sd
Control	none	0.18	0.01	0.19	0.01
Injury					
Group I	IA1	0.24	0.03	0.22	0.09
	IA2	0.24	0.03	0.26	0.08
	IB1	0.23	0.07	0.23	0.06
	IB2	0.20	0.07	0.26	0.08
	IS1	0.24	0.06	0.24	0.06
	IS2	0.24	0.01	0.32	0.08
	IR1	0.22	0.10	0.30	0.07
	IR2	0.23	0.04	0.24	0.04
Group IIa	IS1B1	0.23	0.06	0.24	0.06
	IS2B2	0.25	0.06	0.34	0.04
Group IIb	IR1A1	0.22	0.03	0.28	0.03
	IR1A2	0.26	0.04	0.28	0.02
	IR2A1	0.24	0.03	0.28	0.02
	IR2A2	0.24	0.04	0.29	0.05
	IR1B1	0.26	0.06	0.30	0.04
	IR2B1	0.24	0.05	0.28	0.03
	IR1B2	0.25	0.04	0.30	0.04
	IR2B2	0.25	0.04	0.30	0.04
Group III	IR1B1A1	0.25	0.02	0.30	0.04
	IR2B2A2	0.28	0.04	0.27	0.03

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Table 5.5. Oedema ratios determined by light microscopy for those rats given injurious agents.

agent	*vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IR1A1		none	ns	S
IR1A2		none	S	S
IR2A1		none	S	S
IR2A2		none	S	S
IR1B1		none	S	S
IR2B1		none	ns	S
IR1B2		none	S	S
IR2B2		none	S	S
IR1A1		IR1	ns	ns
IR1A1		IA1	ns	S
IR1A2		IR1	ns	ns
IR1A2		IA2	ns	ns
IR2A1		IR2	ns	ns
IR2A1		IA1	ns	S
IR2A2		IR2	ns	S
IR2A2		IA2	ns	ns
IR1B1		IR1	ns	ns
IR1B1		IB1	ns	S
IR2B1		IR2	ns	ns
IR2B1		IB1	ns	ns
IR1B2		IR1	ns	ns
IR1B2		IB2	S	S
IR2B2		IR2	S	ns
IR2B2		IB2	ns	S
IR1B1		IS1B1	ns	S
IR2B2		IS2B2	ns	ns

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Table 5.6. Oedema ratios. Comparison of carprofen combinations with other injurious agents and combinations.

There were few differences between the ratios induced by single agents and carprofen combinations. The limited number of combinations that did show increased ratios did so mainly in the antrum (6/8). Those combinations that did show an increase in ratios over single agents in the antrum were found slightly more often among the bile combinations. It was only amongst carprofen and bile combinations that there appeared to be higher corporal oedema ratios than were found with the single agents that made up the combination. When the carpofen/bile combinations were compared to the equivalent aspirin/bile mixtures, there was only a difference between

the lower carprofen/bile and asprin/bile mixtures in the antrum.

In group III, rats were given triple combinations of injurious agents (Table 5.5). Again no time effect was evident. No difference was detectable between the corpus and antrum or between the lower and higher concentrations used in the two triple combinations. The lower concentrations were compared with rats given single or double injurious agents at the same concentrations (Table 5.7). No differences were found between double agents and the triple agents as far as oedema was concerned. Comparison with single agents provided sparse evidence of an additive effect. In the antrum, there was a significant difference between those rats given acid or bile and those given carprofen 0.23mM with bile 2.5mM and acid 0.05N, and in the antrum when this combination was compared to bile at 2.5mM. At the higher concentration blend the only significant effect was when this blend was compared to bile in the antrum.

agent	*vs* agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IR1B1A1	IA1	ns	S
	IB1	S	S
	IR1	ns	ns
	IR1A1	ns	ns
	IR1B1	ns	ns
IR2A2B2	IA2	ns	ns
	IB2	S	ns
	IR2	ns	ns
	IR2A2	ns	ns
	IR2B2	ns	ns

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Table 5.7. Oedema ratios. Comparison between triple combinations and single or double agents.

5.3.2.2 White Blood Cells

There appeared to be a time effect as far as infiltration into the submucosa was concerned. However, this was only really apparent after 15 minutes exposure. This time effect was found in 10/20 combinations in both the antrum and the corpus. In general, if a time effect was noted in the corpus, a corresponding time effect was demonstrable in the antrum. The exceptions to this were 0.46mM carprofen and 0.46mM carprofen & 0.05N HCl where no antral time effect was found, and the triple

combinations that had no corporal time effect. Excluding the triple agent combination, there was no time effect in 5/6 combinations that included bile. Time effect was noted in all those combinations where acid was present. There was little difference between the 2 and 5 minute samples, as the time effect was largely restricted to the 15 minute sample.

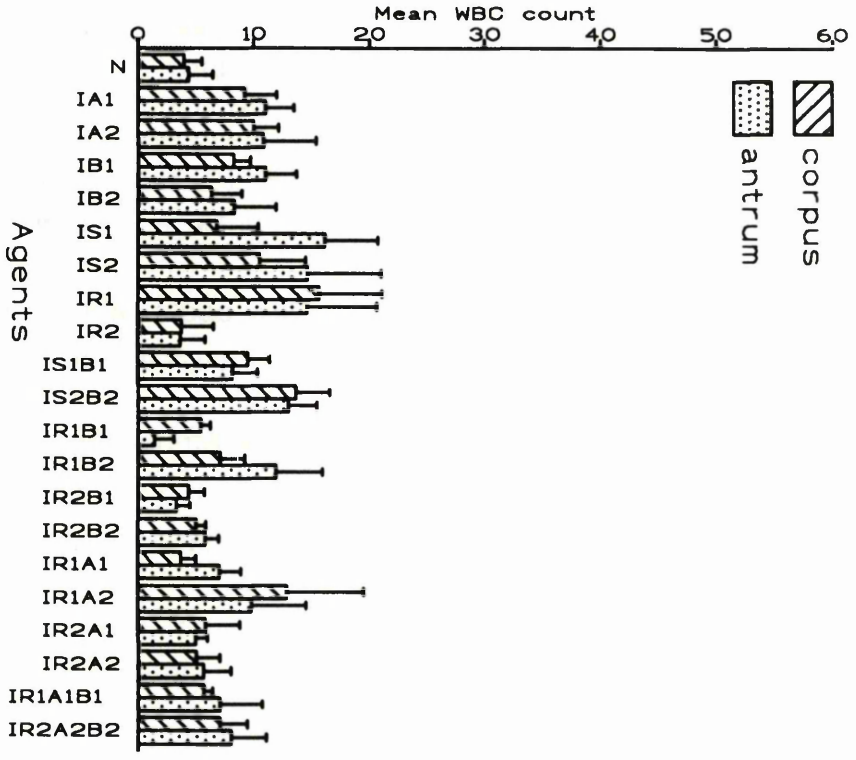
To aid analysis, the WBC counts for each combination were pooled regardless of time effect (Table 5.8). This was to determine if there was any difference between the numbers found in the corpus and the antrum with various combinations. The results are also shown graphically in Figure 5.4.

Group	Agent	corpus	±sd	antrum	±sd
Normal Rat		3.90	1.50	4.38	2.07
Group I	IA1	9.21	2.76	11.00	2.41
	IA2	10.00	2.10	10.80	4.59
	IB1	8.21	1.49	11.00	2.62
	IB2	13.00	2.62	8.29	3.59
	IS1	6.80	3.58	16.10	4.61
	IS2	10.50	3.92	14.60	6.37
	IR1	15.56	5.46	14.50	6.10
	IR2	3.75	2.73	3.63	2.14
Group IIa	IS1B1	9.50	1.82	8.13	2.21
	IS2B2	13.50	3.01	12.90	2.49
Group IIb	IR1A1	3.71	1.26	3.00	1.84
	IR1A2	12.80	6.68	9.75	4.64
	IR2A1	5.75	2.98	4.92	1.04
	IR2A2	5.00	2.00	5.75	2.38
	IR1B1	4.50	0.84	4.46	1.60
	IR2B1	4.38	1.33	3.29	1.17
	IR1B2	7.08	2.09	11.30	4.04
	IR2B2	4.96	0.89	5.64	1.20
Group III	IR1A1B1	5.60	0.78	6.89	3.62
	IR2A2B2	6.99	2.38	8.05	3.03

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Table 5.8. Mean white blood cell infiltrates found with different injurious agents.

Figure 5.4 shows the numbers of WBCs in corpus tended to mirror those found in the antrum. Significant differences were only found with bile (5mM) which had more infiltration in the corpus, whereas both concentrations of aspirin had greater antral infiltrate.



I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen.

Figure 5.4. Bar graph of white blood cell numbers found in rats given injurious agents.

There were significant increases in the number of WBCs in both corpus and antrum when the single agents were compared to the control rats in most instances. In the antrum only those rats given 0.46mM carprofen had similar numbers as the control rats, and similarly in the corpus of those given 20mM aspirin and 0.46mM carprofen (Table 5.9).

agent	*vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IA1		control	S	S
IA2		control	S	S
IB1		control	S	S
IB2		control	S	S
IS1		control	ns	S
IS2		control	S	S
IR1		control	S	S
IR2		control	ns	ns

I=injury, A1 & A2 =0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 =20mM & 40mM aspirin, R1 & R2 =0.23mM & 0.46mM carprofen. S=significant, ns=not significant

Table 5.9. White blood cell infiltrate. Rats given single injurious agents compared to control rats.

When the double combinations were compared with the controls, there was a more varied response, which could not be related to the constituent agents or area. Five of the 12 combinations had a significant increase and the remaining seven did not.

The WBC counts for carprofen were compared with the other single agents. Against carprofen 0.23mM, the numbers found in the other single agents were significantly less. In contrast, against 0.46mM carprofen, the numbers for the other agents were significantly more.

agent	*vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IR1		IA1	S	S
IR1		IB1	S	S
IR1		IS1	S	ns
IR2		IA2	@S	@S
IR2		IB2	ns	@S
IR2		IS2	@S	@S

I=injury, A1 & A2 =0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 =20mM & 40mM aspirin, R1 & R2 =0.23mM & 0.46mM carprofen. S=significantly more, ns=not significant, @S=significantly less.

Table 5.10. WBC counts. Comparison between carprofen and single agents.

The combinations of aspirin and bile from Group IIa were compared with control rats and those given the constituents as individual agents. Against the controls both aspirin/bile combinations produced a significant increase in the number of WBCs

found in the corpus and antrum. In contrast, the effect of 20mM aspirin & 2.5mM bile was no different to that produced by the single agents. When the higher (5mM) concentration of bile was used, there was a significant difference in all areas, except in the antrum when compared with 40mM aspirin alone.

agent	*vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IS1B1		control	S	S
IS2B2		control	S	S
IS1B1		IS1	ns	ns
IS1B1		IB1	ns	ns
IS2B2		IS2	S	ns
IS2B2		IB2	S	S

I = injury, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, S = significant, ns = not significant.

Table 5.11. WBC count. Comparison of aspirin combinations.

agent	*vs*	agent	corpus (p<0.05)	antrum
IR1A1		control	ns	ns
IR1A2		control	S	S
IR2A1		control	ns	ns
IR2A2		control	ns	ns
IR1B1		control	ns	ns
IR2B1		control	ns	ns
IR1B2		control	S	S
IR2B2		control	ns	ns
IR1A1		IA1	@S	@S
IR1A1		IR1	@S	@S
IR1A2		IA2	ns	ns
IR1A2		IR1	S	S
IR2A1		IA1	ns	@S
IR2A1		IR2	ns	ns
IR2A2		IA2	@S	ns
IR2A2		IR2	ns	ns
IR1B1		IB1	@S	@S
IR1B1		IR1	@S	@S
IR2B1		IB1	S	S
IR2B1		IR2	ns	ns
IR1B2		IB2	ns	ns
IR1B2		IR1	S	ns
IR2B2		IB2	@S	@S
IR2B2		IR2	ns	ns
IR1B1		IS1B1	@S	@S
IR1B2		IS2B2	@S	ns

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, S = significant, ns = not significant, @S = significantly less.

Table 5.12. WBC count. Comparison of carprofen with controls, single agents and aspirin combinations.

The comparison between carprofen combinations with controls and other agents is shown in Table 5.12. When the combinations from Group II were compared with control rats, significantly more WBCs were found only in those rats given 0.23mM carprofen & 0.1N HCl and 0.23mM carprofen & 5mM bile. This difference was apparent in both corpus and antrum.

When carprofen plus a second agent was compared with the single agents significantly fewer cells were apparent in a number of instances. Against the individual agents,

three of the four 0.23mM carprofen & acid combinations showed significantly fewer in WBCs in both the antrum and corpus, the exception being carprofen and 0.1N HCl. With the higher concentration of carprofen (0.46mM) and acid significantly smaller counts were noted only in the antrum when compared with 0.05N and corpus with 0.1N HCl.

Fewer WBCs were also found when the carprofen/bile combinations were compared with single agents. This was the case in both the corpus and antrum of those rats given 0.23mM carprofen & 0.05N HCl when compared with carprofen 0.23mM, and 0.05N HCl. When the concentration of bile was increased, change was only noted in the corpus when compared with 0.23mM carprofen, indicating a bile effect. When the carprofen concentration was increased to 0.46mM and compared, lower cell counts were apparent only when compared with bile in both antrum and corpus. No change was evident when 0.46mM carprofen & bile was compared with carprofen alone.

The highest and lowest carprofen/bile combinations were also compared with similar combinations of aspirin and bile. Significantly less WBCs were found in the corpus and antrum of rats given 0.23mM carprofen/2.5mM bile compared to 20mM aspirin/2.5mM bile, but only in the corpus of those rats given the higher concentrations compared with 40mM asprin and 5mM bile.

When the triple combinations were compared with single or double agents there was a distinct lack of change in the WBC numbers. Only when the triple agents at the lower concentrations were compared with 0.23mM carprofen in the corpus and antrum, and compared with 0.23mM carprofen/2.5mM bile was there any difference. In these comparisons it was an increase in WBC counts that was noted. With the triple blend at the higher concentration only when the numbers found in the corpus were compared with 0.1N HCl was a significant reduction found.

agent *vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
IR1B1A1	IA1	ns	ns
	IB1	ns	ns
	IR1	S	S
	IR1A1	ns	ns
	IR1B1	ns	S
IR2A2B2	IA2	@S	ns
	IB2	ns	ns
	IR2	ns	ns
	IR2A2	ns	ns
	IR2B2	ns	ns

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, S = significant, ns = not significant, @S = significantly less.

Table 5.13. WBC count. Comparison between rats administered triple combinations and those given single or double agents.

5.3.2.3 Congestion

The rats dosed with potentially injurious agents were examined for evidence of congestion (Table 5.14). There was little demonstrable evidence of an agent or time effect arising from any of the agents or combinations. Of the 240 areas (120 rats) examined, 175 (73%) had no evidence of congestion, which was most apparent in the corpus.

total = 240 areas	Time 2min				5min				15min			
area	corpus		antrum		corpus		antrum		corpus		antrum	
Congestion	n	%	n	%	n	%	n	%	n	%	n	%
0	26	*10.83*	34	*14.17*	25	*10.42*	32	*13.33*	22	*9.17*	36	*15*
1	14	*5.83*	6	*2.5*	13	*5.42*	6	*2.5*	14	*5.83*	4	*1.67*
2	0	*0*	0	*0*	2	*0.83*	2	*0.83*	4	*1.67*	0	*0*

Table 5.14. Congestion in rats given injurious agents broken done by area and time, and expressed as a percentage of 240 areas.

Congestion was scored as 1 in 57 areas. In these rats congestion was more than twice as likely to be present in the corpus as in the antrum. The most intense congestion (scored 2) was uncommon, being only found in eight areas from different rats, and in six of the eight rats the area congested was the corpus. There was a suggestion of some time effect and some agent effect, in that a score of 2 was not found at 2 minutes, and 4/8 areas were in rats given either aspirin or carprofen in combination with bile. However, the small numbers make the validity of these results uncertain.

5.3.2.4 Apical Damage

	Time					
	2min		5min		15min	
area	corpus	antrum	corpus	antrum	corpus	antrum
Damage						
0	30	24	29	28	29	24
1	10	16	9	12	9	12
2	0	0	2	0	2	6

Table 5.15. Apical damage versus time in rats given injurious agents.

Apical damage was assessed on the degree of disruption of the apical cells in the foveolae and the extent of disruption in the section. The breakdown of the findings of apical damage are given in Table 5.15. There was no evidence of a time or concentration effect in terms of raw numbers. However, when the apical damage (1) was broken down into occurrence in corpus and antrum, it appeared that the corpus was spared apical damage compared with the antrum. The antrum sustained between 30-50% more damage than the corpus at each of the time intervals. Severe damage (2) was not commonly encountered. In addition, bile as a single agent produced twice as many sections with apical damage (41.67%) as did the other single agents, with aspirin producing little apical damage (12.5%). The corpus and antrum were equally affected with bile induced apical damage.

The addition of a second agent increased the apical damage but without an observable concentration effect. Examples of this effect are shown in Table 5.16. The most marked increase was that caused by combining aspirin and bile, when compared with the combination of carprofen and bile.

There was no additive effect demonstrable with the triple combinations which produced apical epithelial damage approximating that induced by some of the single agents.

agent	N	N with damage (score1)	%
IA	24	5	20.83
IB	24	10	41.67
IS	24	3	12.50
IR	24	5	20.83
ISB	24	11	45.83
IRB	48	13	27.00
IRA	48	15	31.25
IRAB	24	4	16.67

I = injury, A = hydrochloric acid, B = bile, S = aspirin, R = carprofen, N = number of areas.

Table 5.16. Apical epithelial damage (score 1) caused by various agents and combinations in rats.

5.3.2.5 Luminal Contents

Accepting the fact that processing the sections will remove much of the normal mucus covering of the mucosa, nevertheless luminal contents were found in a number of sections (Table 5.17).

	Time					
	2min		5min		15min	
area	corpus	antrum	corpus	antrum	corpus	antrum
contents						
NF	26	22	33	28	28	275
TF	6	7	4	6	6	5
NM	3	7	2	2	3	3
TM	5	4	1	4	3	7

NF = little mucus, few cells, TF = thick mucus few cells, NM = little mucus, many cells, TM = thick mucus, many cells

Table 5.17. Luminal contents found in sections of gastric mucosa rats given injurious agents, by time and area.

No obvious time or area effect was apparent. Only in those rats given the carprofen and acid combinations was there a pronounced effect where the mucus was thickened. The cells that were present appeared to be those sloughed by the surface epithelial layer and trapped in the overlying mucus. However, thickened mucus was not always accompanied by the presence of cells embedded in the mucus.

		Luminal Contents							
		Corpus				Antrum			
agents		NF	TF	NM	TM	NF	TF	NM	TM
IA	24	12	0	0	0	10	0	1	1
IB	24	7	0	2	3	5	1	1	5
IS	24	12	0	0	0	9	0	0	3
IR	24	11	0	0	1	8	0	4	0
ISB	24	8	0	4	0	10	0	1	1
IRB	48	20	3	1	0	20	3	0	1
IRA	48	7	13	1	3	6	13	2	3
IRAB	24	9	0	1	2	7	1	3	1

I = injury, A = hydrochloric acid, B = bile, S = aspirin, R = carprofen, NF = little mucus, few cells, TF = thick mucus few cells, NM = little mucus, many cells, TM = thick mucus, many cells, N = number of areas.

Table 5.18. Luminal contents found with some combinations.

There was no obvious correlation between apical damage and the presence of excessive luminal contents. However of the eight areas found to have apical damage with a score of 2, six of these had a thick overlay of mucus containing many cells. This represents only 25% (6/24) of those areas found to have thick mucus with many cells.

In summary (Table 5.19), there was a failure to find a consistent correlation between the findings of gross oedema and histological evidence of oedema. This inconsistency was even more marked when gross erythema and histological congestion were compared, with gross erythema being infrequently substantiated histologically.

Group	Oedema				Erythema/congestion			
	Corpus		Antrum		Corpus		Antrum	
	G	H	G	H	G	H	G	H
Control	-	-	-	-	-	-	-	-
Injury								
IA1	-	-	-	-	-	-	-	-
IA2	-	-	-	-	-	+	-	+
IB1	-	-	-	-	-	+	-	-
IB2	-	-	-	-	+	+	-	-
IS1	-	-	-	-	-	++	-	-
IS2	-	-	-	++	-	+	-	-
IR1	-	-	-	-	-	++	++	-
IR2	-	-	-	++	+++	-	+++	-
IS1B1	-	-	-	-	+++	+	+++	-
IS2B2	-	-	-	++	+++	++	+++	-
IR1A1	-	-	-	-	-	-	+++	-
IR1A2	-	++	-	++	-	++	+++	++
IR2A1	+	++	++	++	+++	++	-	-
IR2A2	+	++	++	+++	+++	-	+++	-
IR1B1	++	++	++	+++	++	-	-	-
IR2B1	++	-	++	+++	++	-	-	-
IR1B2	++	++	++	+++	++	-	+	-
IR2B2	+	++	++	+++	+++	++	+++	-
IR1B1A1	+	++	+	+++	+++	+	+++	-
IR2B2A2	+	+++	+	++	+++	+	+++	-

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, G = gross evidence, H = histological evidence, + = 2 rats.

Table 5.19. Comparison between gross and histological evidence of oedema and erythema/congestion in those rats given injurious agents.

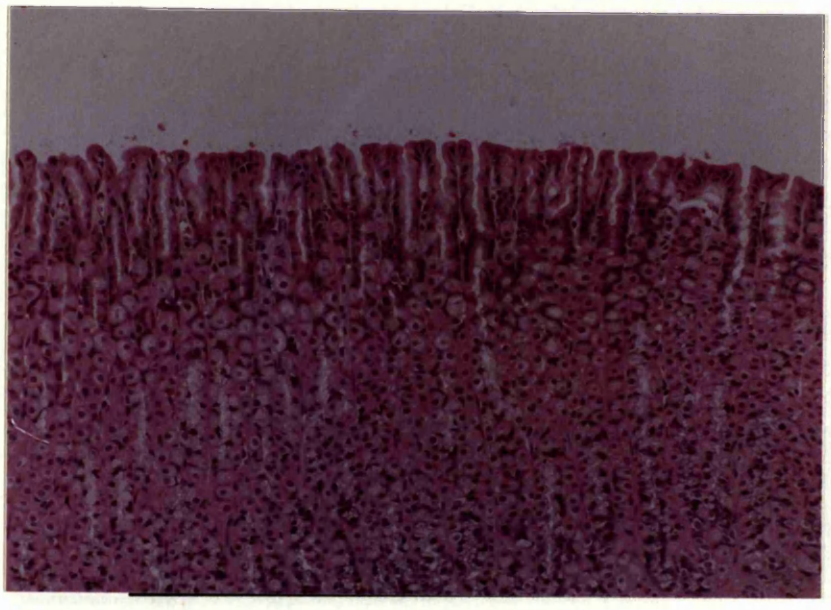


Figure 5.5. Photomicrograph of corpus exposed to 0.1N HCl. There is no obvious change after 5 minutes exposure. (H&E x150)

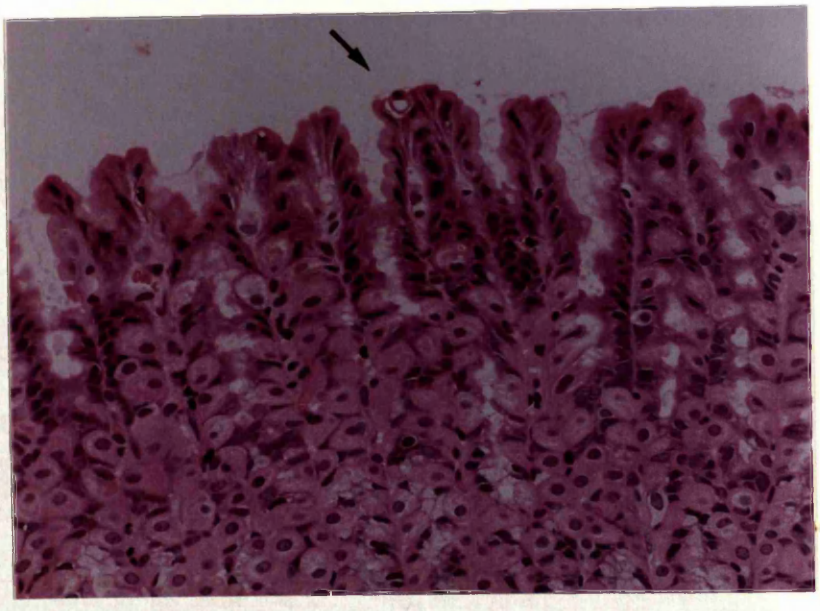


Figure 5.6. Photomicrograph of corpus exposed to 0.1N HCl. There is no obvious change after 15 minutes. An enlarged apical cell can be seen to be in the process of being extruded (*arrow*). (H&E x300)

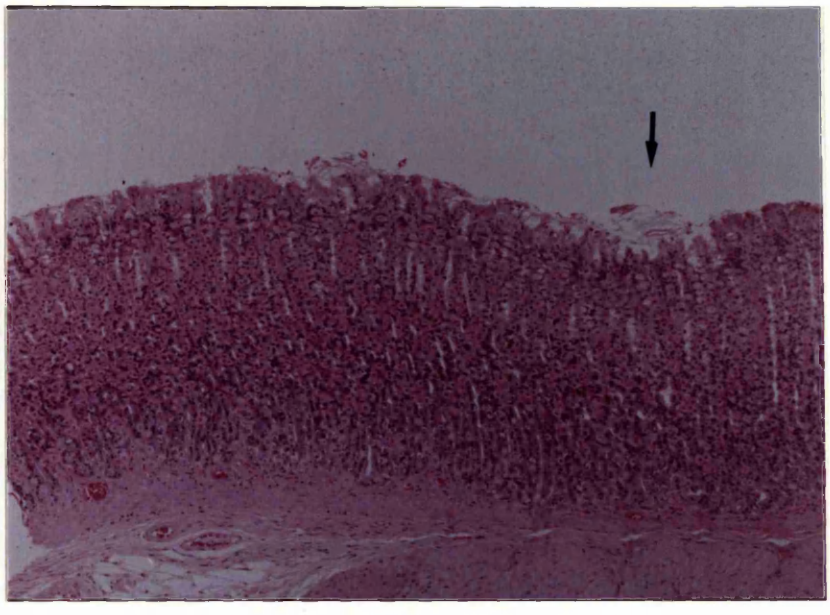


Figure 5.7. Photomicrograph of corpus exposed to 2.5mM bile. After 5 minutes exposure there are traces of debris in the lumen over an area of apical epithelium that is distorted (*arrow*). (H&E x75)

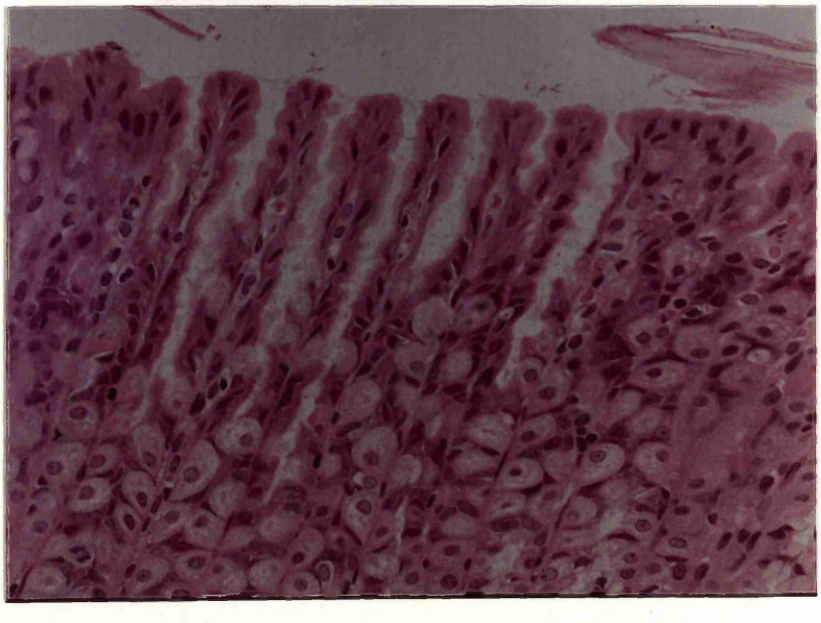


Figure 5.8. Photomicrograph of corpus exposed to 20mM aspirin. After 2 minutes exposure the apical epithelium is unaffected. (H&E x300)

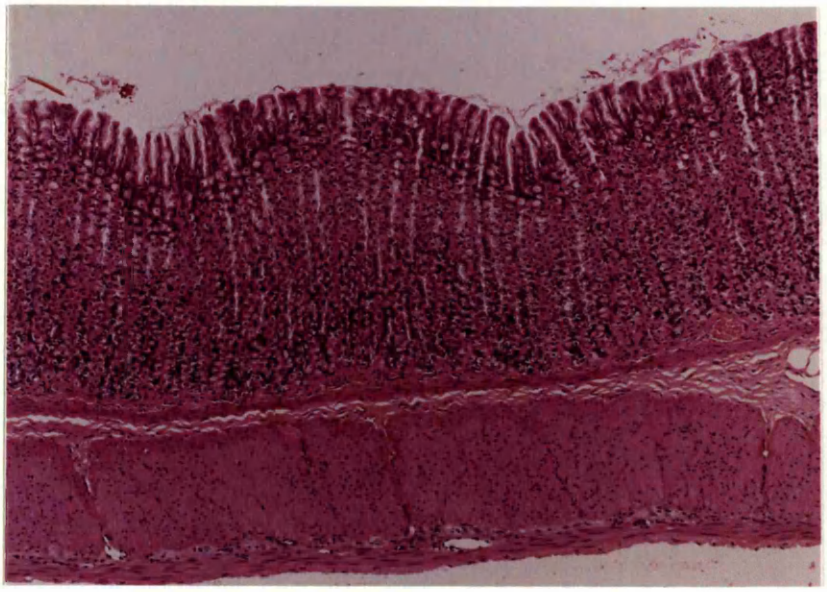


Figure 5.9. Photomicrograph of corpus exposed to 40mM aspirin. After 15 minutes the surface epithelial cells are unaffected, though there is a little mucus on the surface. (H&E x75)

Figure 5.10. Photomicrograph of corpus exposed to 0.46mM carprofen. After 5 minutes exposure the epithelium is unaffected. (H&E x75)

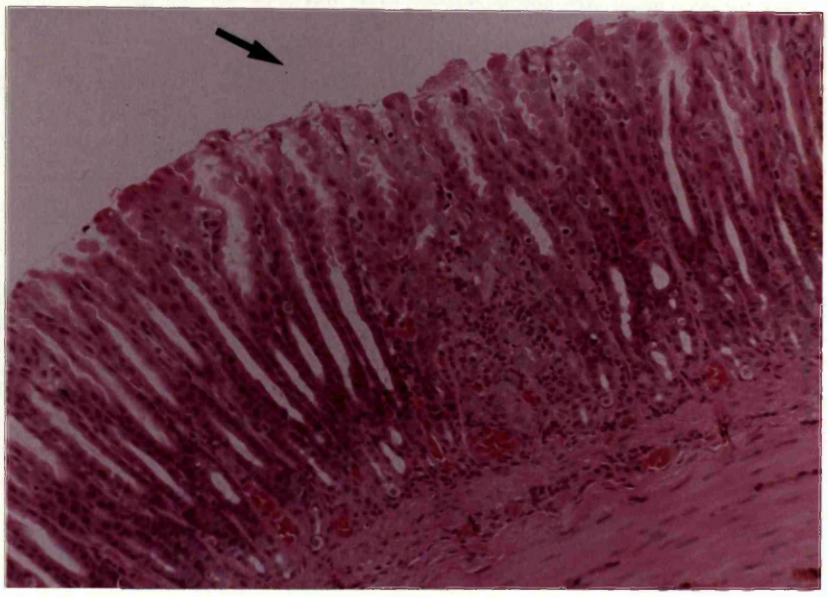


Figure 5.11. Photomicrograph of antrum exposed to 0.46mM carprofen. After 15 minutes exposure there is an area of surface epithelial disruption (*arrow*). There is a modest white blood cell infiltrate and an element of congestion. (H&E x150)

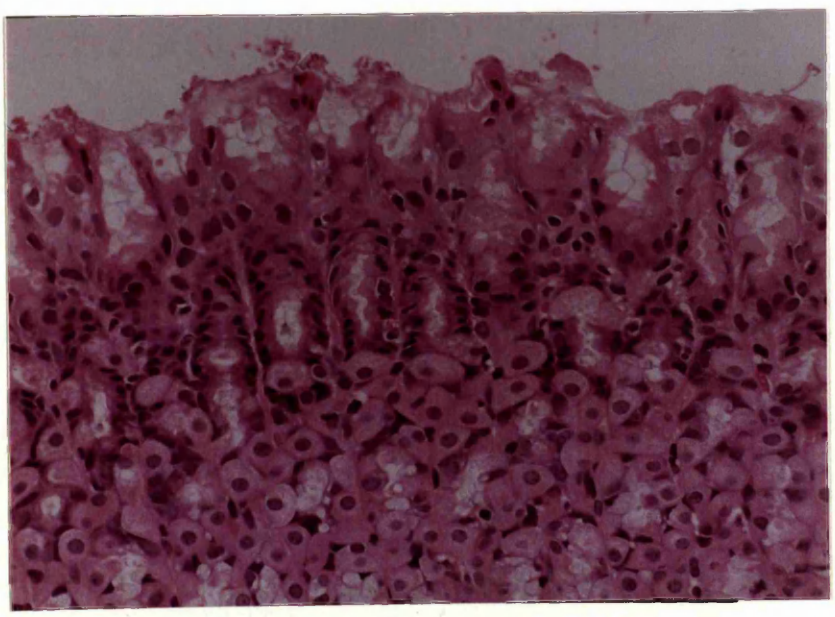


Figure 5.12. Photomicrograph of corpus exposed to 5mM bile. After 15 minutes exposure there is a prominent loss of apical epithelial cells. Overlying the damaged area is a layer of mucus with entrapped cells. (H&E x300)

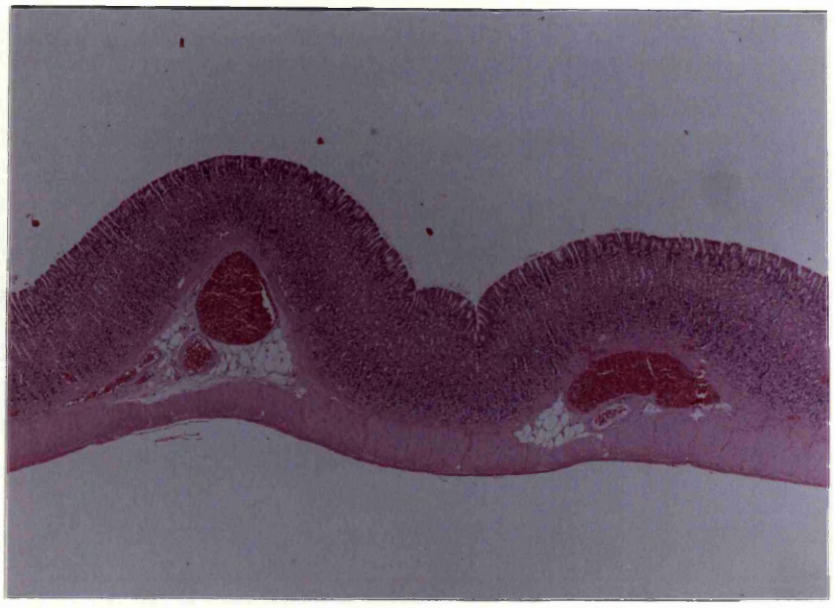


Figure 5.13. Photomicrograph of corpus exposed to 0.23mM carprofen/2.5mM bile. After 5 minutes there is a little lumenal debris. The underlying surface epithelium is unaffected, but the blood vessels are prominent. (H&E x30)

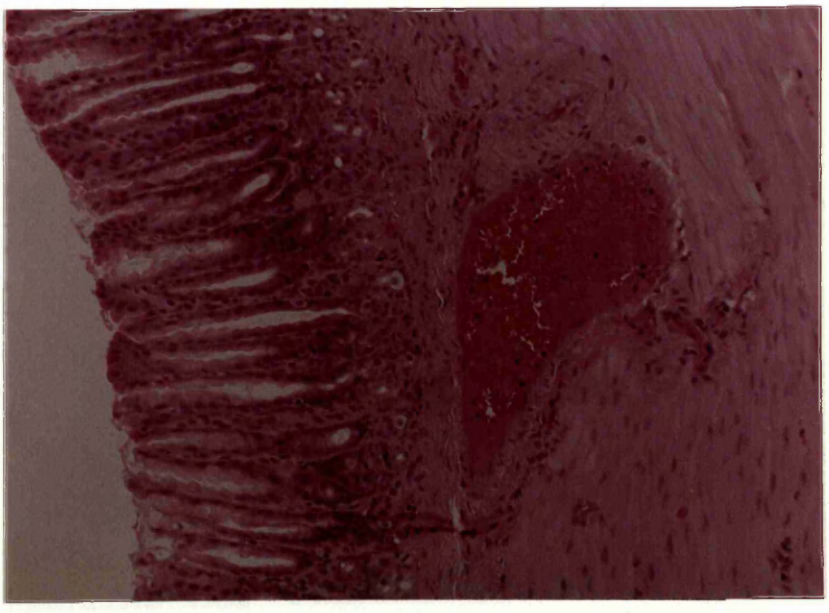


Figure 5.14. Photomicrograph of antrum exposed to 20mM aspirin/2.5mM bile. After 15 minutes exposure there is a little lumenal mucus and a prominent blood vessel present. (H&E x150)

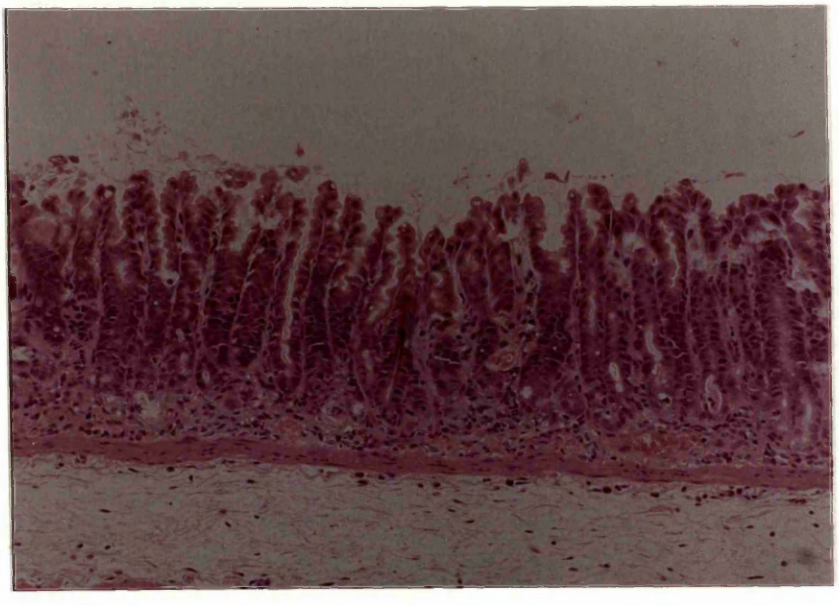


Figure 5.15. Photomicrograph of antrum exposed to 0.46mM carprofen/5mM bile. After 15 minutes there is extensive apical damage and an increased number of cells appear to be being extruded. Underlying this is oedema. (H&E x150)

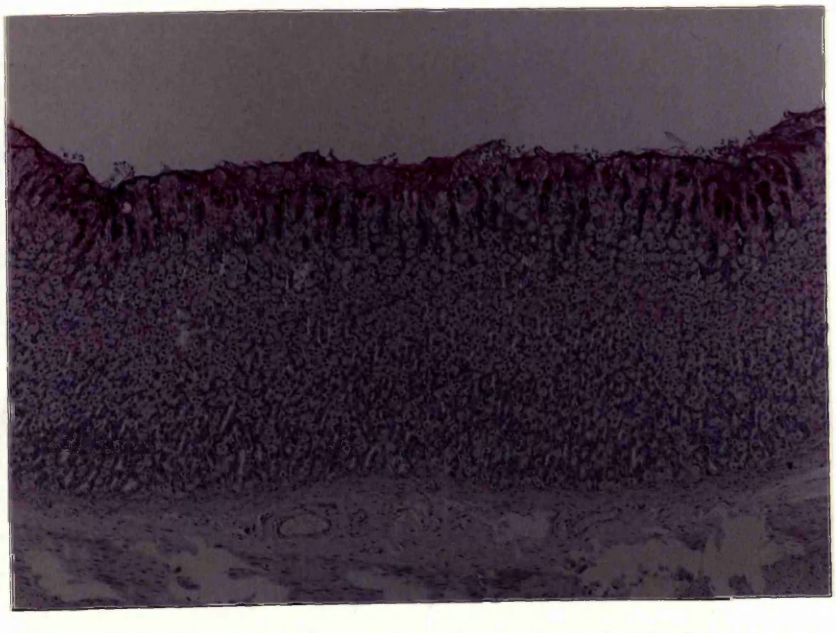
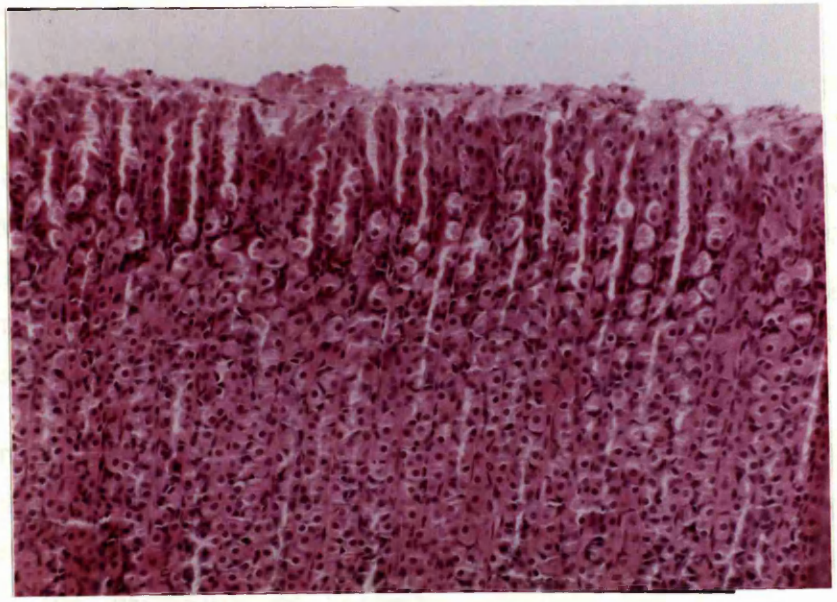


Figure 5.16. Photomicrograph of corpus exposed to 0.46mM carprofen/0.1N HCl. After 5 minutes exposure there is a thick overlying layer of mucus. (PAS/Alcian Blue x75)



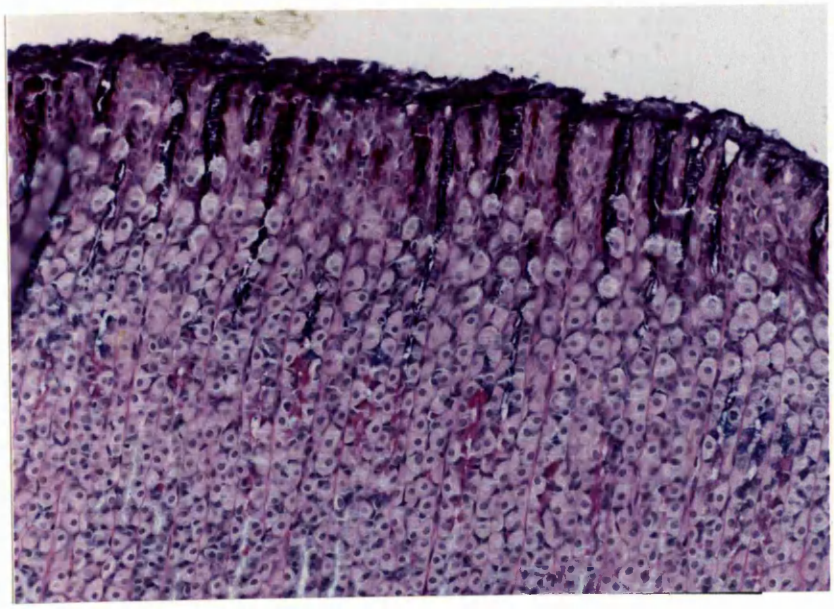


Figure 5.17a & 5.17b. Photomicrographs of corpus exposed to 0.23mM carprofen/ 0.05N HCl/ 2.5mM bile. After 15 minutes there is overlying layer of mucus with a distinct cellular content. (H&E and PAS/Alcian Blue x150)

5.3.3 Scanning Electron Microscopy

When the summed mean scores for general and individual change of the rats given injurious agent/s were compared with normal, the scores obtained from the corpus for general change were essentially similar to those found in the normal rats (see Tables 5.20 and 5.21). Only those rats given 2.5mM and 5mM bile, aspirin 20mM with 5mM bile and 0.46mM carprofen with 2.5mM bile showed a significant increase in score 1 (Figure 5.18). In contrast score 2, which represented individual as opposed to general change, was significantly increased in 15/20 injurious agent/combinations. With the single injurious agents, score 2 was increased with bile and carprofen, but not aspirin (Figure 5.19). Where combinations were applied, only rats given 0.23mM carprofen/5mM bile were spared. Of particular note is that 20mM aspirin/5mM bile also produced marked increase in individual as well as general change.

In the antrum, there was an increase in scores over normal for 10/20 injurious agents. Single agents, with the exception of bile, produced no significant increase in scores. All combinations of agents with carprofen produced significant increases in general change with the exception of 0.23mM carprofen mixed with acid at both concentrations and the higher concentration triple combination (Figure 5.20). The individual scores in the antrum again showed that only bile produced significant increase. All combinations of agents with carprofen produced significantly increased damage with one exception - carprofen and acid at the higher concentrations (Figure 5.21). The combination of 20mM aspirin/5mM bile produced marked increases in both general and individual scores - this effect was similar to that in the corpus (Figures 5.22 and 5.23).

Features	Corpus																		IR1/ A1B1	IR2/ A2B2
	IA1	IA2	IB1	IB2	IS1	IS2	IR1	IR2	IS1B	IS2B	IR1A1	IR1A2	IR2A1	IR2A2	IRIB1	IR1B	IR2B	IR2B		
General																				
mucus covering	3.17	3.17	3.33	2.50	3.50	3.50	2.83	2.83	4.00	2.67	3.50	2.17	2.83	3.00	3.17	2.83	2.33	2.17	3.17	3.33
mucus thickness	3.00	2.33	2.33	2.67	2.33	2.17	2.17	2.17	2.33	3.00	2.67	2.33	2.33	2.33	2.67	2.50	2.50	2.50	2.67	2.67
cell extrusion	1.67	1.83	2.17	3.35	2.00	2.00	1.17	2.00	1.83	2.83	2.00	1.83	1.67	1.50	2.33	2.00	2.00	1.67	1.83	1.67
rosette formation	1.50	1.33	2.17	3.00	1.83	2.00	2.17	1.83	2.17	2.83	2.00	1.67	2.17	1.83	1.83	2.33	2.67	1.83	2.00	2.17
gastric pits enlarged	1.50	1.00	2.17	3.00	2.33	2.33	1.00	1.17	1.83	3.17	1.17	1.17	1.17	1.00	1.33	1.17	1.17	1.83	1.00	1.00
loss of cobblestoning	1.33	2.00	2.17	2.67	1.67	1.67	1.00	1.33	1.17	2.33	1.17	2.17	1.17	1.33	1.83	1.50	1.67	3.00	1.83	1.33
intracellular junctions	1.50	1.17	1.83	1.83	1.50	1.50	1.17	1.50	1.00	1.83	1.67	1.67	1.83	2.50	1.83	2.33	2.33	2.83	2.00	2.33
cell convexity	2.00	1.50	1.50	2.67	1.33	1.00	2.17	2.67	2.33	1.83	3.33	2.50	2.33	2.50	2.00	2.33	2.67	2.83	2.17	3.17
[pooled score 1]	**15.67**	**14.33**	**17.67**	**21.69**	**16.49**	**16.17**	**13.68**	**15.50**	**16.66**	**20.49**	**17.51**	**15.51**	**15.50**	**15.99**	**16.99**	**16.99**	**17.34**	**18.66**	**16.67**	**17.67**
Individual																				
apical ruffling	1.50	1.00	2.17	1.67	1.17	1.33	2.00	3.00	1.83	2.67	2.50	3.17	2.67	2.33	1.67	1.83	2.50	2.33	2.17	2.67
apical holes	1.33	1.17	2.50	2.50	1.50	2.00	2.00	2.50	2.50	2.33	1.83	1.83	1.17	1.50	1.83	2.17	2.17	2.50	1.33	1.83
apical concavity	1.33	1.17	2.50	2.33	1.50	2.00	2.00	1.67	1.33	2.00	2.00	2.00	1.50	1.67	2.17	2.17	2.17	1.83	1.67	1.50
apical erosions	1.50	1.50	2.67	2.67	1.67	1.83	2.67	3.17	1.50	2.50	2.00	3.33	2.50	2.67	3.00	2.67	2.33	3.33	2.33	2.50
intracellular contents	1.33	1.00	2.50	1.33	1.33	1.50	2.17	2.50	1.83	2.00	1.33	2.00	2.00	1.50	1.50	1.67	2.33	2.50	1.17	1.33
empty cells	1.33	1.00	2.33	1.50	1.17	1.33	1.67	2.00	1.50	1.67	1.33	1.67	1.50	1.83	1.67	1.67	1.50	1.67	1.17	1.50
[pooled score 2]	**8.32**	**6.84**	**14.67**	**12.00**	**8.34**	**9.99**	**12.51**	**14.84**	**10.49**	**13.17**	**10.99**	**14.00**	**11.34**	**11.50**	**11.84**	**12.18**	**13.00**	**14.16**	**9.84**	**11.33**

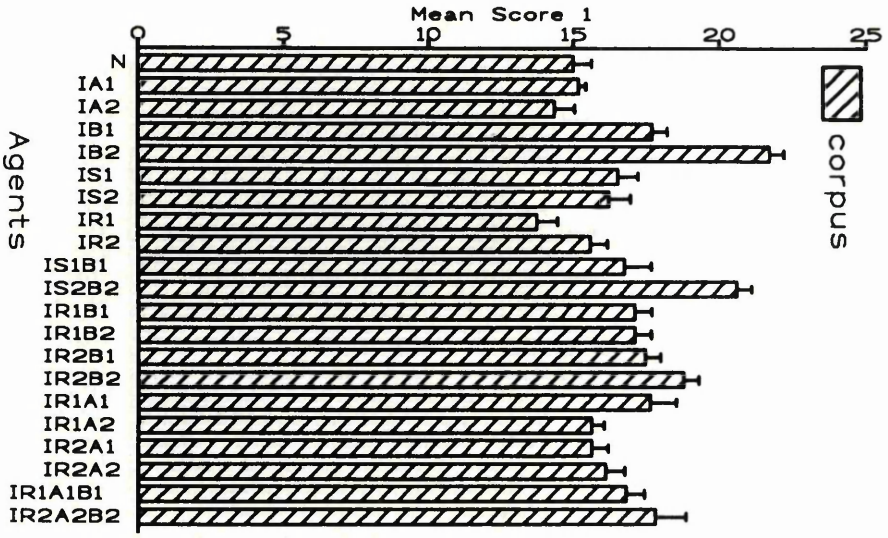
Table 5.20. Corpus: scanning electron microscopy scores pooled for each agent (6rats/agent).

I=injury, A1=0.05N HCl, A2=0.1N HCl, B1=2.5mM bile, B2=5mM bile, S1=20mM aspirin, S2=40mM aspirin, R1=0.23mM carprofen, R2=0.46mM carprofen

	Antrum																		IR1/	IR2/
Features	IA1	IA2	IB1	IB2	IS1	IS2	IR1	IR2	IS1B	IS2B	IR1A1	IR1A2	IR2A1	IR2A1	IRIB1	IR1B	IR2B	IR2B	A1B1	A2B2
General																				
mucus covering	3.00	3.33	3.17	3.67	2.83	3.17	3.00	3.00	3.83	2.00	3.00	3.17	3.33	3.17	3.33	3.17	3.17	2.83	3.33	2.33
mucus thickness	2.83	2.83	2.33	3.17	1.83	2.17	2.33	2.67	2.83	2.83	2.33	2.50	2.50	2.67	3.00	2.33	2.83	2.17	2.83	2.00
cell extrusion	2.00	2.00	2.83	3.00	1.83	1.67	1.33	1.50	1.50	2.33	2.33	1.50	2.50	1.67	1.83	1.67	2.00	1.33	2.33	1.50
rosette formation	1.83	1.17	2.33	2.17	1.50	1.17	1.67	1.50	1.67	2.50	2.00	1.33	2.33	1.67	1.83	1.67	2.33	1.83	2.00	1.67
gastric pits enlarged	1.17	1.33	2.83	2.17	1.50	1.83	1.00	1.00	1.33	2.67	1.33	1.33	1.00	1.00	1.00	1.00	1.33	2.00	1.00	1.00
loss of cobblestoning	1.17	1.50	2.50	2.67	1.33	1.50	1.00	1.17	1.17	2.17	1.17	1.67	1.33	1.67	1.67	1.00	2.00	1.83	1.83	2.17
intracellular junctions	1.00	1.17	2.00	1.33	1.00	1.00	1.50	1.33	1.50	1.83	1.83	2.33	1.67	2.33	1.67	2.33	2.17	2.33	1.83	2.83
cell convexity	1.33	1.50	2.00	1.67	1.00	1.00	3.17	2.17	1.17	2.33	3.00	2.83	2.83	2.83	2.17	3.17	2.33	3.00	3.00	2.67
[pooled score 1]	**14.33**	**14.83**	**19.99**	**19.85**	**12.82**	**13.51**	**15.00**	**14.34**	**15.00**	**18.66**	**16.99**	**16.66**	**17.49**	**17.01**	**16.50**	**16.34**	**18.16**	**17.32**	**18.15**	**16.17**
Individual																				
apical ruffling	1.00	1.00	3.00	1.83	1.33	1.00	1.50	2.17	1.17	1.83	2.00	2.17	1.83	1.83	1.67	2.00	2.17	2.33	1.50	2.83
apical holes	1.50	1.00	2.17	2.33	1.50	1.67	1.83	1.50	2.17	2.17	2.33	2.00	1.50	1.50	1.67	2.00	1.83	1.67	1.50	2.17
apical concavity	1.33	1.17	2.67	1.83	1.33	1.33	1.67	1.17	1.83	2.83	1.67	2.00	1.83	1.50	1.67	2.00	2.00	2.00	1.83	1.67
apical erosions	1.33	1.17	2.33	3.50	1.50	1.67	2.33	2.17	1.50	2.83	2.17	3.33	2.00	1.83	2.00	2.33	1.67	2.50	2.17	2.67
intracellular contents	1.17	1.00	2.50	2.50	1.33	1.17	1.67	1.50	1.67	2.33	1.50	1.67	1.67	1.17	1.50	1.50	1.00	1.50	1.50	1.33
empty cells	1.00	1.00	2.00	2.67	1.00	1.33	1.17	1.33	1.50	2.17	1.50	1.33	1.67	1.33	1.50	1.00	1.33	1.33	1.50	2.33
[pooled score 2]	**7.33**	**6.34**	**14.67**	**14.66**	**7.99**	**8.17**	**10.17**	**9.84**	**9.84**	**14.16**	**11.17**	**12.50**	**10.50**	**9.16**	**10.01**	**10.83**	**10.00**	**11.33**	**10.00**	**13.00**

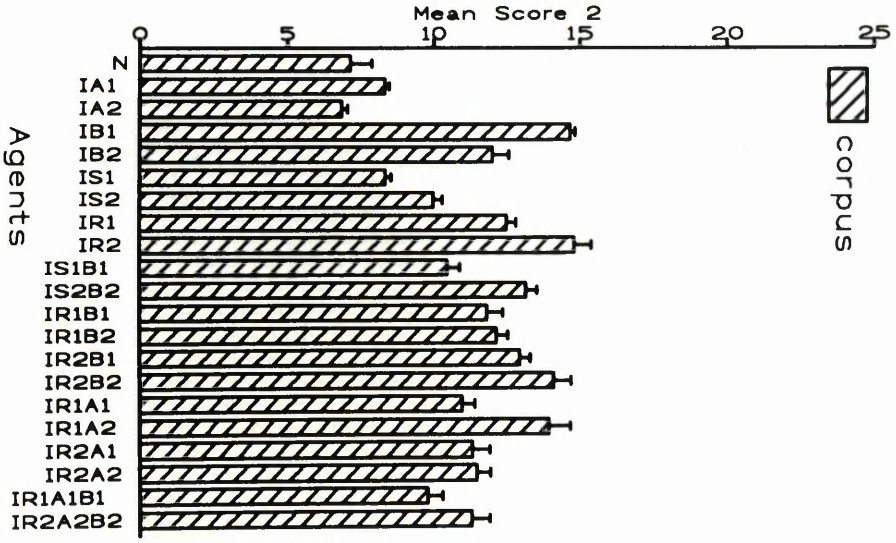
Table 5.21. Antrum: scanning electron microscopy scores pooled for each agent (6rats/agent).

I=injury, A1=0.05N HCl, A2=0.1N HCl, B1=2.5mM bile, B2=5mM bile, S1=20mM aspirin, S2=40mM aspirin, R1=0.23mM carprofen, R2=0.46mM carprofen



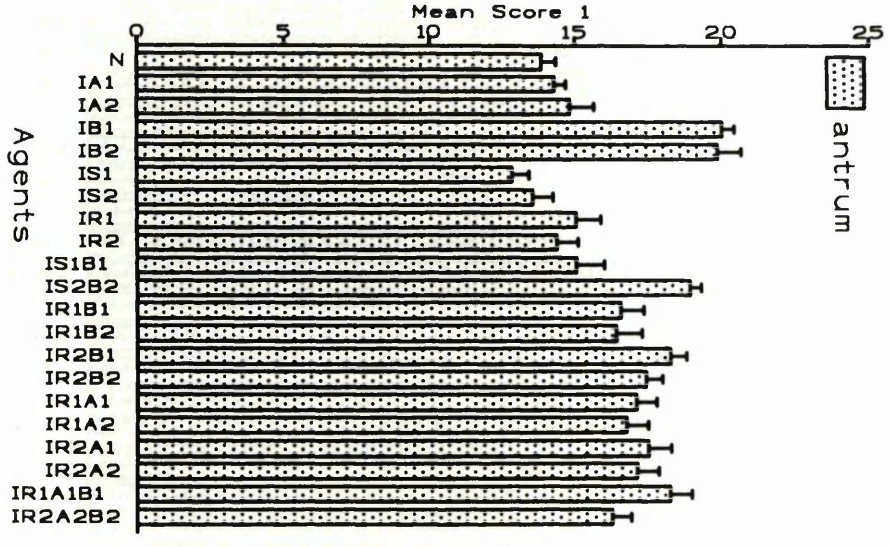
N=Normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.18. Bar graph of corpus scanning electron microscopy general scores obtained from rats given injurious agents.



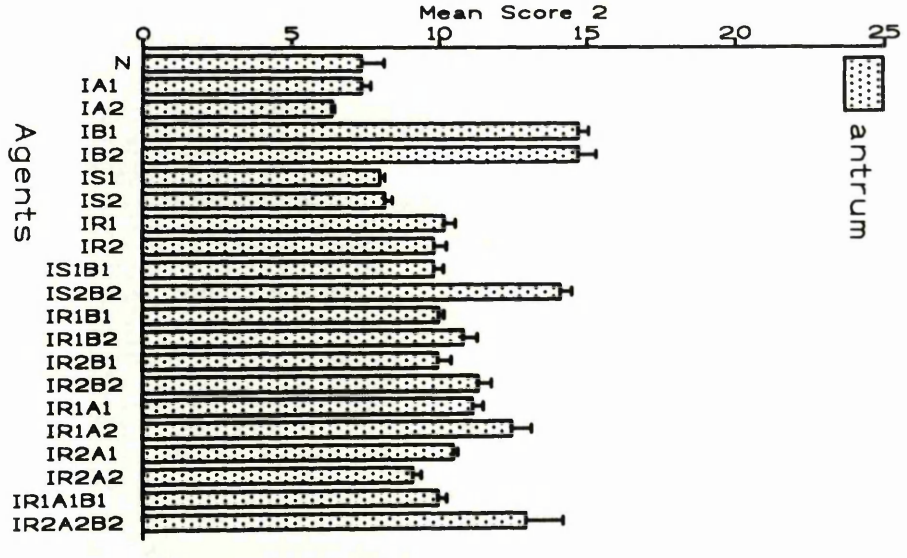
N=normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.19. Bar graph of corpus scanning electron microscopy individual scores obtained from rats given injurious agents.



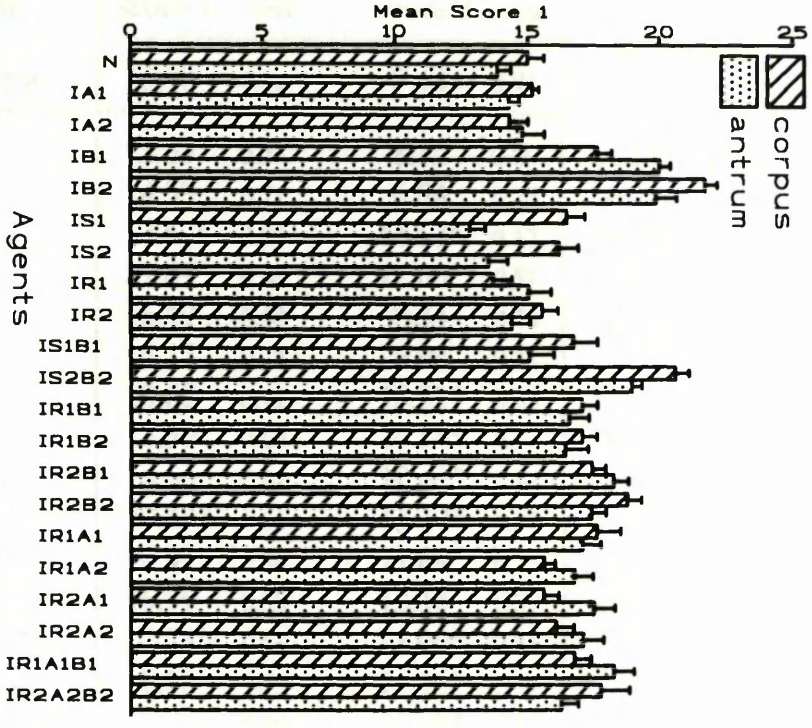
N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.20. Bar graph of antrum scanning electron microscopy general scores obtained from rats given injurious agents.



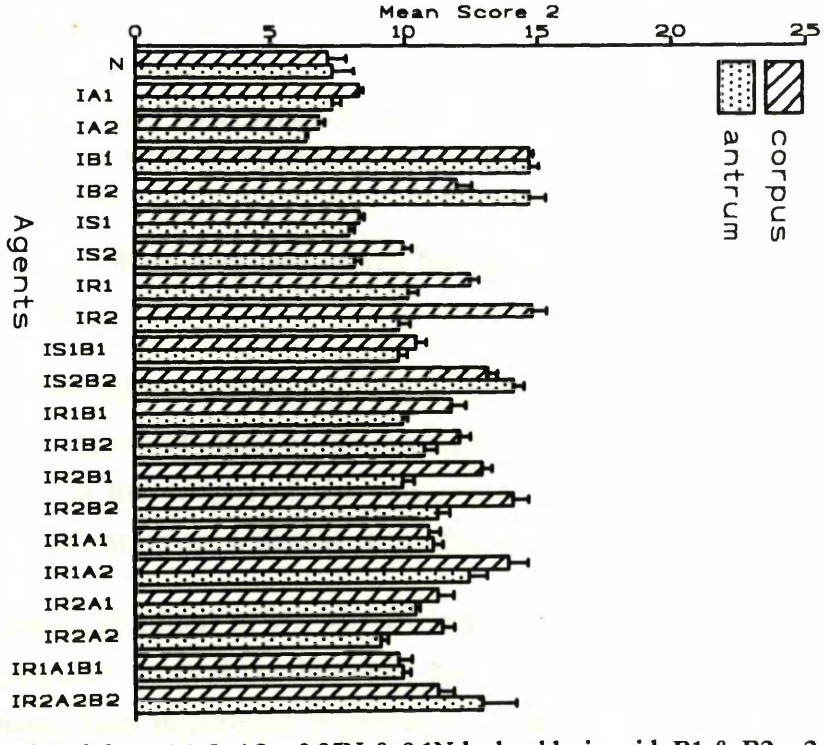
N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.21. Bar graph of antrum scanning electron microscopy individual scores obtained from rats given injurious agents.



N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.22. Bar graph of scanning electron microscopy general scores obtained from the corpus and antrum of rats given injurious agents.



N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 5.23. Bar graph of scanning electron microscopy individual scores obtained from the corpus and antrum of rats given injurious agents.

agent	Corpus score 1	±sd	Corpus score 2	±sd	Antrum score 1	±sd	Antrum score 2	±sd
Normal	15.00	0.61	7.14	0.72	13.86	0.51	7.33	0.80
IA1	15.67	0.25	8.32	0.16	14.33	0.39	7.33	0.33
IA2	14.33	0.71	6.84	0.20	14.83	0.81	6.34	0.09
IB1	17.67*	0.52	14.67*	0.17	19.99*	0.42	14.67*	0.36
IB2	21.69*	0.48	12.00*	0.57	19.85*	0.79	14.66*	0.62
IS1	16.49	0.68	8.34	0.20	12.82	0.59	7.99	0.18
IS2	16.17	0.73	9.99	0.32	13.51	0.72	8.17	0.27
IR1	13.68	0.71	12.51*	0.33	15.00	0.86	10.17	0.38
IR2	15.50	0.60	14.84*	0.57	14.34	0.73	9.84	0.43
IS1B1	16.66	0.92	10.49*	0.42	15.00	0.95	9.84*	0.34
IS2B2	20.49*	0.51	13.17*	0.37	18.66*	0.38	14.16*	0.40
IR1B1	16.99	0.58	11.84*	0.55	16.50*	0.76	10.01*	0.18
IR1B2	16.99	0.55	12.18	0.39	16.34*	0.86	10.83*	0.48
IR2B1	17.34*	0.53	13.00*	0.35	18.16*	0.56	10.00*	0.44
IR2B2	18.66	0.53	14.16*	0.59	17.32*	0.55	11.33*	0.47
IR1A1	17.51	0.90	10.99*	0.45	16.99	0.68	11.17*	0.36
IR1A2	15.51	0.43	14.00*	0.72	16.66	0.72	12.50*	0.68
IR2A1	15.50	0.59	11.34*	0.60	17.49*	0.79	10.50*	0.17
IR2A2	15.99	0.64	11.50*	0.48	17.01*	0.74	9.16	0.27
IR1A1B1	16.67	0.64	9.84*	0.51	18.15*	0.76	10.00*	0.28
IR2A2B2	17.67	1.07	11.33*	0.63	16.17	0.64	13.00*	1.26
no ($p<0.05$)	4		15		10		13	

I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 =20mM & 40mM aspirin, R1 & R2 =0.23mM & 0.46mM carprofen. * indicates were statistically different from normal ($p<0.05$).

Table 5.22. Mean summed scores for variables of change seen by scanning electron microscopy.

Comparison of the summed scores from the corpus and antrum failed to establish a difference based on area of the stomach in nearly all rats. Only the general change (score 1) in the corpus of the rats given 20mM aspirin and the individual change (score 2) of rats given 0.46mM carprofen were statistically greater than in the antrum.

The results obtained at the three time intervals were evaluated, and there was no demonstrable time effect between two and five minutes. However, there was a significant time difference between 15 minute samples and those taken at 2 and 5 minutes with single agents bile, aspirin, carprofen, but not acid. This time effect was also evident with all the combinations.

The scores generated by carprofen are shown in Tables 5.20-22, with the comparison of carprofen with other single agents shown in Table 5.23. When 0.23mM carprofen was compared with other single agents, there was either no significant difference between scores or else 0.23mM carprofen produced lower scores. In contrast, there was no difference in the general scores produced by 0.46mM carprofen when compared with other agents. There was more inconsistency amongst the comparisons of individual scores, where carprofen induced more damage in the corpus than 0.1N acid or 5mM bile but less that aspirin.

			corpus		antrum	
agent*	*vs*	score agent*	1	2	1	2
IR1		IA1	ns	ns	ns	ns
IR1		IB1	@S	ns	@S	@S
IR1		IS1	@S	ns	ns	ns
IR2		IA2	ns	S	ns	ns
IR2		IB2	ns	S	ns	@S
IR2		IS2	ns	@S	ns	ns
($p < 0.05$)						

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, S = significantly higher, ns = not significant, @S = significantly lower.

Table 5.23. Scanning score. Comparison of carprofen and single agents.

There was no significant difference between the scores obtained with 20mM aspirin alone and when in combination with bile (Tables 5.20-22, 5.24). However, 2.5mM bile alone produced higher scores than when in combination with aspirin. Aspirin in combination with 5mM bile produced significantly higher scores than aspirin alone. However, there was no significant difference between aspirin/5mM bile and bile alone, both producing more degeneration and damage than most other agents and combinations (Figures 5.18-5.23).

agent	*vs*	score agent	corpus 1	corpus 2	antrum 1	antrum 2
IS1B1		IS1	ns	ns	ns	ns
IS1B1		IB1	ns	@S	@S	@S
IS2B2		IS2	S	S	S	S
IS2B2		IB2	ns	ns	ns	ns
($p<0.05$)						

I=injury, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, score 1 = general change, score 2= individual damage, S=significantly higher, ns=not significant, @S=significantly lower.

Table 5.24. Scanning scores. Comparison of aspirin combinations.

The comparison of scanning scores of carprofen and other agents is shown in Table 5.25. When carprofen and a second agent combination were compared with the single agents a number of significant changes was apparent, which lacked a consistent pattern.

The addition of acid to carprofen failed to enhance the scores generated in most areas. In four areas where there was an effect on the scores two were positive and two negative. Carprofen on the other hand produced an increase in 7/16 scores when compared to those caused by acid alone. Neither the antrum of the corpus predominated, but the individual score being enhanced in 5/7 where there was an accentuation of damage.

When the results of carprofen and bile combined were compared to the single agents a somewhat different picture arose. Compared with bile, the addition of carprofen made no difference to the general or individual scores obtained from the corpus. In the antrum, this combination produced lower general and individual scores (6/8) than bile alone. Compared to carprofen alone, carprofen/bile combinations caused higher scores (5/16) with no area or bile concentration standing out. However this accentuation of scores was associated with general scores in most cases. Only in the antrum of the rats given 0.46mM bile with 5mM bile were both scores similarly increased.

There was no difference found when the scores generated by the lower concentrations of carprofen/bile were compared with those of aspirin/bile. At the higher concentrations aspirin/bile caused more general change to the corpus and antrum than the corresponding carprofen/bile combination. The individual scores were only found to be different only in the antrum, where aspirin/bile was more damaging than

carprofen/bile (Table 5.25).

agent	score *vs*	agent	corpus 1	corpus 2	antrum 1	antrum 2
IR1A1		IA1	ns	ns	ns	S
IR1A1		IR1	S	ns	ns	ns
IR1A2		IA2	ns	S	ns	S
IR1A2		IR1	ns	ns	ns	ns
IR2A1		IA1	ns	ns	S	S
IR2A1		IR2	ns	@S	@S	ns
IR2A2		IA2	S	S	ns	ns
IR2A2		IR2	ns	ns	S	ns
IR1B1		IB1	ns	ns	@S	@S
IR1B1		IR1	S	ns	ns	ns
IR2B1		IB1	ns	ns	@S	@S
IR2B1		IR2	ns	ns	S	ns
IR1B2		IB2	ns	ns	@S	@S
IR1B2		IR1	S	ns	ns	ns
IR2B2		IB2	ns	ns	ns	ns
IR2B2		IR2	ns	ns	S	S
IR1B1		IS1B1	ns	ns	ns	ns
IR2B2		IS2B2	@S	ns	@S	@S

I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, score 1 = general change, score 2= individual damage, S=significantly higher, ns=not significant, @S=significantly lower.

Table 5.25. Scanning scores. Comparison of carprofen with other agent/combinations.

There was also a lack accentuation and of consistency in the results obtained when the triple combinations were compared with single or double agents (Table 5.26). Against acid alone the triple agents caused higher general and individual scores in the antrum). However, the comparison between triple and double agent combinations showed that supplementation with a third agent did not appear to make any significant difference to the general or individual damage induced.

agent *vs*	score agent	corpus 1	corpus 2	antrum 1	antrum 2
IR1B1A1	IA1	ns	ns	S	S
	IB1	ns	@S	ns	@S
	IR1	S	ns	S	ns
	IR1A1	ns	ns	ns	ns
	IR1B1	ns	ns	ns	ns
IR2A2B2	IA2	S	ns	S	S
	IB2	ns	ns	S	ns
	IR2	ns	@S	ns	ns
	IR2A2	ns	ns	ns	ns
	IR2B2	ns	ns	ns	ns
($p<0.05$)					

I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, score 1 = general change, score 2 = individual damage, S = significantly higher, ns = not significant, @S = significantly lower.

Table 5.26. Scanning scores. Comparison of triple combinations and single or double agents.

More rigourous statistical evaluation by polychotomous logistic regression (BMDP) confirmed the bile, aspirin and carprofen effects, but only the bile effect was further substantiated by generalised linear modelling (GLIM).

5.3.3.1 Individual Components of Scores

Those features such as mucus covering and thickness, rosettes and extrusions, which composed the summed mean scanning scores, found in rats given acid at either concentration were essentially the same in those rats given acid as those found in normal rats (Tables 5.20-21). One feature noted in some of the rats given acid, but not ascribed a score, was that some areas on the stubs showed a flattening of the cells (Figures 5.24 and 5.25). Generally, concentration and time seemed to have no effect on the individual features of cells or cell areas, which were very similar to those found in normal rats.

With bile in the corpus, the surface of mucus appeared to be patchily overlain with precipitated mucus. The mucus thickness and extent of covering were rather similar to that found in the normal rats and in those rats given acid. The gastric pits appeared enlarged, accompanied by a reduction in the cobblestone facade, with accentuation of the intercellular junctions and reduction in the convexity of the cobbles (cells). More cells were found to be extruded and there was a concomitant

rise in the numbers of rosettes seen (Figures 5.26 and 5.27). Individual cell changes such as apical ruffling, erosions and holes were also more apparent. In the antrum, the changes were of the same magnitude, but were slightly more severe when compared with normal antrum. In several rats, where the surface epithelial layer had been stripped away, a more severe damage was manifest as a few foci of exposed lamina propria. Increasing the bile concentration increased the number of rosettes and extruded cells, reduced the cobblestoning and convexity in both the corpus and antrum, and the mucus covering and thickness in the antrum. However, the prominence of intercellular junctions was unchanged. The individual cell change in the corpus was similar to that with the lower concentration of bile. In the antrum, however, apical erosions and empty shells were more frequent in occurrence (Figures 5.28 and 5.29).

With aspirin there was little variation between rats given 20mM and 40mM aspirin in those features that made up the summed scores. The exceptions to this were the increased thickness and covering of antral mucus with 40mM aspirin. Compared with the normal rats the only aspects that markedly differed were an accentuation of the gastric pits and intercellular junctions, and cobblestoning in both corpus and antrum (Figure 5.30). With 40mM aspirin, the number of cells which appeared concave or had apical holes was greater than in the lower concentration of aspirin or in normal rats (Figure 5.31). The addition of bile to aspirin caused little difference at the lower concentrations used. However at the higher concentrations there was an increase in the numbers of rosettes and extruded cells, as well as cell convexity in both the corpus and antrum (Figures 5.37-5.38).

The general changes produced by carprofen were essentially similar in the corpus and antrum of both concentrations. The mucus covering however, was rather more extensive in the antrum, and with both concentrations in both areas the level of cell convexity was much higher than in the normal rats. This accentuated convexity resulted in many of the cells appearing larger than adjacent cells or taking on a rounded, rather than the normal oblong or rhomboid shape (Figure 5.32). Carprofen produced means for score 2 from the corpus that were significantly higher than normal. The particular features that caused this difference were a marked increase in the extent of apical ruffling, holes, erosions and intracellular contents. These features gave many areas of cells a crumpled or imploded appearance. This was more pronounced in the corpus than in the antrum (Table 5.27, Figures 5.33-5.36).

	normal		carprofen 0.23mM		carprofen 0.46mM	
	C	A	C	A	C	A
apical ruffling	1.2	1.1	2.0	1.5	3.0	2.2
apical holes	1.4	1.2	2.0	1.8	2.5	1.5
concavity	1.1	1.1	2.0	1.7	1.7	1.2
apical erosions	1.3	1.4	2.7	2.3	3.2	2.2
intra/c contents	1.1	1.3	2.2	1.7	2.5	2.5
empty shells	1.3	1.3	1.7	1.2	2.0	1.3

C=corpus, A=antrum, intra/c=intracellular

Table 5.27. Individual scanning scores for rats given carprofen.

5.3.3.2 Addition of Acid

The effect of the addition of 0.05N acid in infused solution containing 0.23mM carprofen was to increase the convexity of the surface cells, and the number of rosettes and extruded cells in the corpus and the antrum respectively. These increases did not cause the mean scanning score to be significantly different from that obtained from normal rats. The individual cell damage was significantly different. This was caused by increased apical ruffling, holes, concavity and erosions. Increasing the concentration of acid to 0.1N produced very similar effects to that produced by the lower concentration of acid/caprofen with increased convexity and loss of cobblestoning, but also a reduction in mucus covering. Apical ruffling, holes and concavity were all increased, but the most marked change compared with 0.23mM carprofen/0.05N HCl was the increase in apical erosions. Changing the concentration of carprofen to 0.46mM and adding 0.05N acid also reduced the mucus covering and thickness in the corpus. In the antrum, cell extrusions and rosettes were increased. *In situ* cell features were more commonly encountered with apical ruffling, apical erosions and intracellular contents exposed in both corpus and antrum. In those rats given 0.46mM carprofen and 0.1N acid the mucus covering and thickness were again lower. There was some loss of cobblestoning, accentuation of convexity of the cell surfaces and intercellular junctions. The change in the mucus coat was not mirrored in the antrum, though there was a similar change in cobblestoning, convexity of the surface and intercellular junctions (Figures 5.41-5.42).

5.3.3.3 Addition of Bile

The combination of 0.23mM carprofen & 2.5mM bile showed more extrusions, loss of cobblestoning and convexity than normal. Individual cell injury features were all increased, particularly apical erosions. These changes were evident in both corpus and antrum.

Increasing the concentration of bile to 5mM caused a reduction in the mucus covering, an accentuation of the intercellular junctions and convexity of the cobblestones (cells). Damage included increased apical ruffling, holes, erosions, surface concavity and empty shells. The distribution was similar between corpus and antrum, though the severity was more marked in the antrum.

With carprofen at 0.46mM and bile at 5mM mucus covering was reduced. Rosette formation, gastric pit enlargement, loss of cobblestoning, increased convexity and marked accentuation of the intercellular junctions were all visible. In the antrum there was no change in the mucus covering, but cell extrusion was more prominent than rosette formation.

When both bile and carprofen concentrations were increased to 5mM and 0.46mM respectively, mucus covering in the corpus was reduced, though not in the antrum. The gastric pits were enlarged in both areas, as was surface convexity, but there were more rosettes in the antrum (Figures 5.39-5.40). Marked accentuation of the intercellular junctions was clear. Individual damage was pronounced in all the features noted with the exception of intracellular contents.

5.3.3.4 Triple Combinations

Although score 1 from the corpus of those rats given the lower concentration combination, was not statistically different from the normal rats, some features were more pronounced. There was an increase in the number of rosettes detected and there was some loss of cobblestoning with increased convexity of the cells. From score 2, apical ruffling erosions and concavity of the cell surface were prominently increased. In the antrum extrusions, rosettes and generalised convexity were more obvious. More specific change was restricted to increased number of cells with apical erosions and concavities.

When the higher concentrations were used, the changes in the corpus were similar to those at the lower concentrations, but much more marked as far as prominence of rosettes, intercellular junctions and convexity were concerned. In the antrum the mucus covering was reduced and the mucus thinner. There was loss of cobblestoning, increased prominence of the intercellular junctions and marked

convexity of the cell surface. Apical ruffling stood out accompanied by increase numbers of holes, accompanied by apical erosions and empty shells (Figures 5.43).

	Linear erosion/ Apical damage/ SEM score 2					
	Corpus			Antrum		
Group	G	H	SEM	G	H	SEM
Control	-	-	-	-	-	-
Injury						
IA1	-	-	-	-	-	-
IA2	-	-	-	-	+	-
IB1	-	-	+++	-	++	+++
IB2	-	++	++	-	++	+++
IS1	-	-	-	-	-	-
IS2	-	-	-	-	+	-
IR1	-	+	++	-	+	-
IR2	++	-	++	++	++	-
IS1B1	-	+	+	-	+	+
IS2B2	-	+	++	++	+	+++
IR1A1	-	-	++	-	-	++
IR1A2	-	++	+++	-	++	++
IR2A1	-	+	++	-	+	++
IR2A2	-	++	++	-	+	-
IR1B1	-	+	++	-	++	++
IR2B1	-	-	+++	-	-	++
IR1B2	-	-	-	-	-	++
IR2B2	+	-	+++	+	+	++
IR1B1A1	++	-	+	++	+	+
IR2B2A2	+++	-	++	+++	-	+++

SEM = scanning electron microscopy. I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen, G = gross evidence, H = histological evidence, + = 2 rats.

Table 5.28. Comparison of gross erosion and histological evidence of apical damage in rats given injurious agents.

A comparison of the evidence gleaned from gross, light and scanning microscopy for linear erosions/apical damage shows that there was poor correlation between these examinations (Table 5.28). Scanning electron microscopy was the most sensitive method of detecting damage to the apical membrane of the surface epithelial cell.

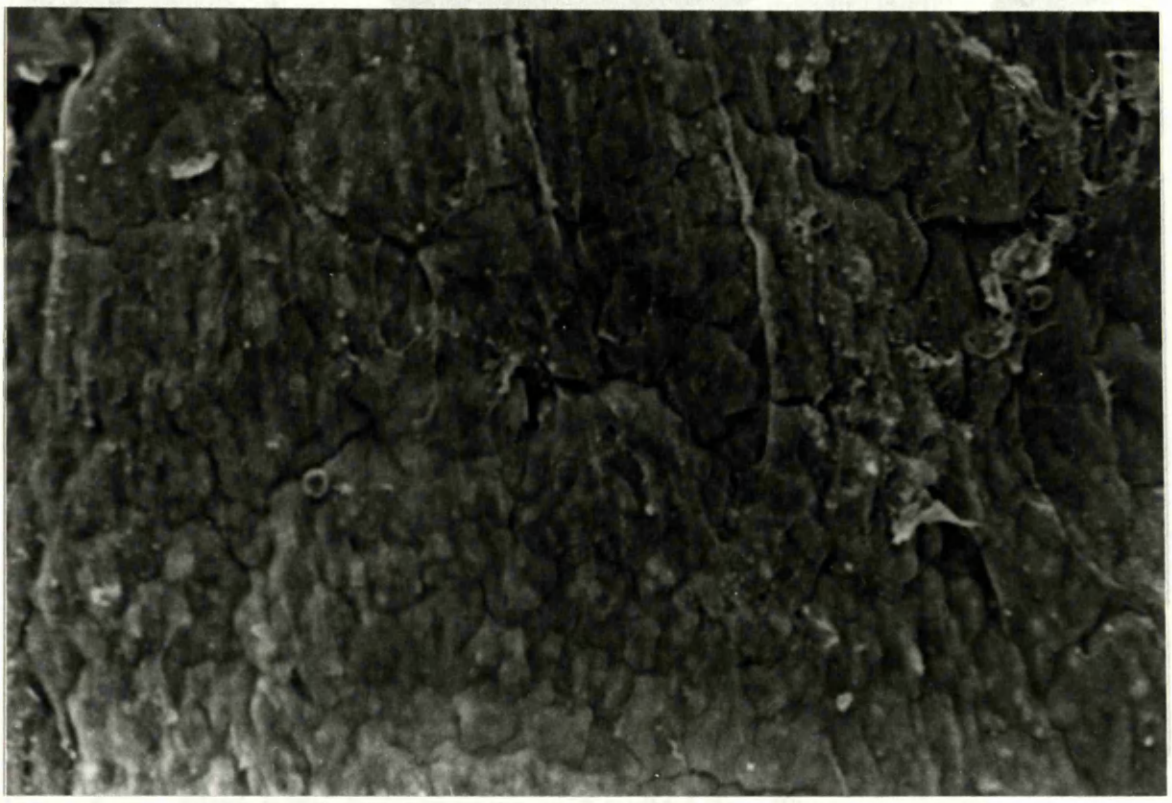


Figure 5.24. Scanning electron micrograph (SEM) following exposure to 0.1N HCl. There is a moderately thick layer of mucus masking the surface epithelial cells. (x360)

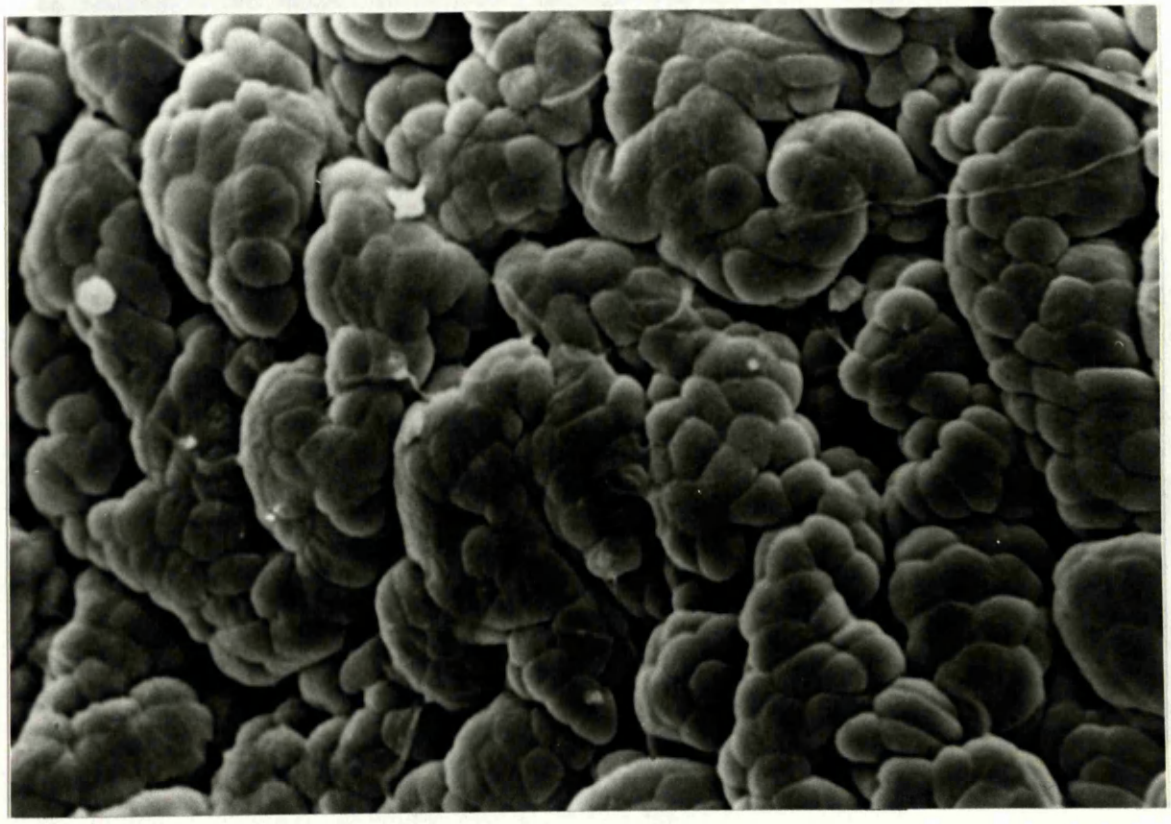


Figure 5.25. SEM following exposure to 0.1N HCl. After 15 minutes there is some flattening of the polygonal cells in the corpus. (x1440)

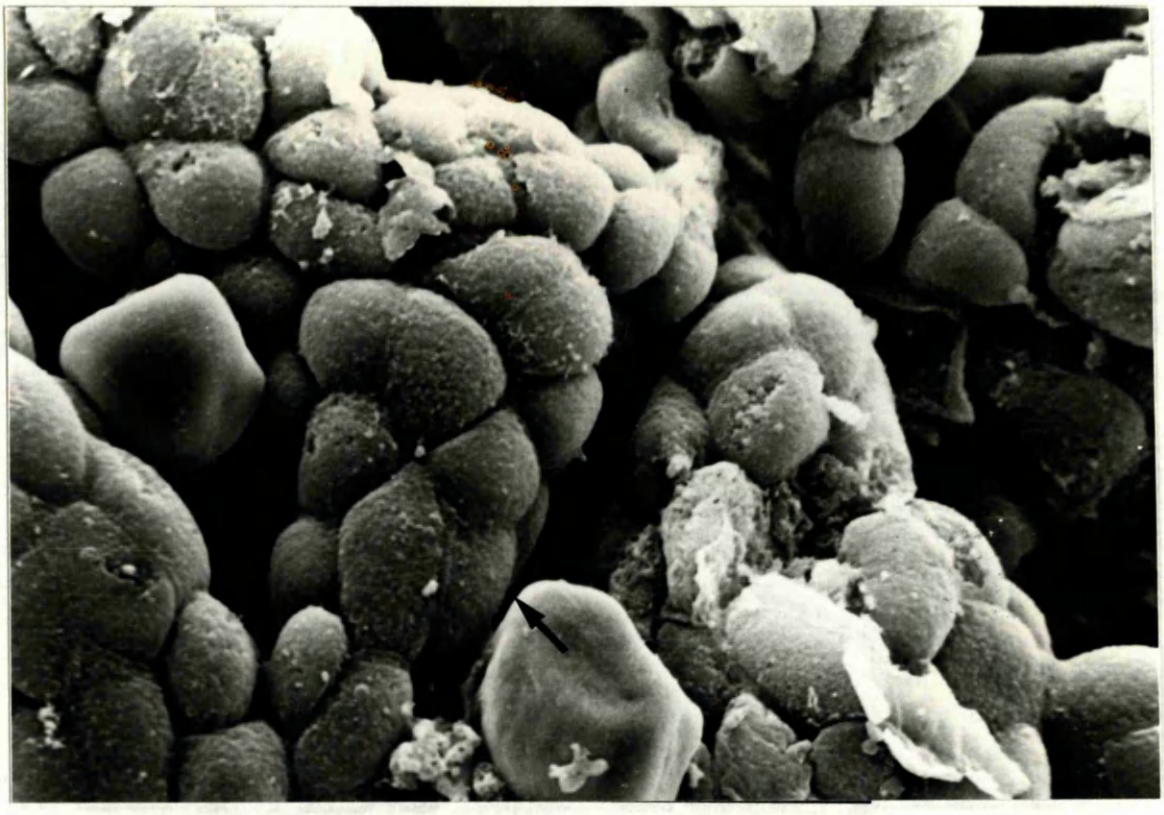


Figure 5.26. SEM following exposure to 2.5mM bile. There is variable swelling of cells some with apical erosions. Large discarded cells lie in the lumen and a rosette is arrowed. Areas of eroded cells are also present. (x2800)

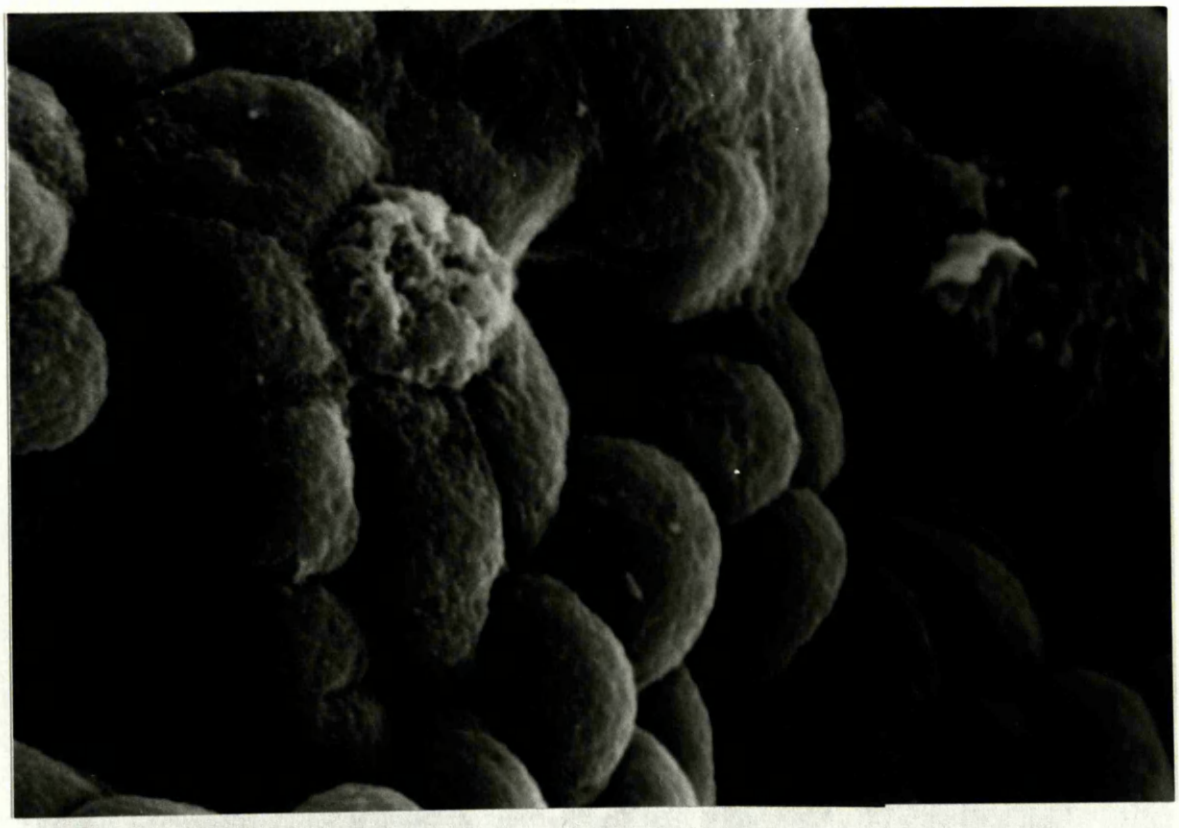


Figure 5.27. SEM following a 5 minute exposure to 2.5mM bile. The cells are swollen and centrally a cell has imploded and is being encroached upon by neighbouring cells. (x5600)

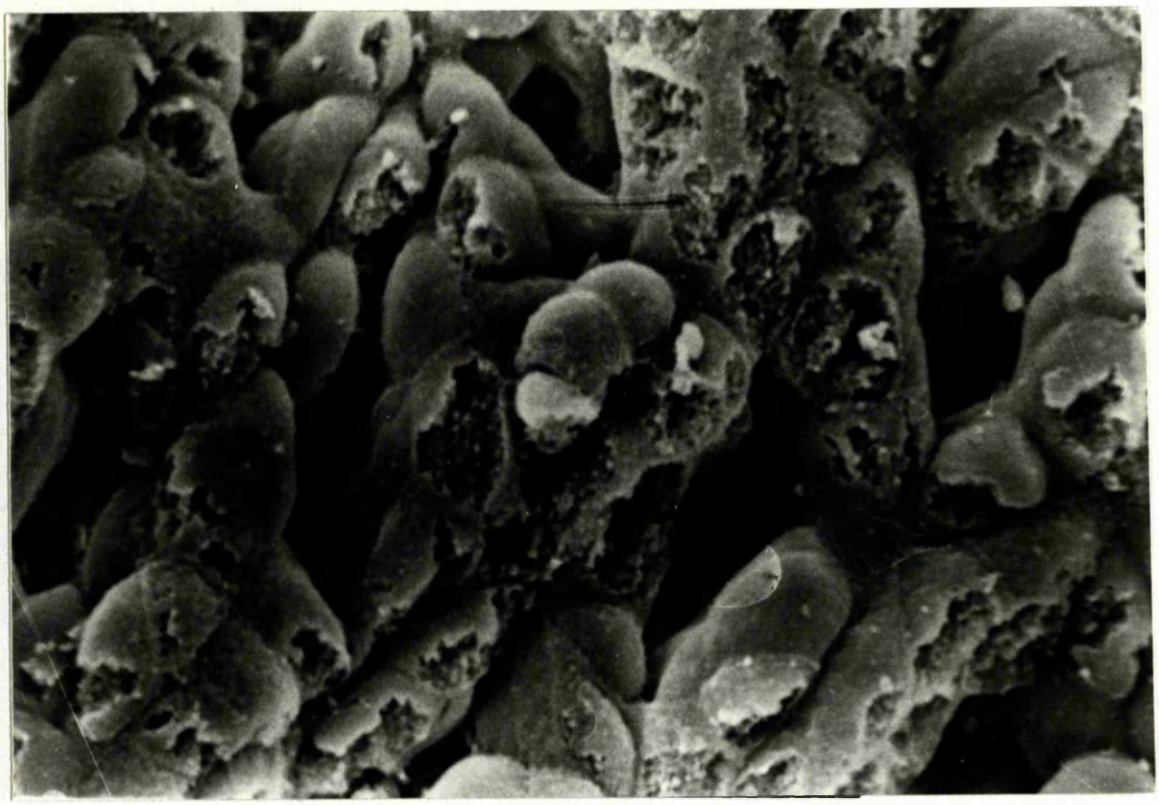


Figure 5.28. SEM following exposure to 5mM bile. After 5 minutes the apical membrane has been lost by many cells exposing intracellular contents. (x2800)

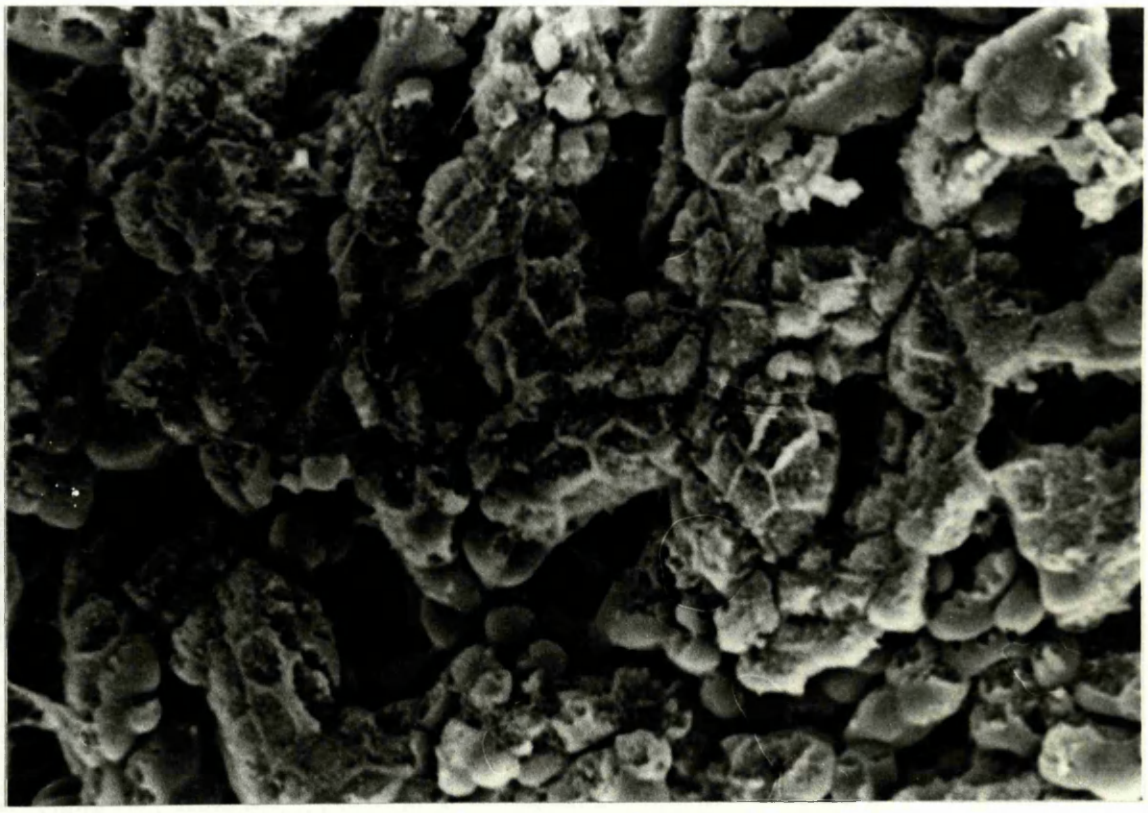


Figure 5.29. SEM following exposure to 5mM bile. After 15 minutes apical membranes have been lost and intercellular junctions are exaggerated. (x1440)

Figure 5.30. SEM following exposure to 20mM aspirin. After 5 minutes there is only some anisocytosis. (x2800)

Figure 5.31. SEM following exposure to 40mM aspirin. A number of cells show apical erosions. Others have lost the apical membrane. (x2800)

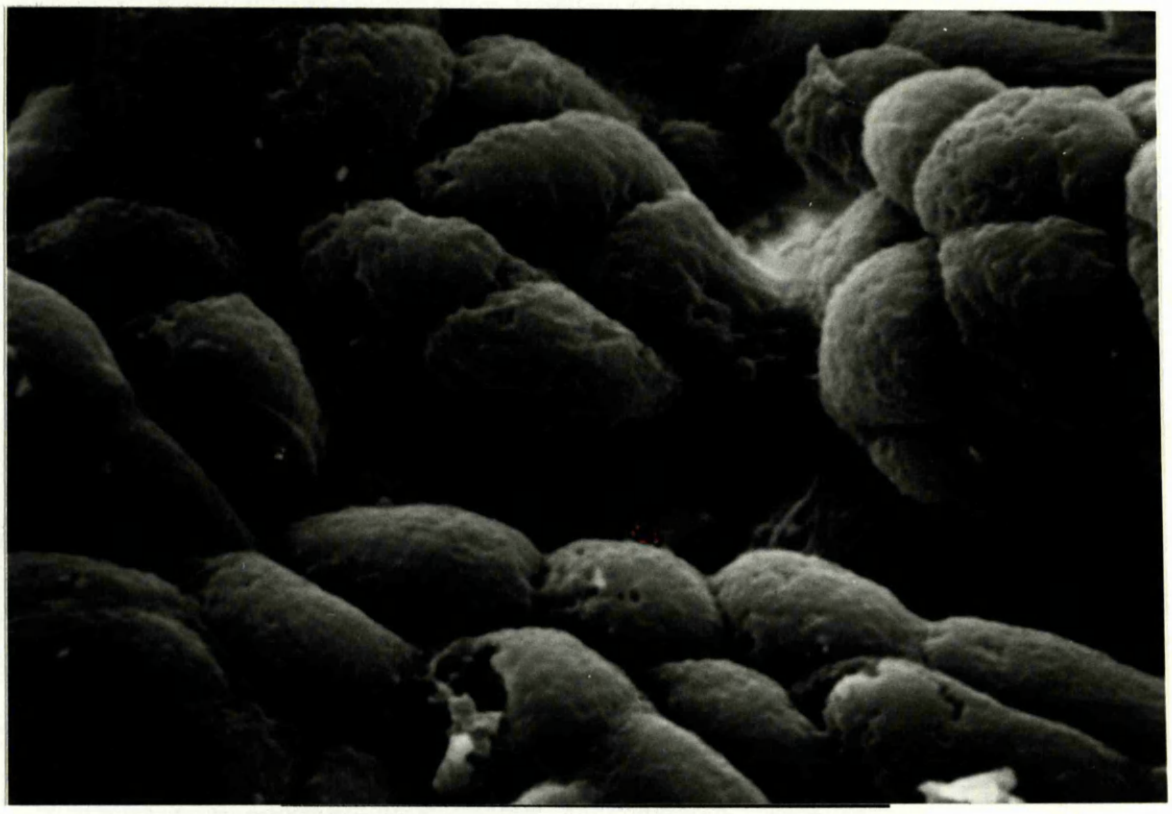


Figure 5.32. SEM following exposure to 0.23mM carprofen. The antral cells are swollen and there is apical ruffling after 5 minutes. (x5600)

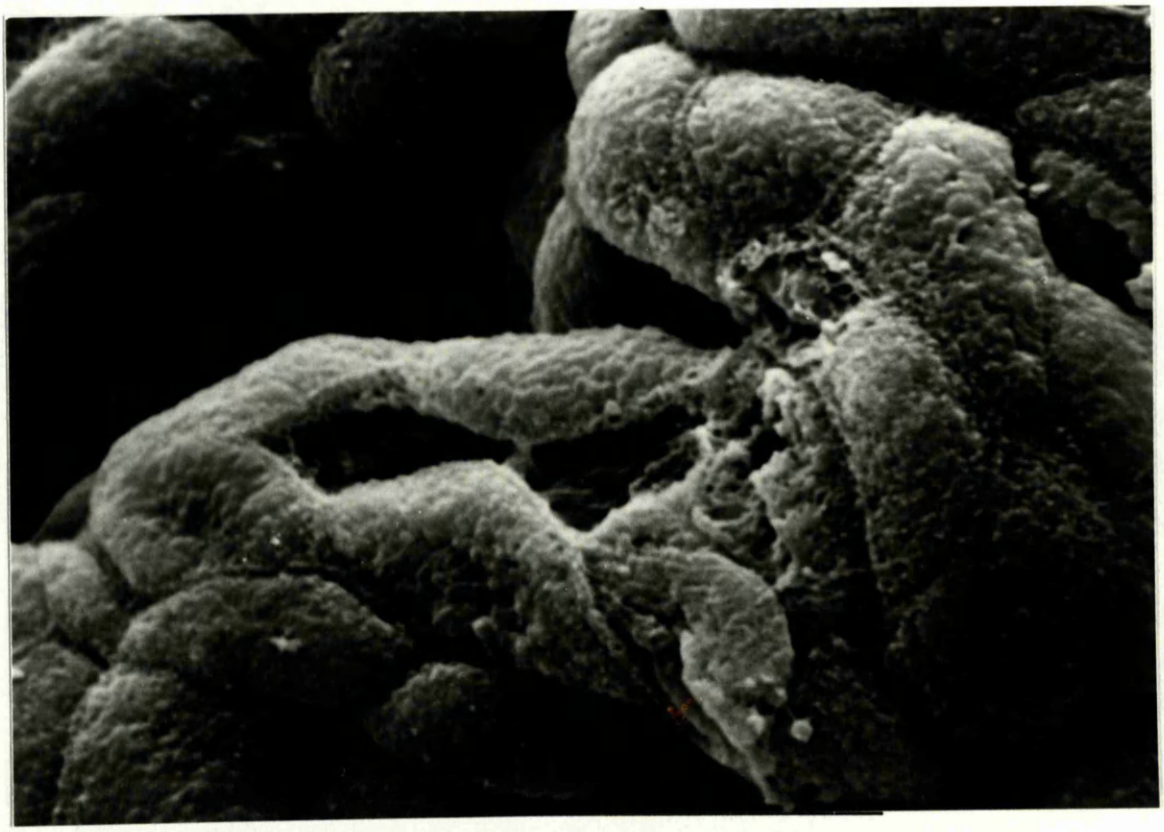


Figure 5.33. SEM following exposure to 0.23mM carprofen. After 5 minutes the corporal cells are swollen with apical ruffling, several present as empty shells. (x5600)

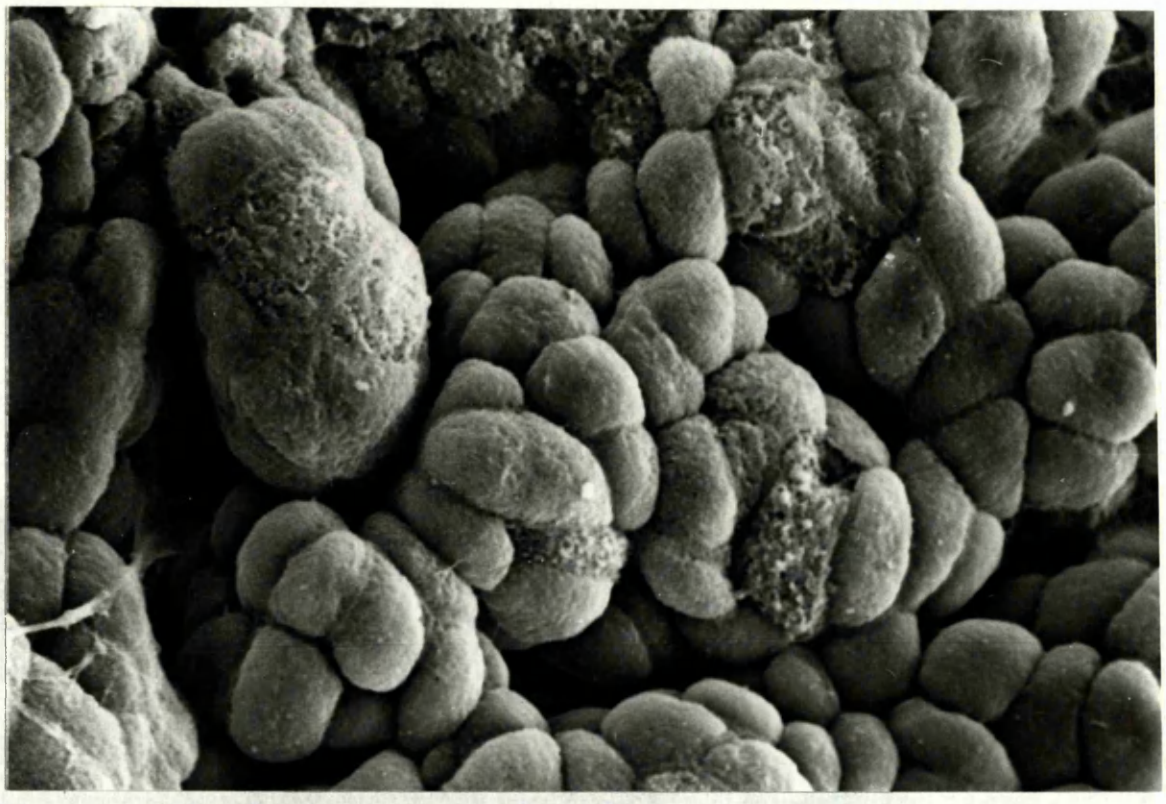


Figure 5.34. SEM following exposure to 0.46mM carprofen. After 2 minutes corporal cells are swollen, some of the apical membranes are peppered with holes and the membrane lost in others. (x2800)

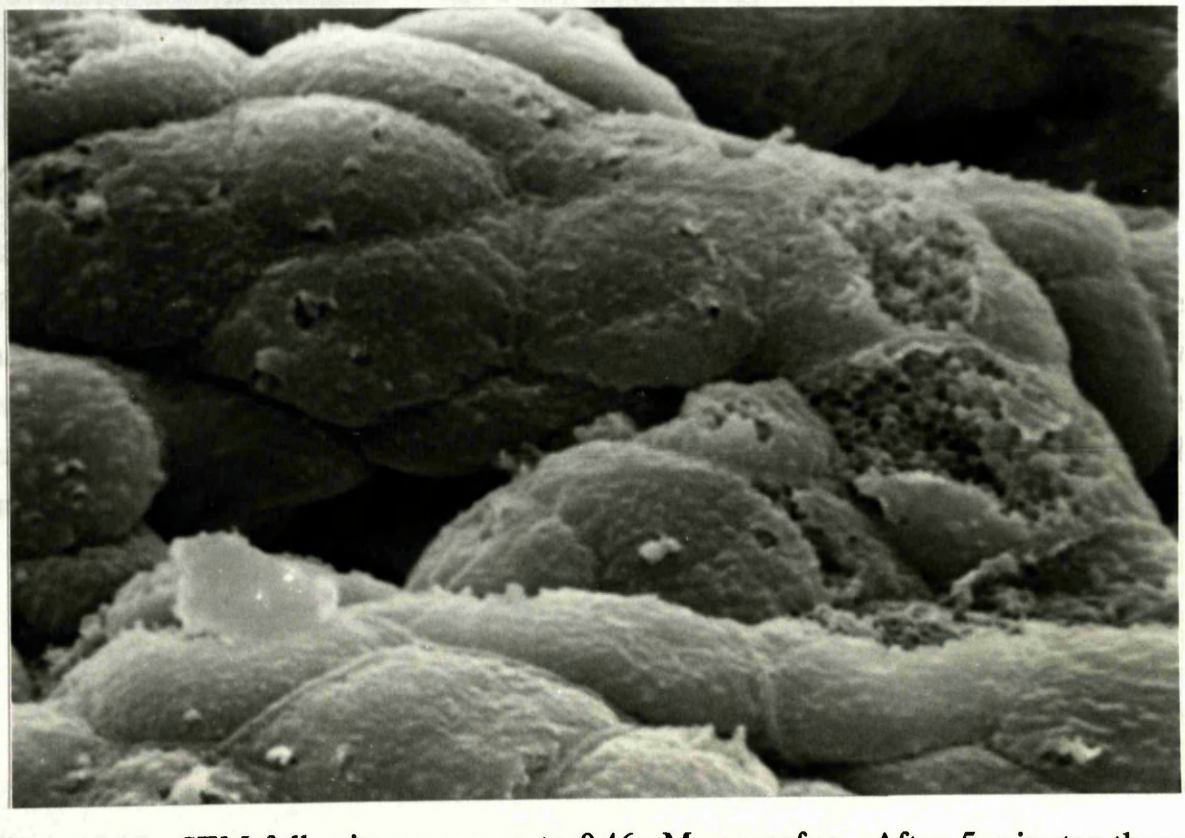


Figure 5.35. SEM following exposure to 0.46mM carprofen. After 5 minutes there are small tears in apical membranes with intracellular contents visible. (x5600)

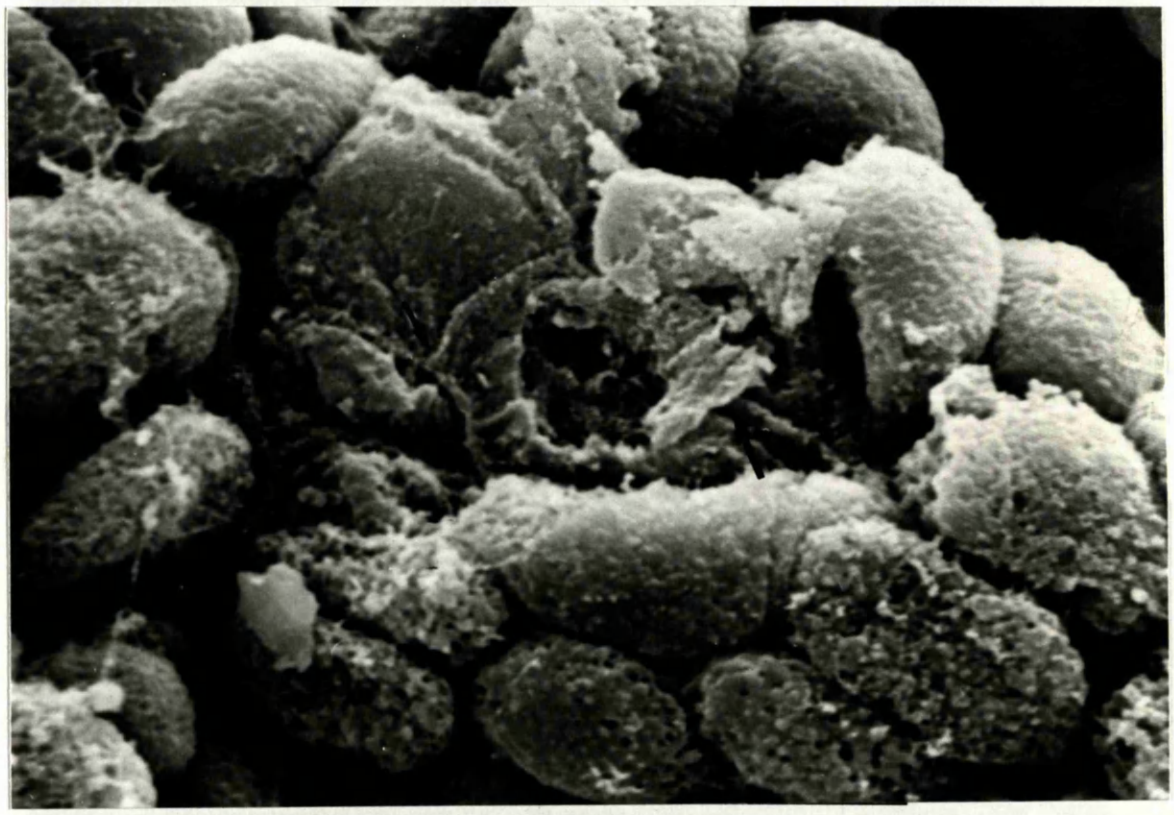


Figure 2.36. SEM following exposure to 0.46mM carprofen. After 15 minutes the antral cells are markedly swollen and distorted. The apical membranes are very ruffled. The intracellular contents are exposed and in one cell the apical membrane can be seen peeled back (*large arrow*). The intercellular junctions are evident (*small arrow*). (x5600)

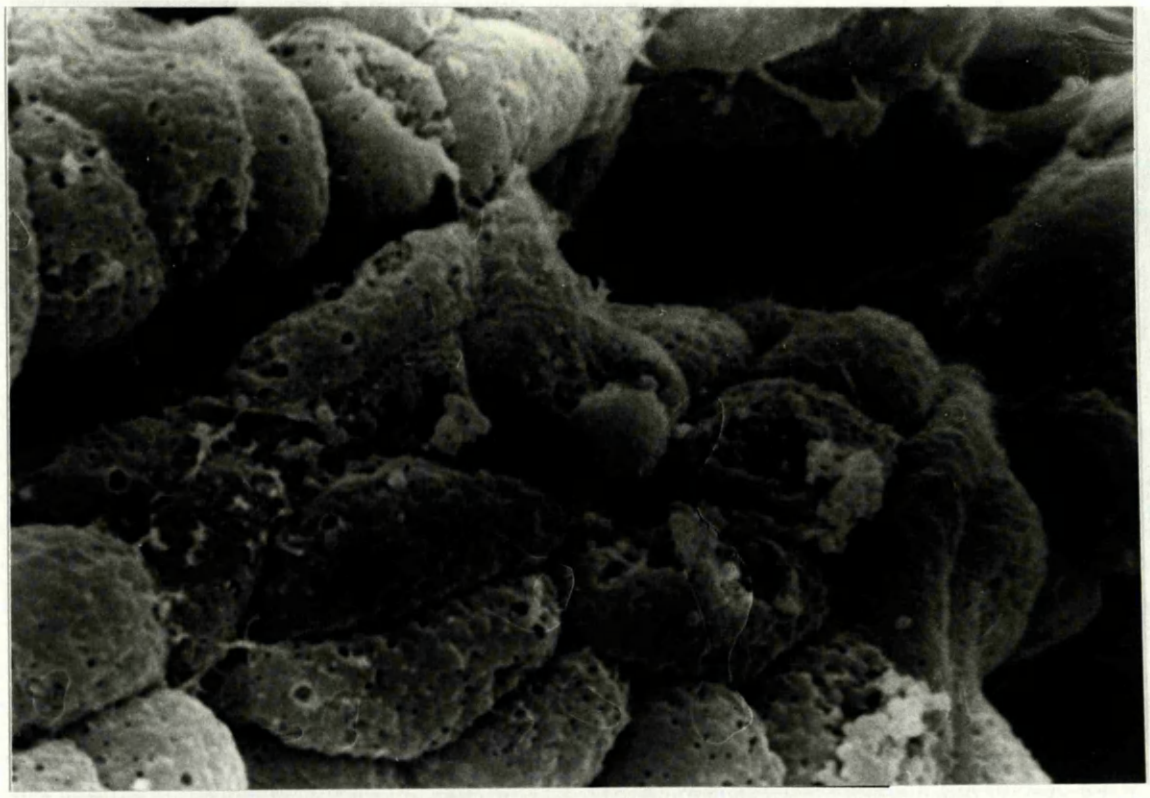


Figure 5.37. SEM following exposure to 40mM aspirin/ 5mM bile. After 15 minutes the corporal cells are enlarged and numerous small holes are present on the apical membrane. (x1440)

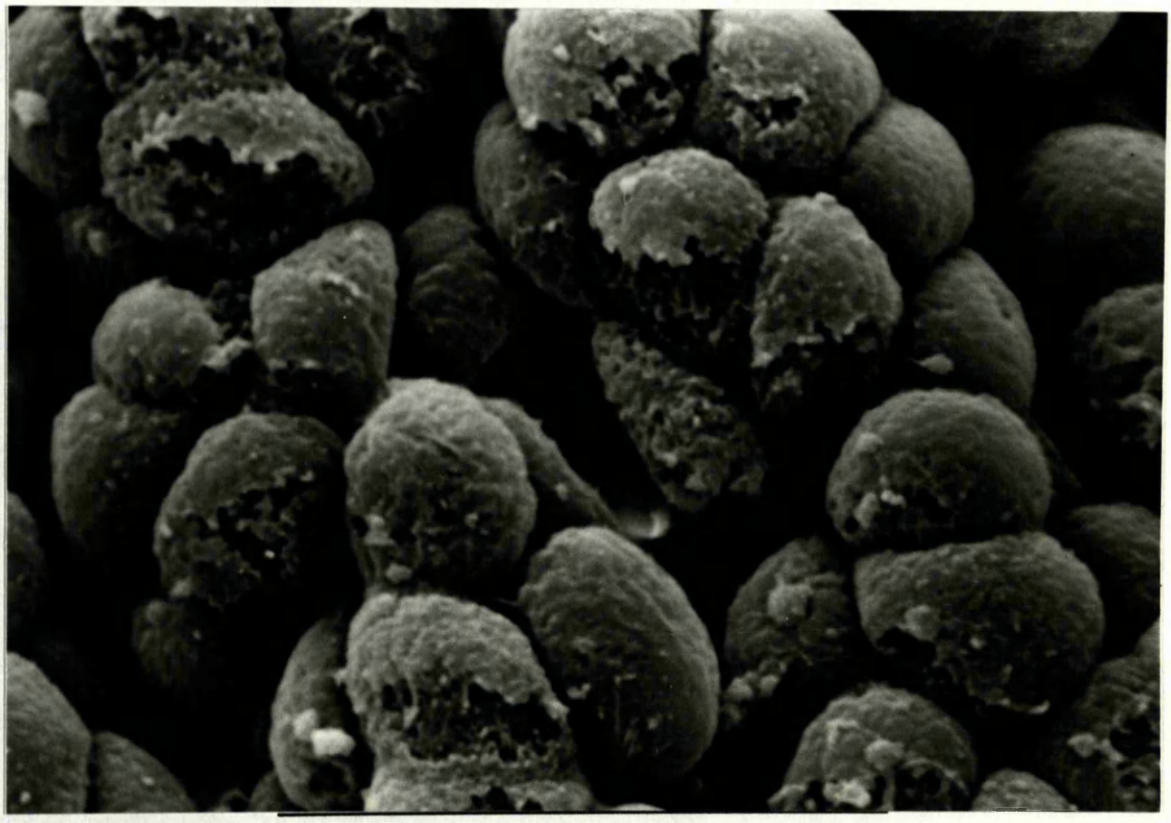


Figure 5.38. SEM following exposure to 40mM aspirin/ 5mM bile. After 15 minutes many antral cells are more convex than normal and the intracellular contents are exposed. (x5600)

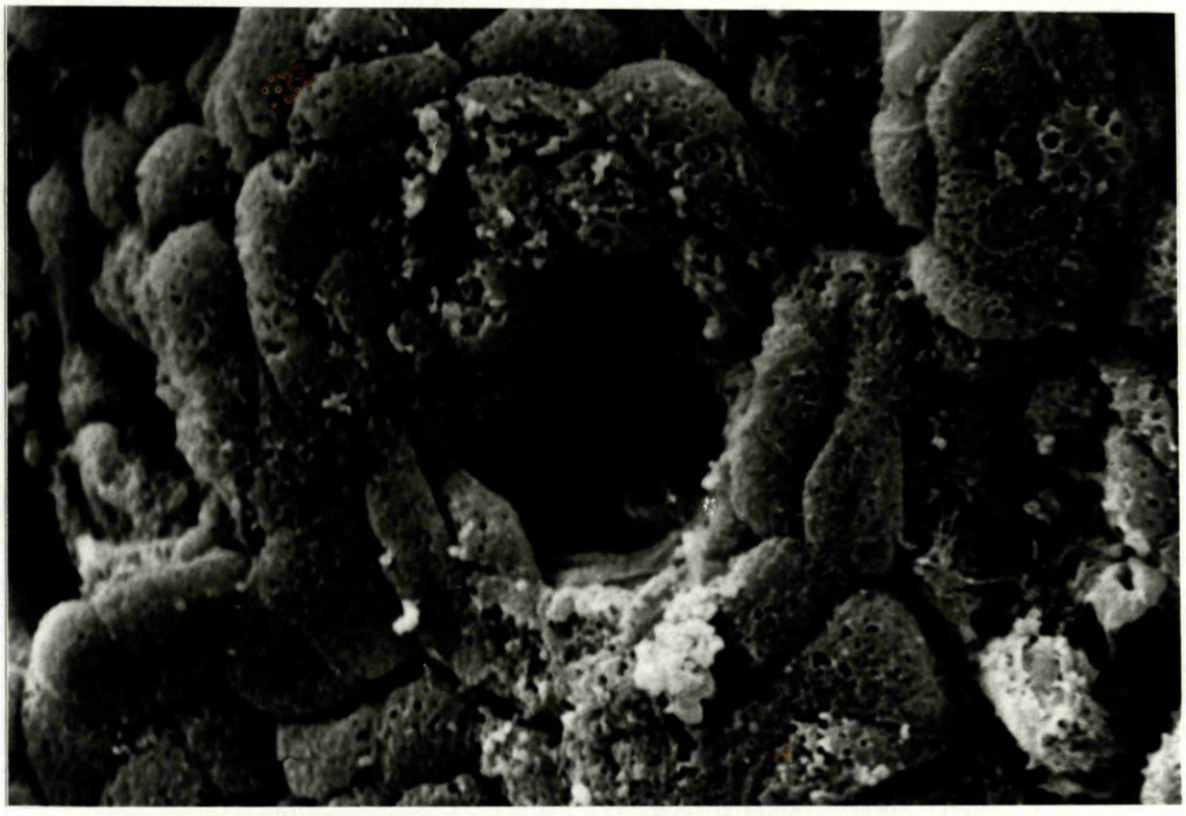


Figure 5.39. SEM following exposure to 0.46mM carprofen/ 5mM bile. After 15 minutes the corporal cells have extensive apical erosions. (x2800)

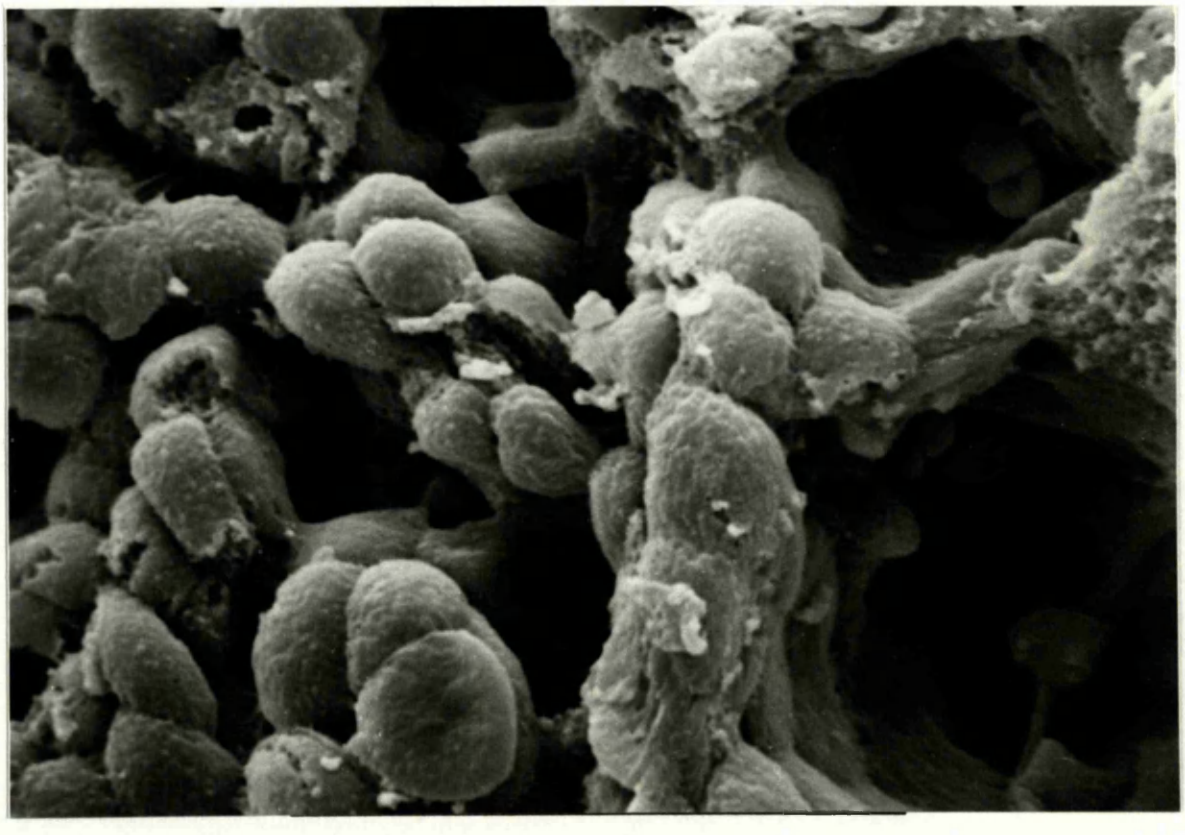


Figure 5.40. SEM following exposure to 0.46mM carprofen/ 5mM bile. After 15 minutes the antral cells are distorted and show apical damage. The normal cobblestone appearance is lost. (x2800).

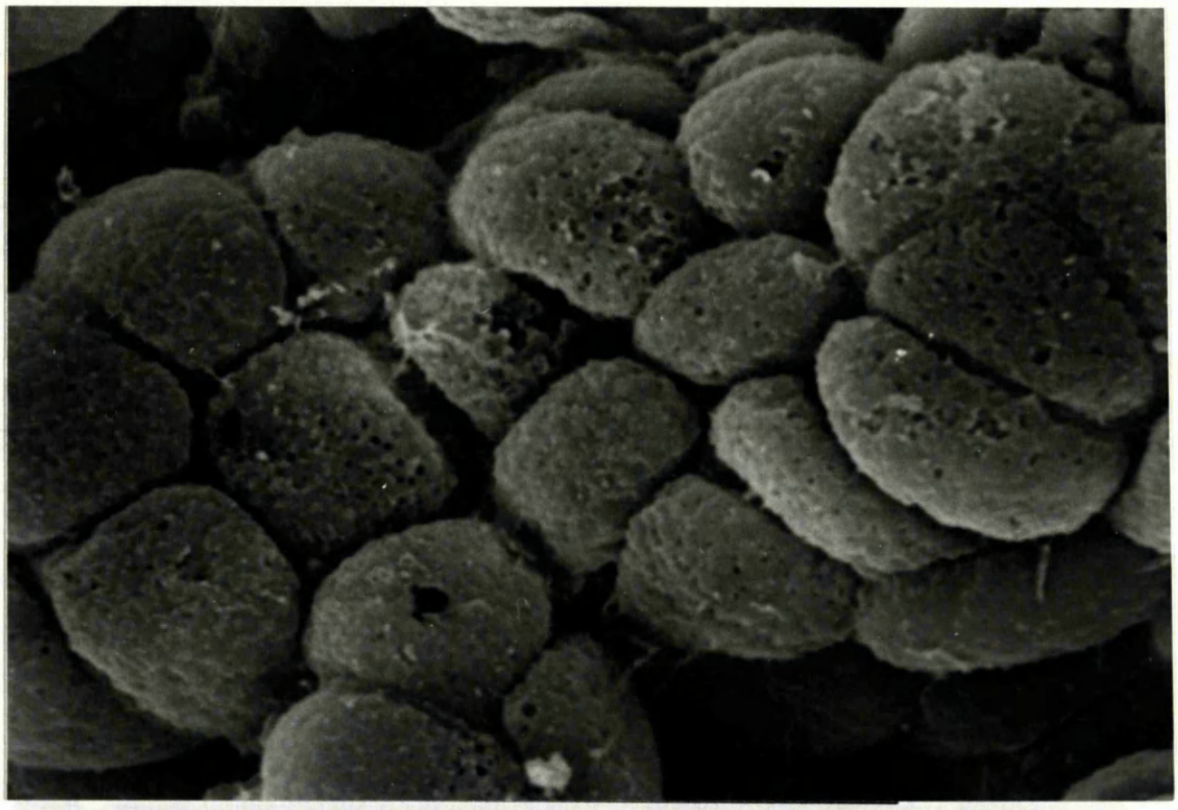


Figure 5.41. SEM following exposure to 0.23mM carprofen/ 0.1N HCl. The cells show large numbers of apical erosions.(x5600)

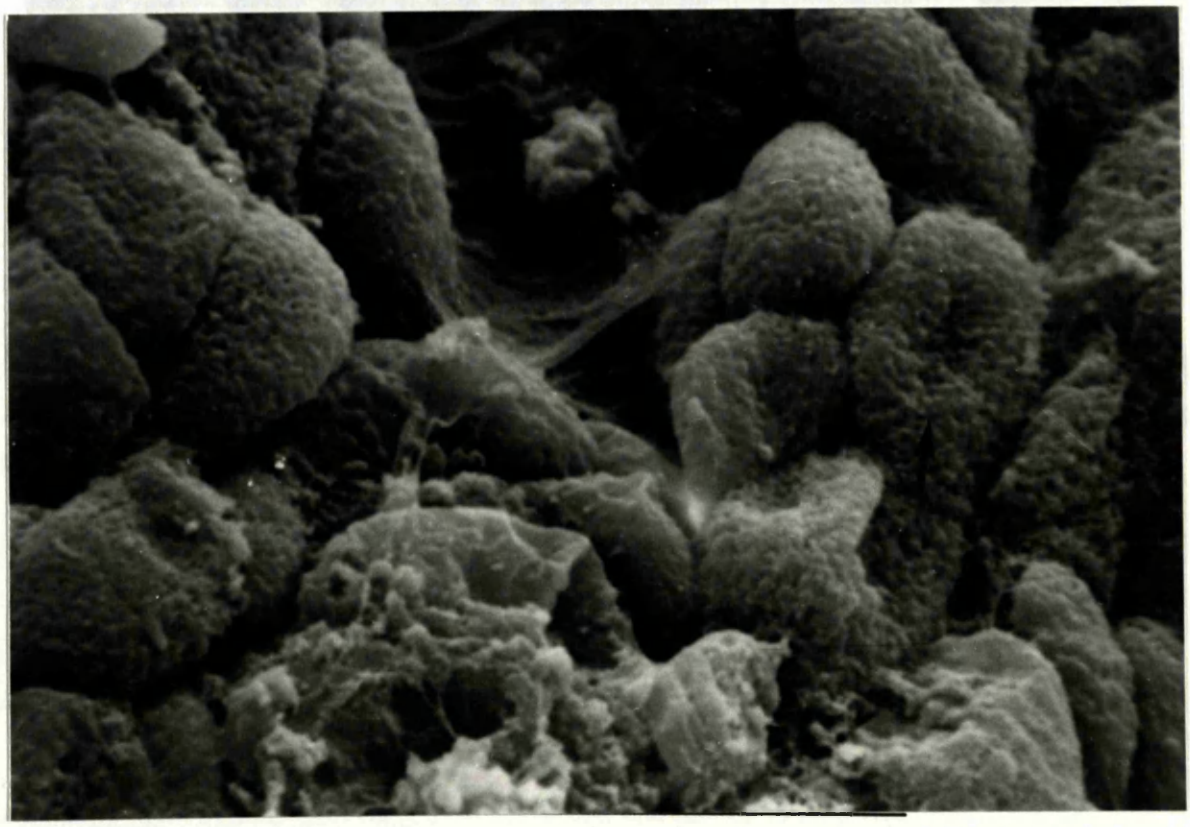


Figure 5.42. SEM following exposure to 0.23mM carprofen/ 0.1N HCl. After 15 minutes the cells are swollen with marked ruffling of the apical cells. One cell has a concave apical membrane (arrow). (x5600)

Figure 5.43. SEM following exposure to 0.46mM carprofen/ 0.1N HCl/ 5mM bile. After 15 minutes the intercellular junctions are extremely prominent. The apical membranes appear to be collapsed. (x5600)

5.3.4 Transmission Electron Microscopy

The surface epithelial cell structure and integrity of those rats given acid were similar to those found in the normal rats (Figure 5.44). In the rats given carprofen or aspirin the change was characterised at 5 minutes by numbers of cells showing swelling and a marked decrease in cytoplasmic and nuclear density, with cytoplasmic vacuolation. The apical mucous granule packs were dissipated but the junctional complexes were intact. Many of these abnormal cells abutted relatively normal looking neighbouring surface epithelial cells (Figures 5.45 and 5.46). At 15 minutes, those cells showing decreased cytoplasmic density appeared to have imploded with apical membrane rupture with more marked loss of mucous granules. The nuclear chromatin was more marginated (Figure 5.47). Bile produced essentially similar change, though in places the severity of cell disruption was more marked (Figure 5.48). The combination of acid or bile with carprofen or aspirin caused more extensive and more severe damage to the surface epithelial cells. This progression of damage was most pronounced where the nsaid was paired with bile (Figures 5.49-5.52).

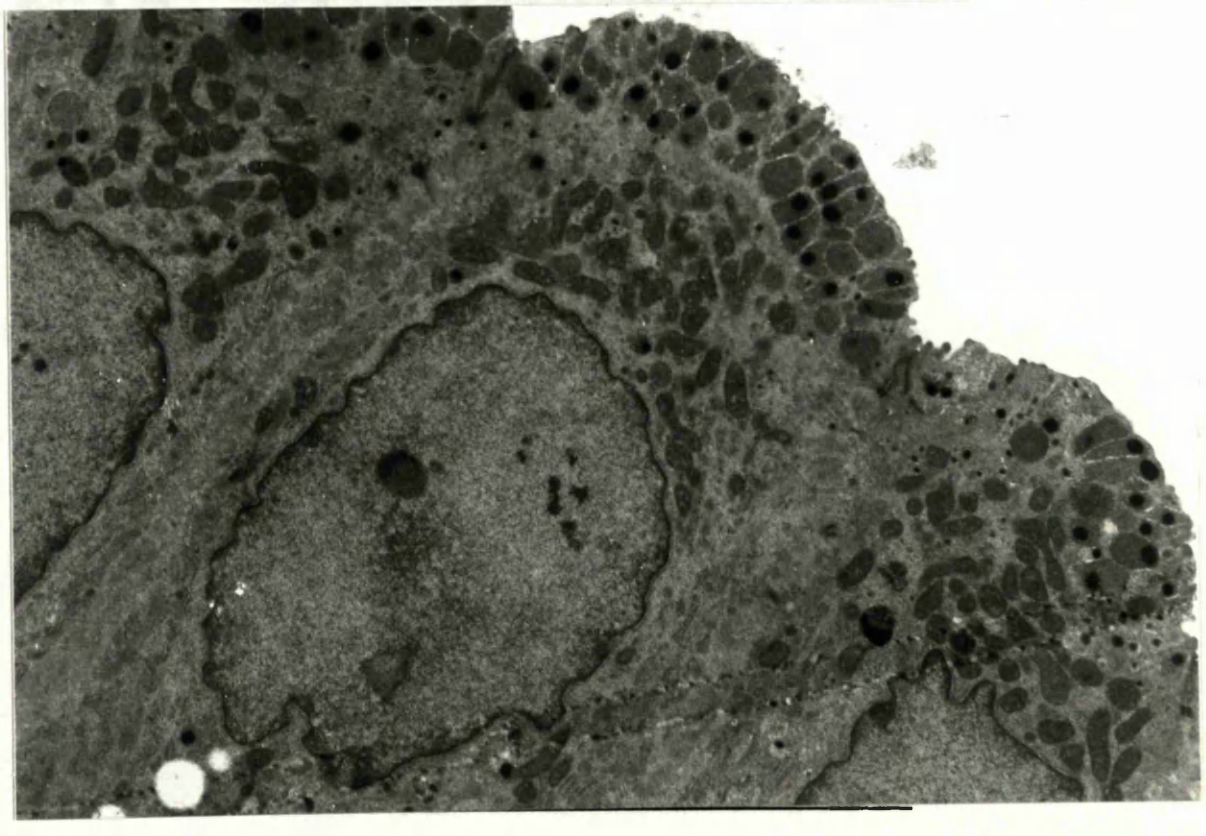


Figure 5.44. TEM of surface epithelium following exposure to 0.1N HCl. The distribution of organelles, nucleus, cytoplasmic density, junctional complex and mucous granule pack are normal (x8000).

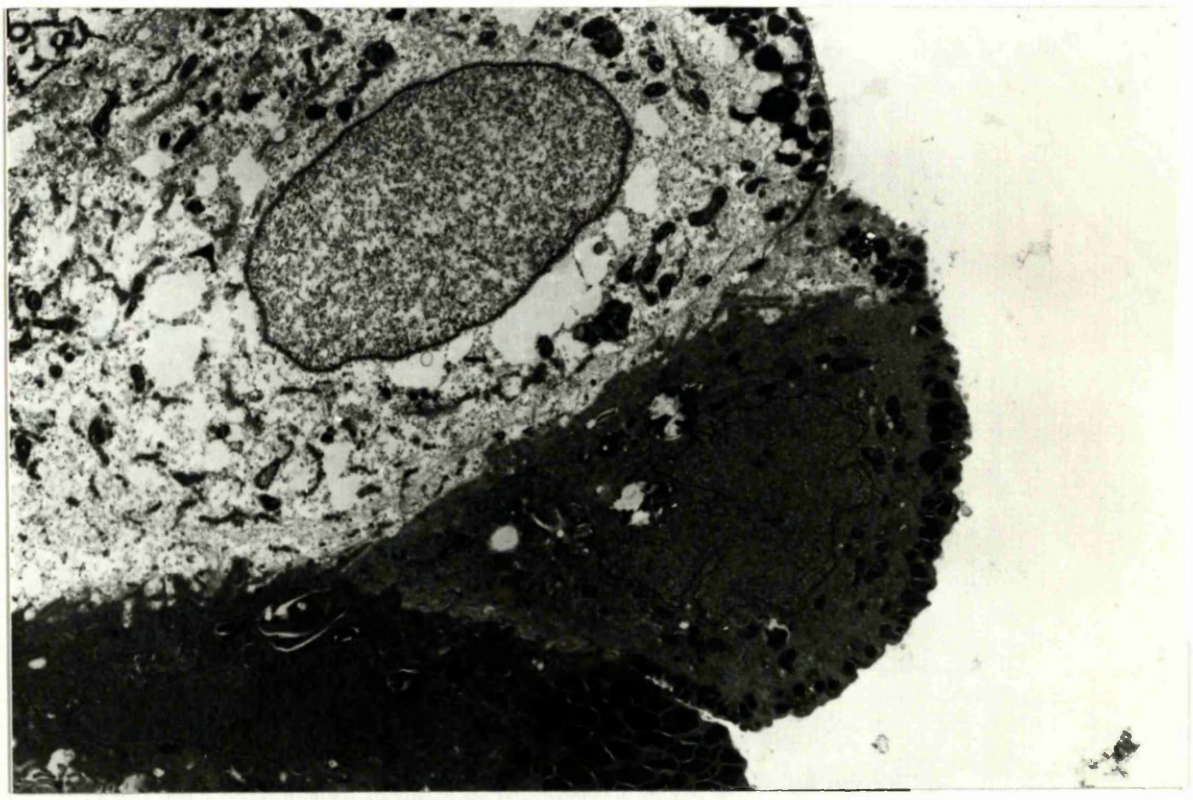


Figure 5.45. TEM of surface epithelial cells following 5 minutes exposure to 40mM aspirin. The cell shows intracellular swelling with a decrease in cytoplasmic. There are organelle remnants dotted throughout and a loss of apical mucous granule aggregations (x5400).

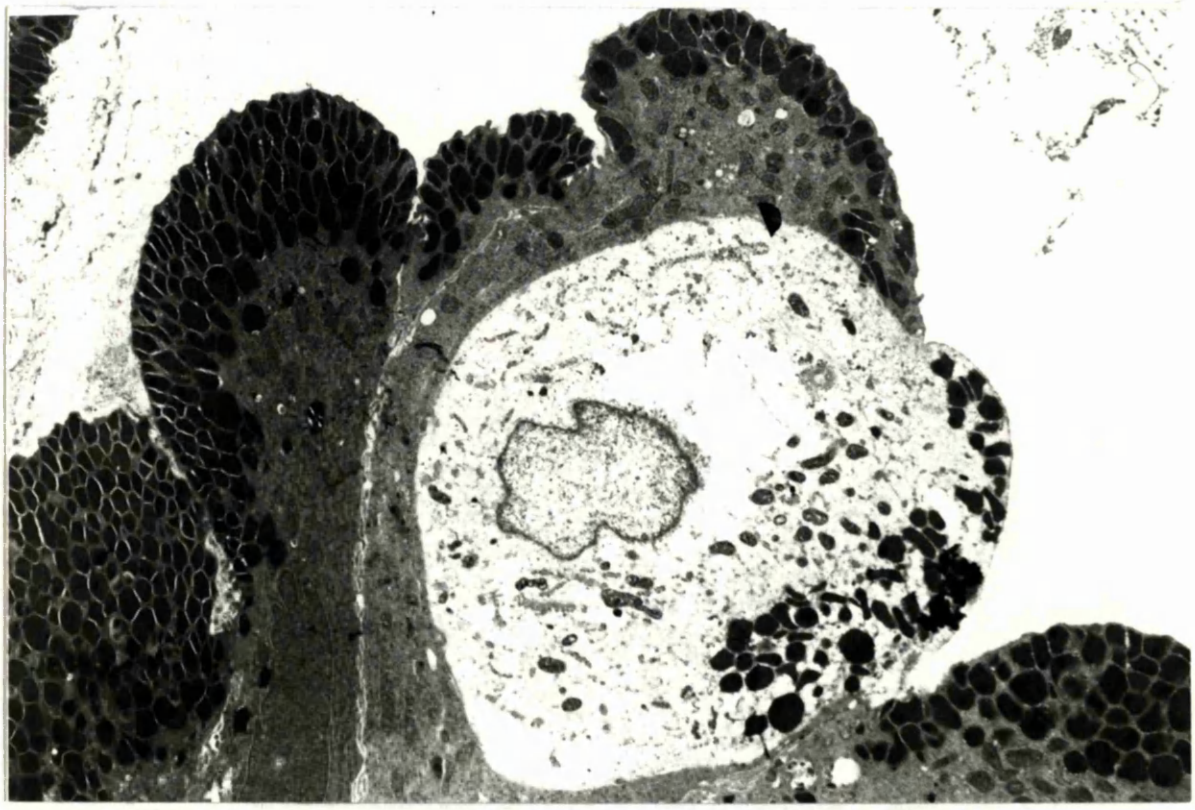


Figure 5.46. TEM of surface epithelial cells following 5 minutes exposure to 0.46mM carprofen. The cell shows intracellular swelling with a decrease in cytoplasmic density. There are organelle remnants dotted throughout and a loss of apical mucous granule aggregations. The junctional complex with the adjacent cell is normal, but this cell also shows some decrease in cytoplasmic density with a few cytoplasmic vacuoles formed. There is only a thin line of mucous granules beneath the apical membrane (x5400).

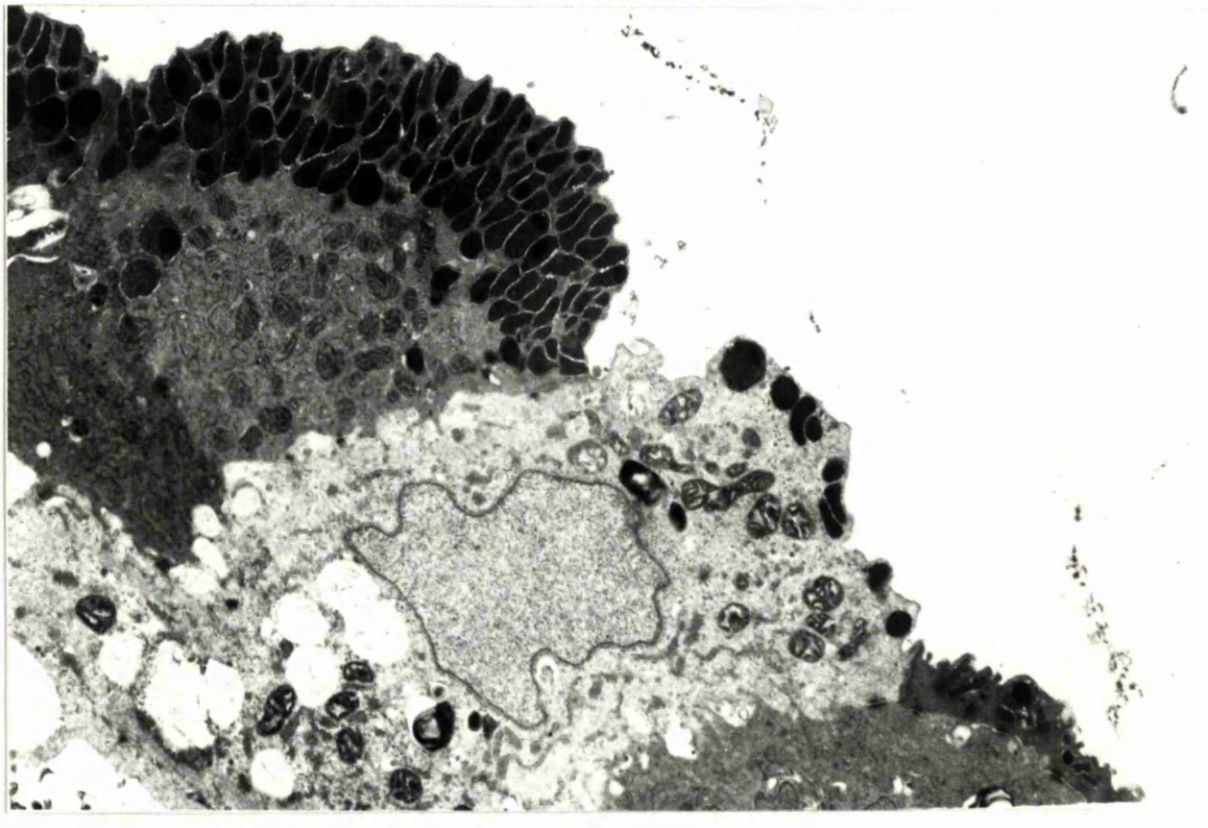


Figure 5.47. TEM of surface epithelium following 15 minutes exposure to 0.46mM carprofen. There is surface debris beneath which is a collapsed cell showing loss of cytoplasmic density and vacuolation. The apical membrane appears disrupted and there are few mucous granules present (x8000).

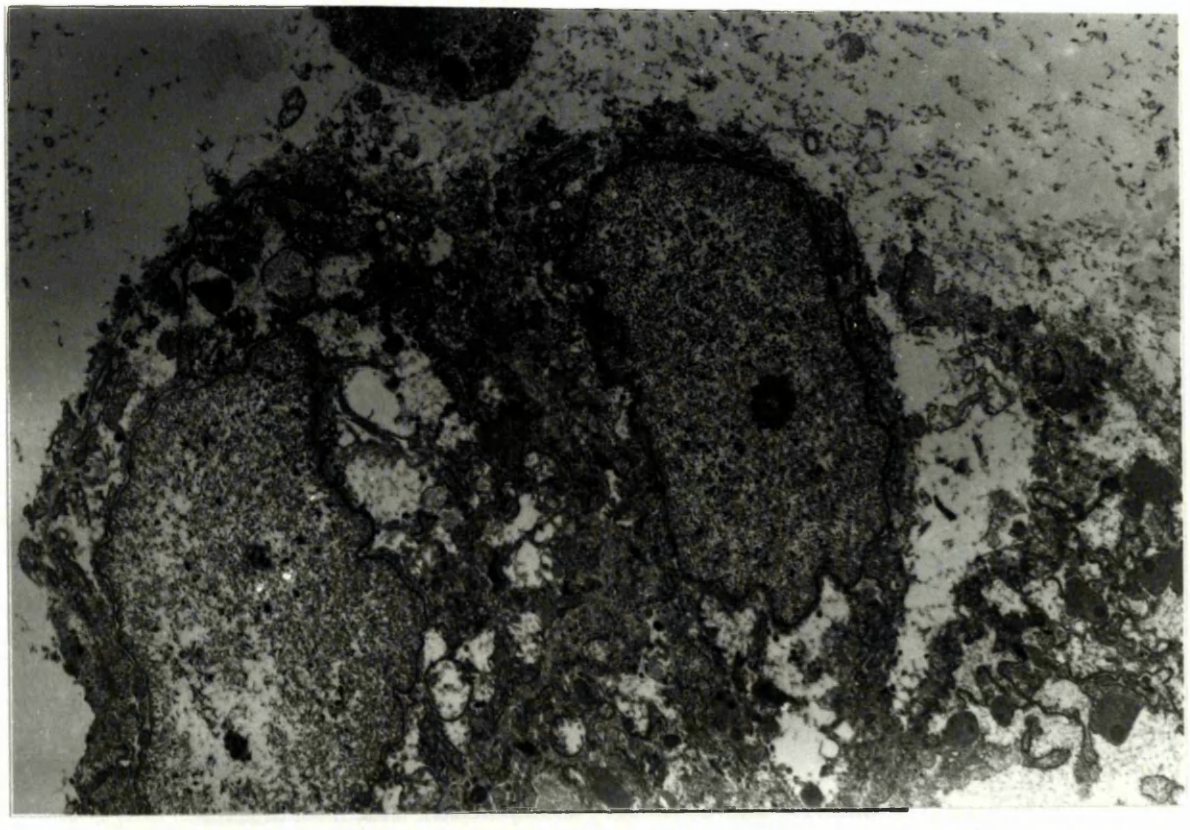


Figure 5.48. TEM of surface epithelium following 5 minutes exposure to 5mM bile. The nuclei seem relatively unharmed, but there is extensive disruption of the cytoplasm, and absence of the apical membrane and mucous granules. The cytoplasm appears to be confluent with the lumen (x8000).

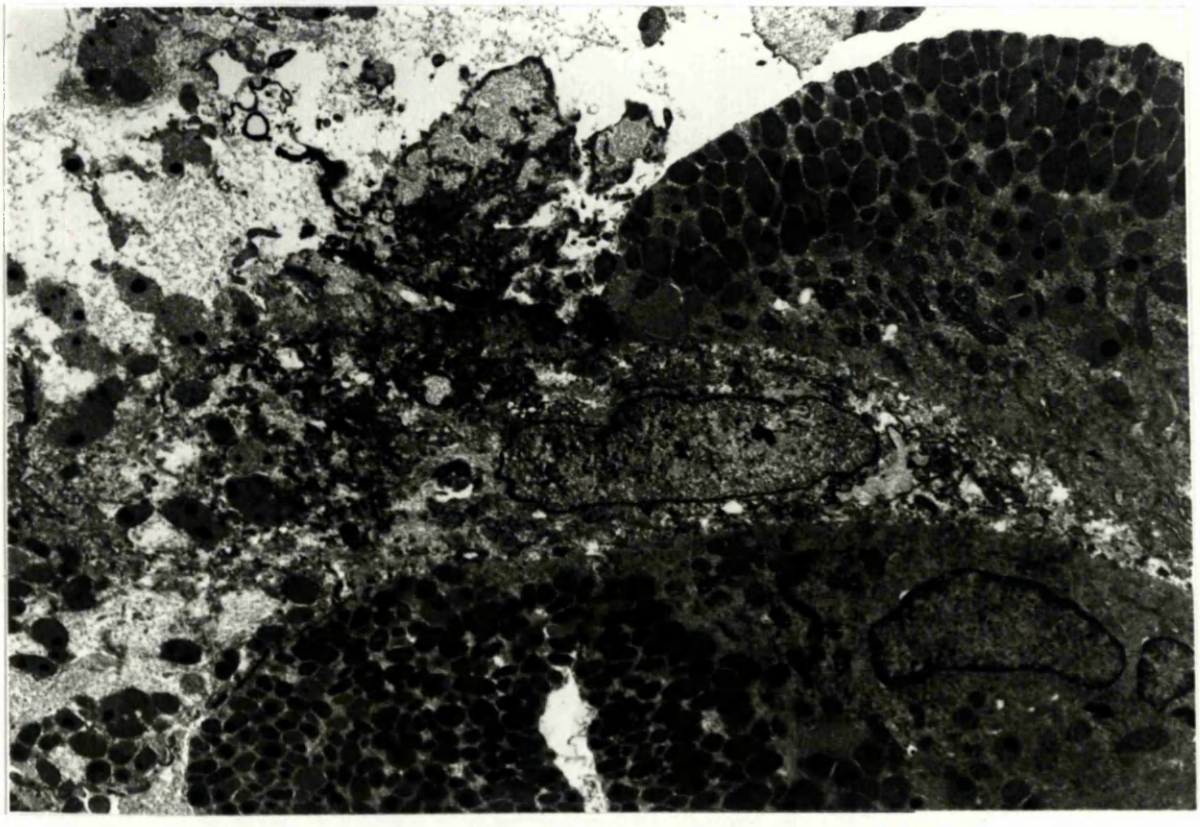


Figure 5.49. TEM of surface epithelium following 5 minutes exposure to 40mM aspirin/5mM bile. The cytoplasm is disorganised, has lost its homogeneity and appears to have exploded into the lumen. The neighbouring cells appear to be encroaching to close this defect in the epithelial integrity (x5400).

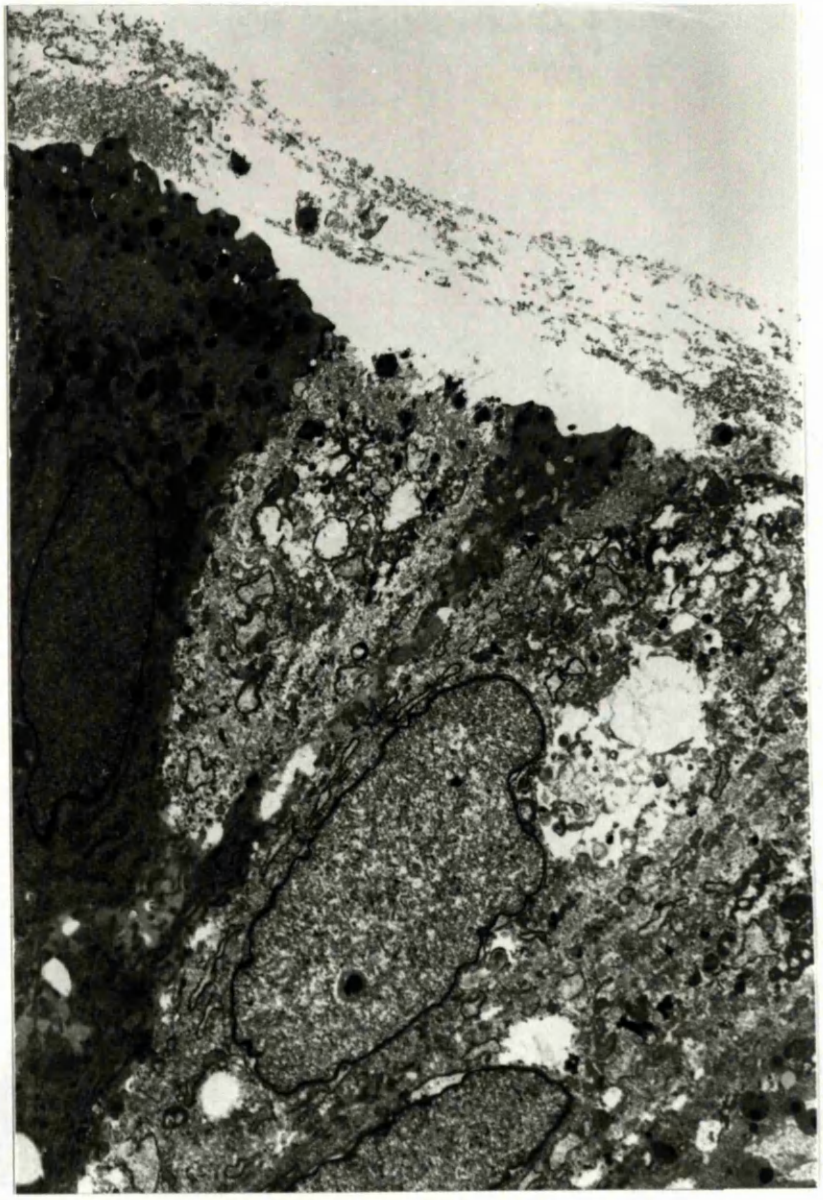


Figure 5.50. TEM of surface epithelium following 15 minutes exposure to 0.46mM carprofen/0.1N acid. There is a thick acellular lumenal layer covering the cells. Several cells are seen to have been disrupted *in situ*, though the junctional complexes are still visible and intact. The one relatively normal cell has lost the bulk of its mucous granules (x5400).

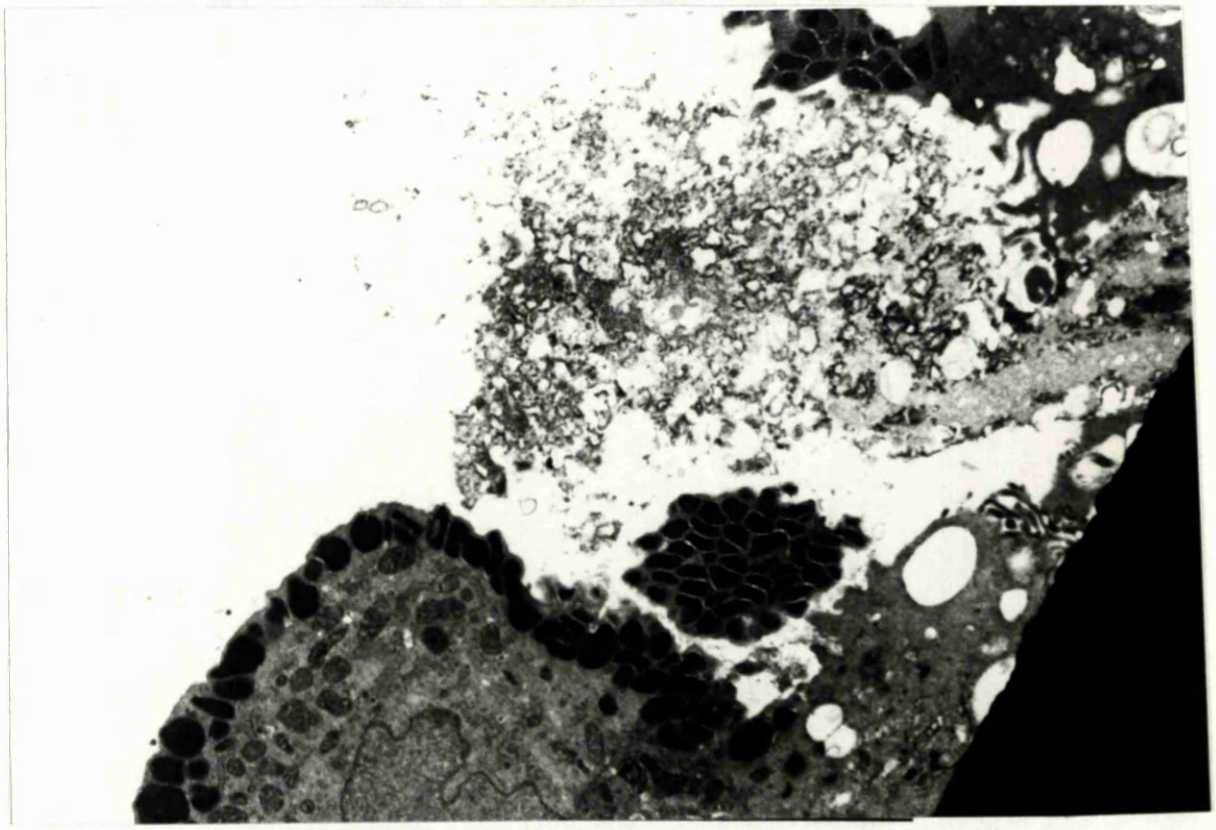


Figure 5.51. TEM of surface epithelium following 5 minutes exposure to 0.46mM carprofen/5mM bile. The apical membrane has been stripped away exposing the cytoplasm to the lumen. The cytoplasm shows numbers of large vacuoles and a decrease in density. The adjacent cell, whilst still having an apical membrane, shows a loss of mucous granules with the remaining granules forming as thin peripheral layer (x8000).

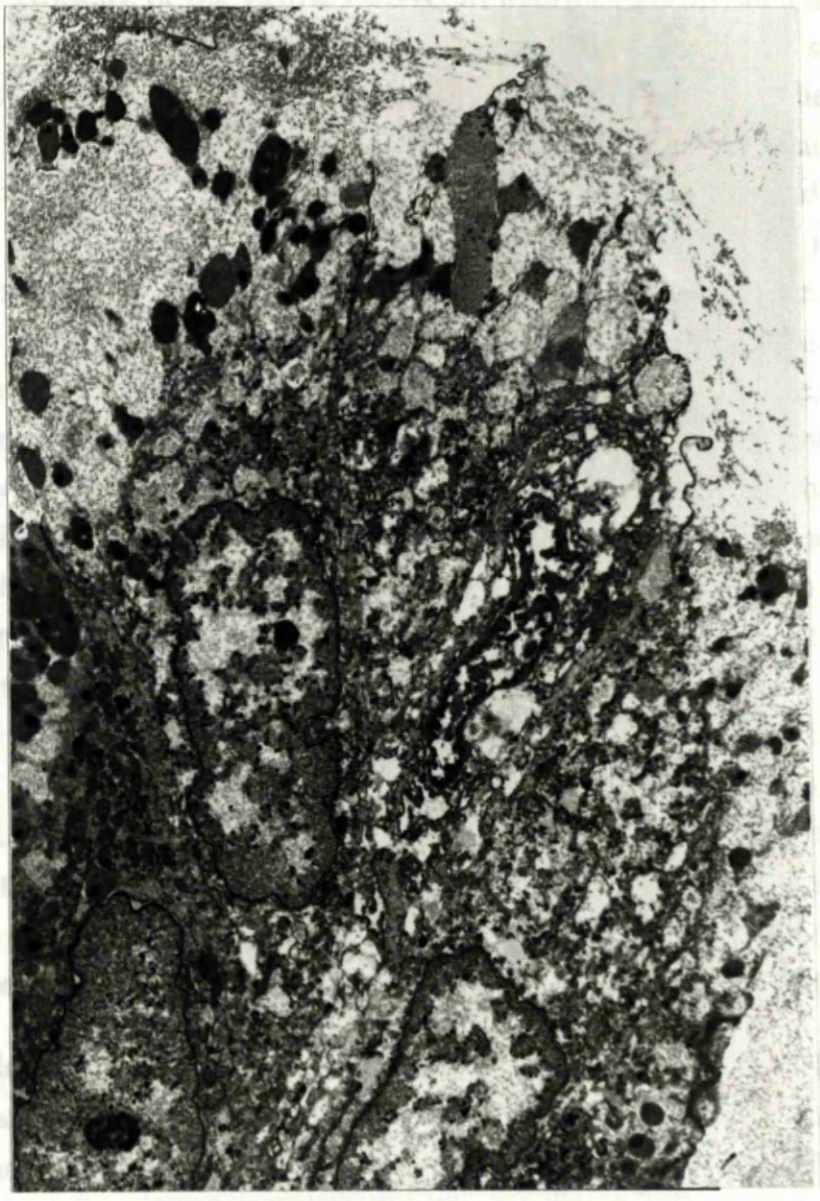


Figure 5.52. TEM of surface epithelium following 15 minutes exposure to 0.46mM carprofen/5mM bile. Extensive destruction and damage to a number of cells is visible. The junctional complexes still appear to be intact (x5400).

5.4 Discussion

In the rat the exposure of the gastric mucosa to injurious agents such as aspirin and ethanol has been reported as inducing reddening and erosions of the surface within two minutes of application (Morris and Harding 1974, Szabo 1987). The reddening is caused by an increase in blood flow and a reduced venous return resulting in stasis and congestion in the post-capillary blood vessels (Guth 1984, Pihan and others 1986). An increase in blood flow to the gastric mucosa can be induced by the release of histamine arising from cell damage (Szabo 1987). Grossly visible erosions are considered to represent defects in the epithelial layer. Pin-point areas of reddening or erosion may also be the result of submucosal petechial haemorrhage, which occurs quite commonly in man following administration of aspirin (Cohen and MacDonald 1982). If more necrotising agents, such as ethanol, are used then haemorrhaging erosions develop and even ulceration with sloughing of large areas of the mucosa. In these cases the colour of the surface varies between a deep red to almost black (Tarnawski and others 1985a). Similar changes can occur in the gastric mucosa as the result of other injurious agents - examples are dogs treated with NSAID drugs (Chvasta and Cooke 1972, Ewing 1972), and in man and dog with bile (Byers and Jordan 1962, Davenport 1968, Kivilaakso, Fromm and Silen 1978, Houghton and others 1986, Lipowitz, Boulay and Klausner 1986). However, changes of a superficial nature are not likely to be visible to the naked eye (Lacy 1987) where there is no focal accumulation of erythrocytes. Further, minimally injured cells may not be detectable by light microscopy (Parisio and Clementi 1976, Schmidt and others 1985).

The distribution of lesions throughout the stomach can be variable. Reports in the literature tend to suggest that the corpus region is more often affected than the antrum (Bommelaer and Guth (1979). One explanation suggested is that there are many more parietal cells present in the corpus than in the antral region of the stomach (Berger 1934, Read and Johnstone 1961, Ritchie, Barzilai and Delaney 1966). Thus the greater energy and oxygen demands required for acid production may leave the corpus more prone to attack that impairs the blood flow (Cheung and others 1978, Fromm 1981). However, parietal cells induce a lowering in pH by the production of acid, and in view of the evidence that active acid secretion protects the mucosa one might expect that the antrum would be more easily damaged than the corpus (Fromm, Silen and Robertson 1976, Bugat and others 1976, Smith and others 1977). Furthermore, the antrum is more permeable and thus will absorb more H^+ ions than the corpus, again making it more prone to back-diffusion induced damage (Dyck and others 1969). On the other hand, Davenport (1967a) and Whittle (1976) found that certain agents, for example NSAID drugs, required the presence of exogenous luminal acid to stimulate marked lesions suggesting that acid had some

damaging capabilities. Further it has been shown that both NSAIDs aspirin and phenylbutazone cause a rise in luminal acidity (Kirsner and Ford 1955ab). The disparate conclusions reached in publications may reflect different experimental methods, for example the authors quoted above have found either corpus or antrum more prone to damage, whilst Cooke and Kienzle (1974) found that both areas responded in a similar fashion. Thus the reason why more lesions are found in the corpus than the antrum of the stomach in some situations is as yet not fully understood.

Erosions of the gastric mucosa tend to develop in a linear pattern on the ridges of the rugal folds, the explanation being that they are further from the blood vessels and therefore have poorer perfusion (Mersereau and Hinchey 1982, Lipowitz, Boulay and Klausner 1986). The effect of blood supply in the types of lesions produced in the stomach has been shown to be quite important in man and the experimental animal. Stress ulceration occurs in man after severe spinal trauma, burns or shock when there is hypotension (Lucas 1981). The effect of curtailing the blood supply, and the lack of or decreased effect that potentially injurious agents exhibit in the presence of a normotensive stomach in some experimental situations has been demonstrated clearly in the rat and canine gastric mucosa (Ritchie 1975, Cheung and Chang 1977).

In contrast to several previous reports, which include Harding and Morris (1984), no very obvious gross changes were found in the majority of the rats to which individual injurious agents were given in this study. This could be associated with the fact that, apart from the ligation of the pylorus, the stomachs of these animals were not otherwise tampered with in contrast to previous workers who, for example, manipulated the stomach to produce a gastric chambers. In addition some workers used larger volumes (10ml) of the substances tested (Harding and Morris 1984) than those used in the investigations described in this thesis.

The results of the application of diverse agents to the different regions of the stomach can be very variable and depend on the concentration of the agent used, and the length of exposure. Ethanol, for example, will produce almost total denudation of all areas of the stomach (Tarnawski and others 1985a). Bile, on the other hand, has been reported as producing gross lesions restricted to the corpus (Fringes, Lorenz and Oehlert 1985). Considering each of the specific agents used in the investigations described in this thesis, first the application of HCl acid either as 0.05 or 0.1N to the rats' gastric mucosa did not cause any obvious marked effects on gross examination. The acid concentration used (0.1N) in the rats investigated was no higher than the stomach is capable of generating but even when Mersereau and Hinchey 1973, Moody and others (1978) experimentally covered the gastric mucosa of rats with higher

concentrations of HCl (0.15N), they did not find any gross changes such as reddening or oedema. A similar lack of change was noted by Ritchie (1981). These observations may be related to there being no experimental interference with prostaglandin formation and release (Osada and others 1990). The concentration of acid used in the investigations discussed here was chosen because it was hoped, by exposing the gastric mucosa of the rats to these concentrations of acid for different periods of time, to identify preliminary changes caused by application of acid alone. In the one rat in which generalised erythema was seen it was assumed that this particular rat had gastric problems prior to the beginning of the experiment. The consistent lack of change in the rats to which acid was applied to their gastric mucosa was confirmed by light and scanning electron microscopy where almost all the parameters assessed were not significantly different from those found in the control group of rats. However, one notable change was the increased number of white cells found in the antral and corporal areas of the stomachs of the acid-challenged rats. It is difficult to explain why the relatively innocuous concentrations of acid should trigger white cell infiltration in view of not finding any other abnormalities, and indeed the numbers of white cells seen may be of little significance. However, the liberation of histamine from damaged cells sets in train the body's inflammatory defense mechanisms against damage. This includes the migration of white blood cells and so the invasion of the gastric mucosa by increasing numbers of white cells could represent an actual response by the gastric mucosa when exposed to any abnormal circumstances. The white blood cells themselves may contribute to mucosal injury by producing large amounts of reactive oxygen metabolites (Grisham and Granger 1988, Wallace and Granger 1992). However, using agents as mild irritants Robert and others (1978) found that infiltration of white blood cells was detectable at 24 hours but not at one hour after exposure. Thus the finding of increased WBC infiltrate with a number of injurious agents reported in this thesis is at odds with some previously published work. One explanation that may be advanced for this is that the numerical distribution of WBC numbers in the rat gastric wall varies widely and this finding with particular agents may be merely coincidental. Evidence of congestion was seen on histological examination of gastric mucosa sections in two of the rats given 0.1N acid. Congestion being a change associated with inflammation may lend support to the inference that the increased white cells found may indeed indicate change caused by the acid.

When the sections of gastric mucosa from the rats subjected to the acid treatment were examined by scanning electron microscopy one further interesting feature was seen. This was the general flattening of the apical membrane of many of the cells causing them to loose their convexity and to produce a loss of the normal cobblestone appearance. This feature has been reported before by Fringes, Lorenz and Oehlert

(1985) in rats given higher concentrations of acid (0.2N). No explanation was offered by these workers for this appearance and they did not report finding any cellular abnormality of this region on examination with light microscopy. No swelling of the cells were detected in either investigations. The apical flattening of the cells in these circumstances is at odds with the changes produced by certain other injurious agents that induce a loss of normal convexity caused by swelling of the cells. This also results in a loss of the cobblestone appearance. An explanation for this flattening may be that the luminal acid caused shrinkage of the cell and reduction of the contact surface area. Alternatively, the increase in hydrogen ion concentration may stimulate the release of large volumes of bicarbonate from the superficial cells (Konturek and others 1984b) resulting in shrinkage of the cell. The scanning electron microscopic appearance did not resemble that reported after administration of 0.1N HCl by Fujita, Tanaka and Tokunaga (1981)l, who found what appeared to be marked anisocytosis, nor the loss of apical membrane after 0.15N HCl reported by Wood and Dubois (1983). Therefore, although there were no dramatic changes seen in the rats investigated with low concentrations of acid used for the length of time the rats' mucosa was exposed to this agent, there were some changes seen at microscopic level which may indicate that some minor degree of damage was produced. This could explain some of the findings recorded when acid was given in conjunction with other agents and one cannot overlook the possibility that with time the damage might have become more severe and changes may have become apparent.

Bile was the other single endogenous product that was used in this study as it has been recognized as being injurious to the gastric mucosa in a number of species including man (Domellöf, Reddy and Weisburger 1980, Houghton and others 1986), the dog (Black, Hole and Rhodes 1971, Moody, Zalewsky and Larsen 1981) and the cat (Smith 1914). Bile causes the release of histamine (Rees and others (1977b). On gross examination, exposure of the rat gastric mucosa to even the higher concentration of bile used for up to five minutes produced inconsistent results. However, after exposure for 15 minutes, mild reddening was a consistent finding even at the lower of the two concentrations of bile applied. When sections were examined microscopically the erythema seen on gross examination of the corpus in some of the rats mucosa was corroborated by finding evidence of congestion in the sections prepared from this region of the stomach of rats treated with bile for 15 minutes. Furthermore, there was also histological evidence of congestion in the corpus of two rats given the lower concentration (2.5mM) of bile, again after exposure for at least 15 minutes. These results tend to suggest that the length of time that the gastric mucosa is exposed to bile is probably more important than the concentration used. The concentrations of bile chosen for this study were those frequently used in experimental models and are similar to those present in stomachs with

duodenogastric reflux (Ritchie 1975 and Ritchie and Shearburn 1976), in which situation gastric ulceration is reported. Although ulceration of the gastric mucosa was absent in the rats treated with bile in this study, both concentrations of bile induced a significant WBC infiltration compared to the controls. In those rats where 2.5mM bile was administered, there were greater numbers of white cells in the antrum than the corpus but the converse was true when 5mM bile was used, but these findings were not statistically significant, though perplexing. Robert and others (1978) have shown that bile will stimulate the infiltration of neutrophils into the gastric mucosa and the overall results in this study suggest that there is not a regional effect. The longer the mucosa is exposed to the injurious agents the more histamine will be released and stimulate greater white blood cell movement, the results in these rats suggests that it takes longer than 5 minutes for this effect to be occur when bile is the agent used. It has been reported that acid plays a role in the formation of "bile" ulcers (Rees and Bowen 1982, Bénichoux, Durlik and Mainard 1986). The gastric acid environment in the rats exposed to bile may not have been that required to produce erosions or ulcerations.

Interference with mucosal blood flow has been shown to cause a back-diffusion of hydrogen ions, which again has been suggested as part of the mechanism involved in the production of the ulcerated lesions associated with bile in the dog (DenBesten and Hamza 1972, Cheung and Chang 1977, Moody, Zalewsky and Larsen 1981). As already stated no deliberate attempt was made in the experiments reported in this thesis to interfere with the blood flow to the gastric mucosa. Another aspect that must be considered in translating the results of the bile experiments is that when consistent superficial ulceration and submucosal haemorrhage was induced - it was after a five hour exposure (Mann 1976). In the rats described here the longest period of exposure to bile was 15 minutes. Failure to identify oedema, and consistent erythema in these studies of rat gastric mucosa could therefore have been due to a too short exposure of bile at these concentrations.

Although no dramatic changes were apparent on gross examination, microscopic examination revealed more damage by bile to the apical membrane of the gastric cells than with any other single agent. Nearly 42% of the rats treated showed varying degrees of this type of damage when bile was present. The explanation for this may be because bile, in addition to being capable of reaching the basal lamina beneath the cell, also acts as a detergent destroying the lipid membrane of the surface cell (Davenport 1968, Duane and Weigand 1980). Although bile has detergent properties and is able to diffuse through the mucus layer, there has been some debate over whether it is capable of disrupting and damaging the adsorbed layer of mucus (Allen, Bell and McQueen 1984, Turnberg and Ross 1984). However, the apical changes

seen were not as marked as those described in the literature where the damaged cells were reported as forming a luminal arcade or arch over the exposed *in situ* cells (Lacy and Ito 1984), or where the necrotic cells were scattered haphazardly over the intact cells (Schmidt and others 1985). The results here were mainly concerned with the surface epithelial cell, it s apical membrane and the damage that bile might exert compared to that already reported (Davenport 1967b, Davenport 1968, Duane and Weigand 1980, Eastwood 1975). Certainly, exfoliating cells were present in larger numbers than with any of the other single agents and this was most marked in the corpus and antrum of those rats given 5mM bile. This increase in cell extrusion was accompanied by increased rosette formation, which represents the stage following complete extrusion of cells (Pfeiffer 1970b). Fringes, Lorenz and Oehlert (1985) found that the lesions in their rats, where bile was the agent used, were largely restricted to the corpus and that apocrine secretion with the associated loss of the apical membrane was a constant feature. Whilst the results found in the present work concurred with the corporal damage, there was also a similar degree of damage in the antrum in contrast to the results reported by the above workers. It is difficult to explain this difference on the basis of the concentration of bile used as they used a considerably higher concentration of bile (80mM). One explanation could be that the above authors examined their rats after two hours, whereas in these studies the rats were sacrificed after 15 minutes. The longer period of time may have allowed the antrum to recover by cellular restitution. Work by Morris and Wallace (1981) and Lacy and Ito (1984) supports this hypothesis, as they showed that restitution is virtually complete after one hour. The scanning electron microscopy demonstrated apical ruffling, cell extrusion, apical membrane swelling and apical erosions. These findings are in agreement with those reported by Winborn, Guerrero and Hodge (1978) and Fringes, Lorenz and Oehlert (1985). The latter authors obtained their results using a much higher concentration of bile, as already stated. However, rats given 2.5mM bile showed many cells with ruffled apical membranes, and although this was not such a feature in those given 5mM bile, these had many cells with apical erosions, which might be the preliminary stage leading to the ultimate loss of the apical cell membrane (Forte, Silen and Forte 1976, Easmann and others 1984). It has been suggested that the loss of the apical cell membrane may result in an apical expulsion of mucus, representing an epithelial response to injurious agents, where exocytosis fails to meet the mucus requirements of the mucosa. This could explain the finding of cells containing no inclusion bodies as described by Zalewsky and Moody (1979). Thus although the mucosa of the rats examined after treatment with bile alone showed only limited gross changes, mainly erythema, microscopic examination both at light and electron microscopic level revealed varying degrees of cellular damage including extrusion and death of cells, apical membrane collapse and erosion, and yet the junctional complexes were intact concurring with previous work

(Forte, Silen and Forte 1976, Eastwood 1985). These findings indicate the capability of bile to enhance degeneration of the gastric mucosa. These specific changes that can be attributed to bile on its own are of interest particularly in understanding the changes that subsequently occurred when a mixture of agents including bile was applied.

The concentrations of aspirin selected for this study were similar to those used previously to study its effect on the gastric mucosa and gastric mucosal barrier (Davenport 1964, Hingson and Ito 1971, Garner 1978, Bommelaer and Guth 1979). Again, as with acid and bile, aspirin produced no marked gross changes. Blanching reported by Morris and Harding (1974) and ulceration (Konturek and others 1981b) were not seen. Time and concentration cannot be used as an explanation for the failure to repeat the above results, because these findings were obtained shortly after exposure. The probable explanation is that the exposure of rat mucosa to aspirin reported here was not supplemented with acid as is frequently done in other experimental situations (Brodie and Chase 1967, Morris and Harding 1974). Indeed Gottschalk and Menguy (1970) showed that achlorhydric rats did not develop gastric erosions after receiving large doses of aspirin for two days.

Oedema was demonstrated histologically in the rats challenged with aspirin, especially in the antral region of the stomach of those given 40mM. When the gastric mucosa is exposed to damaging agents, the release of histamine causes the leakage of fluid into the interstitium giving rise to oedema (Davenport 1966, Johnson and Overholt 1967). Davenport (1969) proposed that this fluid released from the damaged mucosa would dilute and neutralise endogenous acid helping to protect the mucosa from the luminal acid. The release of histamine will also lead to vasodilation and increased blood flow (Moody 1967, Gerber and Nies 1982). In this series some of the rats given aspirin demonstrated congestion of the gastric mucosa. The inconsistent finding of congestion in the face of recognised damaging concentrations of aspirin may again be explained by the lack of exogenous acid present in the stomachs (Augur 1970).

All the rats treated with aspirin showed increased white blood cell migration with the highest numbers found in the antrum. Apart from these features, in general the epithelium looked substantially normal without any real evidence of erosions as has been reported by previous workers. St. John and others (1973) found that a single dose of aspirin was sufficient to produce erosions, which were largely restricted to the corpus of the stomach in their groups of rats. A possible explanation for this regional effect is that aspirin exerts most of its effect on areas of the mucosa which secrete acid because then the aspirin is more likely to be in an undissociated form. Eastwood and

Quimby (1982) found chronic administration of aspirin to rats caused epithelial proliferation in the corpus and not the antrum, and suggested that the failure of antral proliferation was the reason why ulcers were usually located there. Antral lesion predeliction was noted by Bugat and others (1976) following intravenous administration of aspirin to cats. However, this does not explain the preferential corporal damage that has been reported in the literature. The reason may be that there are in fact two separate responses. The initial acute effect may cause cell degeneration in the corpus of the stomach, followed in the long term by epithelial proliferation to replace the cell loss induced by aspirin-like substances. The antrum on the other hand may be able to resist the initial assault, possibly by its ability to produce more prostaglandin than the corpus (Konturek and others 1981c).

However, the development of acute lesions in aspirin treated rats may also be related to the length of exposure, which in the case of St. John and others (1973) was four hours. Rainsford (1978), on the other hand, found that maximum lesion development had taken place after two hours of exposure. In the rats treated with aspirin in these investigations, there was little evidence of the exfoliation of cells or loss of the thick mucus layer over damaged areas as has been described by Morris and Harding (1974) after only five minutes exposure. Nor was there any evidence in the rats discussed in this thesis, of the more severe superficial layer detachment described by Yeomans, St. John and de Boer (1973).

The results of the studies using scanning electron microscopy were similar to those obtained from normal rats. In those rats given 40mM aspirin there appeared subjectively to be more cells with holes and somewhat concave apical membranes than were found in normal rats or those given 20mM aspirin possibly indicating some increased mucus production. In contrast, Frenning and Öbrink (1971) found in the cat, that 17mM aspirin caused exaggeration of the normal convexity of the surface epithelial cell, but they supplemented the aspirin with acid therefore enhancing intracellular absorption of the aspirin and ions.

Scanning electron microscopy showed that the mucus layer was thinner in the antrum than was found with other single agents, though this was not as definite in the corpus. The most likely reason why the mucus appeared to be thinner is that aspirin had to a certain extent affected the hydrophobic layer of adsorbed surface-active phospholipids on the luminal aspect of the surface epithelial cells (Lichtenberger, Richards and Hills 1985). The effect of damaging the hydrophobic layer would be to make it more soluble and thus allow it to be dissolve in the gastric juice and not contribute to the mucus layer that is maintained during processing of the samples and present on the stubs available for scanning electron microscopy.

Overall the response of the gastric mucosa in the rats treated with aspirin was not as expected from previous reports in the literature in that although as with exogenous acid there were changes which could represent the early stages of cell damage they were not consistent and were mainly of a minor nature.

It is well recognised that the use of non-steroidal anti-inflammatory drugs produces gastric mucosal damage (Dodd, Minot and Arena 1938, Douthwaite and Linott 1938, Muir and Cossar 1955, Lanza and others 1979, Fromm 1981). Carprofen is said to have less harmful effect on the gastric mucosa as measured by effect on gastric potential difference, microbleeding, and prostaglandin generation in healthy human volunteers and those with peptic ulcer disease (Konturek and others 1982b, 1983). This was one of the agents used in the studies of the rats mucosa when single agents were applied. The dose of carprofen used was at the lower end of the therapeutic range quoted for the dog (McKellar and others 1990). Previous workers using the same dosage claimed that a reduction in the inflammatory swelling and an analgesic effect was achieved in cases of adjuvant arthritis in rats (Strub, Aeppli and Müller 1982). In contrast to the rats given aspirin the gross appearance of the gastric mucosa of the rats given carprofen was of consistent antral reddening, and even a suggestion of linear streaking and erosion in a few animals where the higher concentration of carprofen was used. The duration of exposure to carprofen did not appear to make any difference to the type or number of changes produced. The linear erosions seen on gross examination in a few rats were not substantiated histologically. There are several possible explanations for this disparity. First, the sections prepared may have failed to include these specific areas. Second, the linear erosions may in reality have been hyperaemic areas and not actual epithelial damage. Supportive evidence for this supposition comes from work in man, where the ingestion of sub-therapeutic doses of aspirin gave gross change of linear and punctate erosions that failed to be confirmed on histological examination - loss of mucus was the main finding (Cohen and MacDonald 1982). Similarly in the dog, the endoscopic finding of hyperaemia was not always substantiated by examination of endoscopic biopsies (Roth and others 1990). Histological examination of the antrum showed oedema, with lower frequency in the corpus. In many instances the oedema evident on microscopic examination was not apparent on gross examination and as already stated, repeated evidence of gastric erosion was lacking. This could be due to acid secretion present in normal circumstances but not present in these starved rats, where only basal acid secretion would have been present. The development of erosive gastritis in association with injurious agents has been reported to require the presence of acid (Moody, Zalewsky and Larsen 1981). As with the other single agents carprofen (0.23mM) resulted in an increase in white cells in the tissues of both the corpus and antrum. However, this feature was not so apparent in the mucosa of the

rats treated with the higher dose of carprofen used. It is rather difficult to find a suitable explanation for the discrepancy as the higher dose is unlikely to have suppressed WBC infiltrate. With both concentrations of carprofen some cellular damage was evident on scanning electron microscopy. This consisted of swelling of the cells which was different from the shrinkage of the cells that resulted from the treatment with acid alone. The swelling of the cells led to a loss of convexity and has been attributed to the accumulation of the injurious agents intracellularly (Hingson and Ito 1979). Such an effect has been reported previously in rats to which aspirin was applied - contrary to the findings in the rats in this study. Again cell damage was seen most often in the corpus and the damage to the cells was the same as that seen with aspirin and bile. However, the mucus layer of the corpus area of the gastric mucosa in the rats treated with carprofen was generally thinner overall than with any of the other single agents used, suggesting that carprofen was producing a greater degree of change than the other agents investigated, possibly by affecting mucosal hydrophobicity as aspirin has been shown to do (Goddard, Hills and Lichtenberger 1987).

The single agents used in these studies did not produce the superficial epithelial damage that has been reported by previous workers (Grant 1945, Hingson and Ito 1971, Yeomans, St. John and de Boer 1973). The various reasons presented in explanation for these different findings are the concentrations used, the length of time of exposure, and the fact that the rats were starved beforehand and therefore their mucosa was not actively producing acid. Thus the most important finding from the experimental studies presented here with regard to the use of single injurious agents such as bile, aspirin and carprofen at the concentrations used is that the length of time that the stomach is exposed to these agents would appear to dictate the extent of the damage done to the gastric mucosa. Finally the role of acid, a normal secretory product of the stomach, may be controversially one of protection (Smith and others 1977) or not (Hansen, Aures and Grossing 1978), and acid alone, in a concentration similar to those found in the normal stomach, is apparently harmless.

Much as been written in the literature of the effects of combinations of agents on the gastric mucosa of man and animals (Gottschalk and Menguy 1970, Semple and Russell 1975). The combinations investigated in the work reported in this thesis were aspirin/bile, carprofen/acid and carprofen/bile, and carprofen/acid/bile. Of particular interest were the combinations including the NSAID drug carprofen as this drug has the potential to be used increasingly in the treatment of animals. It is believed to be associated with minimal gastric abnormalities (Randall and Baruth 1976), and certainly not the major effect of inducing gastro-duodenal ulcers (O'Brien and Bagby 1987). The lower dose of carprofen with the addition of acid caused

reddening of the mucosa in the antrum in particular, though increasing the concentration led to reddening in the corpus as well. The higher concentration of carprofen with the addition of acid also gave rise to reddening of the mucosa around the corporo-antral junction in particular, with more involvement of the corpus as the time of exposure increased. However, this degree of reddening was not subjectively that much more dramatic than that obtained from carprofen alone in the antrum, but the addition of acid appeared to cause extension of this reddening to the corpus. This spread to the corpus, which was not present with the single agents alone, suggests that the addition of acid was enhancing the change induced with carprofen. This effect would be due to the provision of exogenous acid for back-diffusion of H^+ ions into the mucosa as suggested by Davenport (1967a) and Whittle (1976).

Doubling the concentration of carprofen increased the hyperaemia and induced gross appearance of oedema in one of the rats. The changes appeared to originate in the antrum and spread from there to the corpus. In these circumstances the fact that the antral area of the stomach is not normally in as much contact with acid compared to the corpus, and does not secrete acid, may play a part in the way in which these areas of the stomach responded to the addition of acid to carprofen. Those rats given the higher concentrations of both carprofen and acid did have the most severe changes suggesting that either carprofen or acid at the higher concentrations was enough to maximise the effect.

It has already been elaborated that acid enhances the damaging effects of barrier breakers. Davenport (1967ab) having noted the fact that the mucosa, if damaged by salicylates, became more permeable, wondered what would happen if bile was present in such a situation. He found that with luminal concentrations of taurocholate from 10mM that bile could be found in the venous blood of dogs with Heidenhain pouches. He concluded that bile salt was absorbed when the mucosal barrier was broken by salicylates. Bile itself will not only be absorbed but will induce back-diffusion of hydrogen ions (Ivey, DenBesten and Clifton 1970, Werther and others 1970), but that erosive lesions required an additional agent such as acid (DenBesten and Hamza 1972).

The combination of bile and aspirin in this series of experiments produced a gross reddening that was extensive in the corpus, but focal in the antrum. This appearance was not evident when the agents were used singly implying that there may have been a synergistic action. The effect of increasing bile concentration gave rise to an enhancement of reddening at 15 minutes suggesting that there was both a time and concentration effect. The finding that 2.5mM bile was able to enhance the gross change induced by aspirin compliments the work of Semple and Russell (1975), who

used a similar concentration of bile and found that it was sufficient to significantly amplify the mucosal lesions detected in rats given aspirin. However, these workers and Kauffman (1984) found that the production of haemorrhagic lesions was dependent on the concentration of aspirin. Thus in the rats used in this study it is suggested that the concentration of aspirin used was not sufficient to induce severe lesions, but enough to induce to evidence of mucosal response. Similar evidence of the synergistic action was demonstrated by Djahanguiri, Abtahi and Hemmati (1973) by testing the hypothesis in a different way. They ligated the pylorus to prevent reflux of bile and showed that aspirin-induced ulcerations were prevented. Similarly, in this study, the pylorus was ligated to prevent the exposure of the gastric mucosa to regurgitated endogenous bile and confirmed the combined effect of bile and aspirin on the gastric mucosa.

The evaluation of oedema and WBC counts demonstrates that a concentration effect was evident when bile and aspirin were used together, which again supports the findings of Semple and Russell (1975). Whilst oedema was only observed grossly in one rat, it was a feature when the tissues were examined by light microscopy where corporal oedema was present. However, the higher concentrations did evince oedema more frequently in the antrum which confirms the work of Ritchie (1981b) that bile has a concentration effect on the gastric mucosa as far as cytotoxicity is concerned.

Scanning electron microscopy confirmed the damaging effect caused by the combination of aspirin and bile. The individual scores in the both corpus and antrum of the lower concentrations were the only scores that were not significantly higher than control rats. The general scores were enhanced by cell extrusion and rosette formation and by enlargement of the gastric pits and cell swelling. Evidence that damage was caused was seen in the increased numbers of cells showing holes in the apical membrane, and with increased concentration produced more cells with erosions and concave membranes suggesting collapse of the cell. This was substantiated by the changes to the cytoplasm and mucous granules, which is supported by previous work (Hingson and Ito 1971, Rainsford 1975, Baskin and others 1976)

The combination of carprofen and bile induced the same degree of hyperaemia caused by carprofen and acid except that the reddening originated in the corpus and extended into the antrum as the concentration of agents used increased. Reflux of bile can occur in the normal animal and healthy man and the area of the stomach most likely to come into contact with that refluxed bile is the antrum (Faber and others 1974, Sonnenberg and others 1980, Ehrlein 1981, Happé and Van den Brom

1982, Mattioli and others 1990). The antrum may therefore be more capable than the corpus of withstanding exposure to bile through prior cytoprotective adaptation (Chaudhury and Robert 1980, Scheurer and others 1981). In the experiments undertaken in this thesis bile flooded the corpus and antrum equally, producing the initial reaction in the corpus and, speculatively, only after overcoming the natural barrier in the antrum did this area of the stomach react.

The evidence of apical damage in more sections obtained from rats given aspirin and bile than given other combinations suggests that regardless of other features carprofen may well be less destructive to the apical cell.

In addition, it would appear from the studies undertaken here that there is still some confusion over the area effect of damage. Experimental work has implicated the corpus as being more weak, yet the antrum is more permeable and it is this region where peptic ulceration occurs (Ruding 1967) and is exacerbated by administration of NSAIDs indicating that this area demonstrates a weakness in this line of defence. The results from scanning electron microscopy confirmed this effect and showed that carprofen/bile caused less damage than did aspirin/bile, at the higher concentrations only. There were no differences at the lower concentrations.

The application of mixtures of carprofen, acid and bile produced the most consistent and obvious gross change seen with any of the agents or combinations. The reddening of the mucosa and presence of linear erosions and pin point corporal erosions are typical of those described when asprin/acid has been used in other experimental situations (Cooke 1976). However, this appearance was not substantiated when sections of tissue were examined histologically for congestion of apical damage indicating that gross evaluation can be suspect as far as hyperaemia/erythema is concerned. These triple combinations failed to produce higher oedema scores or, in general, WBC infiltrate than was obtained with either carprofen/acid or carprofen/bile indicating that the addition of either bile or acid to double combinations of carprofen did not enhance inflammatory change. Despite this lack of aggravation seen at light microscopy level, some features detectable with scanning electron microscopy were affected, but the effect was mainly restricted to the antrum. In the corpus the features were little different from that obtained with carprofen and acid or bile combinations.

In the antrum, the addition of bile at the lower concentrations accentuated the number of extruded cells and convexity of the cells, but other than these features the supplementation of the challenging mixture with a third agent made no difference. The higher concentrations of triple agents appeared to reduce the thickness and coverage of the mucus layer and exaggerated the intracellular junctions, but left the

other general features unaltered. However, the number of cells demonstrating apical ruffling and presence of apical membrane damage such as holes and erosions, and loss of intracellular contents was increased over those rats given just carprofen with acid or bile.

Thus the triple combinations whilst producing apparently more spectacular gross change did not have light and microscopic evidence to support this finding. The exception to this was the accentuation of antral damage of individual cells possibly by the alteration in mucus layer that was demonstrated by thinning and poorer coverage. However, this antral damage is what might be expected in the clinical situation where the combination of acid, refluxed bile and ingested NSAIDs come together either to cause erosive gastritis and hence peptic ulceration, or aggravate pre-existing peptic ulcers by attacking the peptic ulcer base (Semple and Russell 1975, Cooke 1976).

Thus in this series of experiments it was found that aspirin and bile produced more obvious damage than carprofen mixed with bile and that the creation of an environment with three agents did enhance damage produced by double combinations in the antrum only.

6. Pre-treated Rats

6.1 Introduction

Drugs that are used to manage gastric lesions are generally designed to interfere with the auto-destructive capabilities of the gastric mucosa, and thus be able to counteract the gastric effects of endogenous and exogenous noxious agents.

Early drugs were antacids, which were basic compounds and neutralised the secreted acid. However, several limiting factors are inherent in this type of drug since it is rendered ineffective once it has neutralised the secreted acid, and further antacid is needed for the next wave of acid secretion. Following administration, the drug, like any other ingestant, is emptied from the stomach, possibly before the full antacid potential of the drug has been realised. This drawback may be compounded by the particular formulation allowing a slow acid-base reaction - magnesium trisilicate is an example of this.

Amongst the basic anions used are bicarbonate, trisilicate and hydroxide. The metallic ion used in combination with the basic anion is very important in determining the efficacy of the antacid. Aluminium and magnesium, in the hydroxide forms, are those most commonly used. The advantage of magnesium hydroxide is that the compound is not very soluble and the hydroxyl ions are not available to corrode the stomach mucosa, yet are still very reactive with hydrogen ions.

Commercial preparations are usually a mixture of magnesium and aluminium. This is because the combinations have a greater effect than would be expected from the simple sum of each drug's effect. Aluminium hydroxide will adsorb pepsin (Berstad 1982), stimulate mucus secretion (Caspary 1982) and promote prostaglandin release (Berstad and others 1987) and will also adsorb bile and lysolecithin. The delay in gastric emptying induced by Al^{3+} is countered by the Mg^{2+} (Brunton 1990).

The underlying flaw in the use of compounds to neutralize acids in the lumen is that they are short acting; 1-2 hours at the most (Brunton 1990). An alternative approach is to choose a compound capable of protecting the injured stomach by forming a layer adherent to the top of damaged epithelium. One such compound is sucralfate, a complex formed from sucrose octasulphate and polyaluminium hydroxide. If the pH is less than 4, there is extensive polymerisation and crosslinking of the sucralfate, and it forms a sticky and viscid whitish gel (Brunton 1990). The

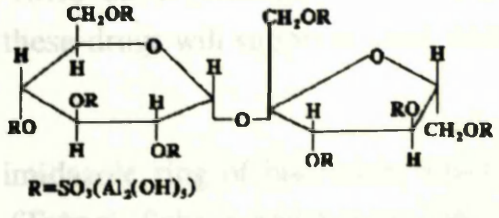


Figure 6.1. Structure of sucralfate.

efficacy of sucralfate is substantially increased if the intra-gastric environment has a pH of less than 4 (Konturek and others 1991b).

A separate approach to counteract the deleterious effect of agents in the stomach is to suppress acid secretion, as it plays such a significant role as an aggravator of mucosal damage. Histamine is a mediator in the stimulation of gastric acid and its release occurs during the early phases of gastric secretion. Acetylcholine and gastrin are also involved in the release of acid. However, the histamine H_2 receptor would appear to be the major controller of the parietal cell hydrogen ion production line (Code 1977). Cimetidine was the first of the commercially successful antagonists. It attaches to the H_2 receptor and blocks the activation of the parietal cell (Brimblecome and others 1975, Burland and others 1975). However, there are now recognised side-effects to cimetidine such as pruritis and gynaecomastia, male sexual dysfunction and mental confusion (Delle and others 1977, Van Thiel and others 1979, McMillen, Ambis and Siegel 1978, Galbraith and Michnovicz 1989). Many of the side-effects and drug interactions of cimetidine can be traced to the inhibition of cytochrome P_{450} (Peura and Freston 1987). However, from a patient compliance point of view, one of the drawbacks of cimetidine is the requirement for it to be taken four times daily. Ranitidine, another H_2 antagonist, has overtaken cimetidine in market share because it lacks the side-effects, but importantly needs only to be taken twice daily (Bohman, Myren and Larsen 1980, Woodings and others 1980). Both these drugs will suppress basal and stimulated acid secretion (Ostro 1987).

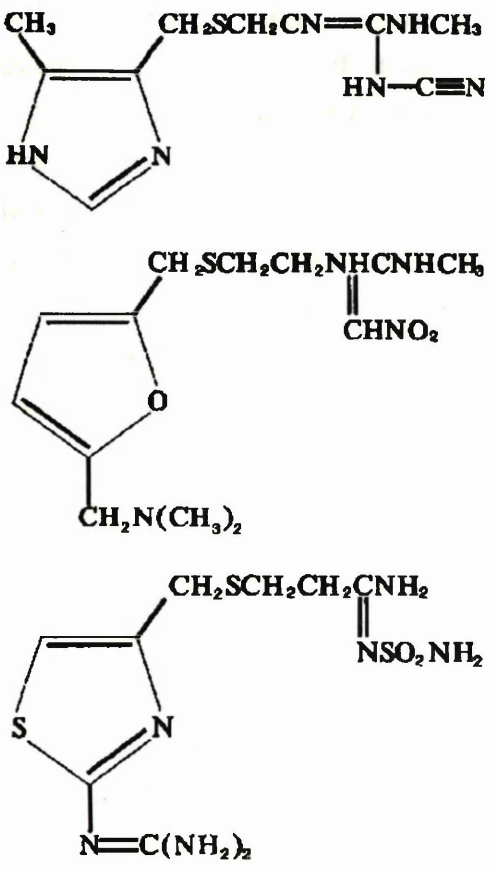


Figure 6.2. Structure of H_2 antagonists (*from top*) cimetidine, ranitidine and famotidine.

A newer H_2 antagonist, famotidine has had the imidazole ring of histamine, which was retained in cimetidine, replaced by a thiazole (Takagi, Takeda and Maeno 1982) (Figure 6.2). It offers further opportunities for improved patient/ owner compliance, since it need only be given once daily in the evening (Dammann and others 1987), and has been shown to be as effective as other H_2 receptor antagonists at permitting ulcer healing (Cochran and others 1989). Further, it has been demonstrated to be more

potent as an acid suppressor than cimetidine (Bertaccini and others 1986, Ostro 1987).

There have been a number of studies comparing the efficacy of sucralfate and the older H_2 antagonists such as cimetidine. In experimental feline oesophagitis pre-treatment with sucralfate was shown to be more effective than cimetidine (Clark and others 1987). In a similar study of the naturally occurring disease in man, sucralfate and cimetidine were found to be comparable when assessed endoscopically (Tytgat 1987). In the stomach of rats challenged with 100% ethanol, cimetidine was ineffective in reducing the number and extent of the induced lesions compared with sucralfate where there was a significant reduction (Kuwayama, Miyake and Matsuo 1987).

Aim

The aim of the work presented in this chapter is to compare the effect on the gastric mucosa of the administration of famotidine and sucralfate to rats for five days. The effect of challenging these rats with several injurious agent/combinations, including carprofen, was also examined. This was done with a view to compare the two drugs and any differences between the effects of the injurious agents on pre-treated rats.

6.2 Material and Methods

Batches of six Sprague-Dawley rats were housed and fed as described in section **3.1.** Rats were given either 1ml famotidine ($0.25mgml^{-1}$) or 1ml sucralfate ($200mgml^{-1}$) for five days. The drugs were given fresh as a mixture of drug and distilled tap water and the bottles were always well agitated prior to drawing up the mixture to be given to the individual rat. The drugs were administered *per oesophagus* using a syringe and Spreull needle.

The rats were starved, anaesthetised and the pylorus ligated as described in section **3.1.** Control rats were given either the drug or were sham-tubed with 1ml saline daily and killed after 5 days. Groups of six rats had carprofen, carprofen & bile, aspirin and aspirin & bile infused into their stomachs (Table 6.1).

agent	concentration
aspirin	40mM
aspirin & bile	40mM & 5mM
carprofen	0.46mM
carprofen & bile	0.46mM & 5mM

Table 6.1. Injurious agents used in rats given protective drugs.

Pairs were killed at 2, 5 and 15 minutes post-infusion. The method of gross examination and description of the harvesting of tissues is detailed in section **3.2.**

6.3 Results

6.3.1 Gross

6.3.1.1 Controls

No abnormalities were seen on the mucosa of the rats given either the protective agents alone or which had been sham tubed.

6.3.1.2 Sucralfate pre-treatment followed by

6.3.1.3 Aspirin

Aspirin alone produced little or no gross change to the mucosa. In one rat there was coating of the antrum with sucralfate.

6.3.1.4 Aspirin & Bile

When a mixture of bile and aspirin was used there was widespread reddening of the mucosa in the corpus, with spread of this erythema to the antrum in 3/6 rats. There was minimal coating of the mucosa with sucralfate, but it was more obvious in the corpus than the antrum.

6.3.1.5 Carprofen

After the infusion of 0.46mM carprofen, sucralfate was found as an adherent lacey layer in the antrum of all six rats. Some hyperaemia in the corpus was found in two rats, at 2 and 5 minutes.

6.3.1.6 Carprofen & Bile

In contrast to carprofen on its own, when bile (5mM) was added there was much less antral binding of sucralfate in 2/6 rats (Figure 6.3). Corporal hyperaemia was found in all six rats, either quite generalised or restricted to the greater curvature, with sparing of the lesser curvature.

6.3.1.7 Famotidine pre-treatment followed by

6.3.1.8 Aspirin

There was widespread reddening of the corporal mucosa in 4/6 rats, in the other two it was more localised to the dorsal and ventral surfaces of the mucosa. In the two 15 minute rats there was also involvement of the antrum with focal reddened areas on the ridges of the rugal folds.

6.3.1.9 Aspirin & Bile

With the infusion of both bile and aspirin there was widespread erythema in two rats after 5 and 15 minutes. In the other four the reddening was restricted to small areas on the dorsal or ventral surfaces. Alteration in mucosal colour was only found in the

antrum in one rat as focal change on the rugal ridges.

6.3.1.10 Carprofen

Moderate erythema was present in 5/6 rats following infusion of 0.46mM carprofen. This was evident in the corpus only, which although generalised in three of the rats was most pronounced on the greater curvature. In two rats there was oedema of the antrum and corpus, both of which had a few linear erosions on the antral rugae.

6.3.1.11 Carprofen & Bile

Erythema was less pronounced in the corpus of those rats exposed to bile and carprofen, again most noticeable in the corpus along the greater curvature (4/5 rats). Little or no gross change was evident after 15 minutes.

Figure 6.3. Gross mucosal appearance - sucralfate// carprofen/bile. The mucosa of the corpus and antrum is reddened and sucralfate can be seen as a white lacy covering.

6.3.2 Light Microscopy

(see Figures 6.5-6.19)

6.3.2.1 Oedema

No obvious time effect was found in either the corpus or antrum when the oedema ratios obtained from each of the combinations were examined. There was no difference in oedema ratios detectable between normal rats, sham-tubed rats and those unchallenged rats given famotidine and sucralfate (Table 6.2). The exception to this was the antrum of the rats given sucralfate. The antra of these rats had significantly higher ratios than the corpus and those rats forming the rest of the protection control subgroup.

The effect of sucralfate and famotidine under challenge conditions was compared with normal and sham-tubed rats. All rats, with the exception of those pretreated with famotidine and challenged with carprofen and carprofen/bile, had significantly higher ratios in the corpus, and all had more oedema in the antrum compared to the normal and sham-tubed rats.

In the sucralfate group, the corpus of the rats challenged with NSAID alone had higher ratios compared to the control sucralfate rats. In the antrum, all the challenged rats had significant evidence of being oedematous compared to the pre-treated controls. Of the famotidine rats, the antra of all challenged rats produced higher oedema ratios than the pre-treated famotidine controls. In the corpus there was no difference between controls and challenged rats, with the exception of the rats given aspirin/bile (Table 6.3).

Group	Agent	mean corpus	±sd	mean antrum	±sd
Control	NC	0.20	0.02	0.20	0.01
	CT	0.21	0.03	0.19	0.03
	CS0	0.22	0.03	0.28	0.03
	CF0	0.22	0.04	0.24	0.03
Injury	IS2	0.24	0.01	0.24	0.08
	IS2B2	0.25	0.06	0.34	0.04
	IR2	0.23	0.04	0.24	0.04
	IR2B2	0.25	0.04	0.30	0.06
Challenge	SS2	0.30	0.04	0.36	0.03
	SS2B2	0.25	0.04	0.37	0.05
	SR2	0.26	0.03	0.33	0.04
	SR2B2	0.25	0.07	0.34	0.04
	FS2	0.26	0.02	0.29	0.04
	FS2B2	0.29	0.02	0.28	0.03
	FR2	0.22	0.03	0.27	0.02
	FR2B2	0.24	0.03	0.29	0.02

NC = normal, CT = sham-tubed, CS = unchallenged sucralfate, CF = unchallenged famotidine, prefix F = famotidine, prefix S = sucralfate, R2 = 0.46mM carprofen, B2 = 5mM bile, S2 = 40mM aspirin.

Table 6.2. Oedema ratios of rats given protective drugs, and those obtained from rats given injurious agents.

The effect of the different challenging agents on the oedema ratio obtained from the rats was evaluated, and also a comparison was made between the effect of famotidine versus sucralfate (Table 6.3). The different combinations of challenging agents made no statistical difference to the oedema ratios from either those rats given famotidine or sucralfate. When sucralfate and famotidine were compared there was no demonstrable effect in the corpus. The antra of rats challenged with 0.46mM carprofen/5mM bile had significantly more oedema if they had been pre-treated with sucralfate rather than famotidine. Sucralfate rats given aspirin or aspirin/bile had higher ratios than comparable famotidine rats, again only demonstrable in the antrum.

drug	*vs*	agent	corpus ($p<0.05$)	antrum
SR2		CS	S	S
SR2B2		CS	ns	S
SS2		CS	S	S
SS2B2		CS	ns	S
FR2		CF	ns	S
FR2B2		CF	ns	S
FS2		CF	ns	S
FS2B2		CF	S	S
SR2		SR2B2	ns	ns
SR2		SS2	ns	ns
SR2B2		SS2B2	ns	ns
SS2		SS2B2	ns	ns
FR2		FR2B2	ns	ns
FR2		FS2	ns	ns
FR2B2		FS2B2	ns	ns
FS2		FS2B2	ns	ns
SR2		FR2	ns	ns
SS2		FS2	ns	S
SS2B2		FS2B2	ns	S
SR2B2		FR2B2	ns	S

NC=normal, CT=sham-tubed, CS=unchallenged sucralfate, CF=unchallenged famotidine, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin.

Table 6.3. Oedema ratios. Comparison between challenged rats and unchallenged, between different injurious agents, and between different protective drugs.

When the pre-treated and challenged rats were compared to those that had been given comparable injurious agents in the injury group there was little evidence of any effect on the corporal oedema ratios. In the antrum of those rats pretreated with sucralfate the oedema ratio was higher in those rats given carprofen, carprofen/bile or aspirin/bile. No trend was evident between those given famotidine and those from the injury group that were given no pre-treatment. Famotidine caused a lowering of the ratio compared to those given aspirin/bile in the injury group.

drug	*vs*	agent	corpus ($p<0.05$)	antrum ($p<0.05$)
SR2		IR2	ns	S
SR2B2		IR2B2	ns	S
SS2		IS2	S	S
SS2B2		IS2B2	ns	ns
FR2		IR2	ns	ns
FR2B2		IR2B2	ns	ns
FS2		IS2	ns	S
FS2B2		IS2B2	ns	@S

Prefix F=famotidine, prefix S=sucralfate, I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significantly higher, ns=not significant, @S=significantly lower.

Table 6.4. Oedema ratios. Comparison between pre-treated and challenged rats.

6.3.2.2 White Blood Cells

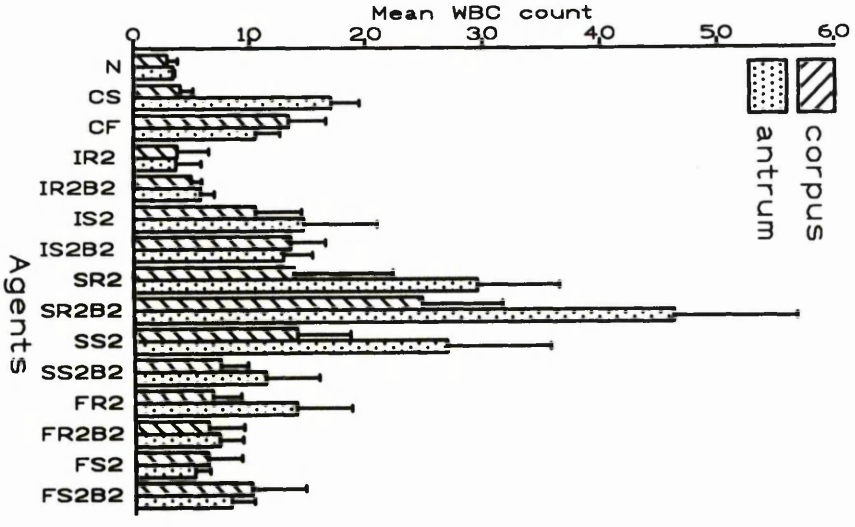
There was no difference in the infiltration of WBCs in those rats that were not tubed and those sham-tubed. Nor was there any difference in the numbers found in the corpus of the control rats given sucralfate and the normal rats whether sham-tubed or not. However, the numbers found in the antrum of the control sucralfate rats were significantly higher. In both the antrum and corpus of rats given famotidine alone, the numbers of cells were higher compared with both the normal and sham-tubed rats.

No time trend was apparent. As was done with the results from the injurious groups (4.3.2), the WBC counts for each combination were pooled. This was to establish if there was any difference between corpus and antrum with the various combinations of protective drug and challenging agents. There was substantially no difference between the numbers found in the corpus and antrum of the sham tubed, control rats and those given famotidine. The rats given sucralfate as controls had much higher numbers of WBCs in the antrum than in the corpus (Figure 6.4).

	Agent	mean corpus	±sd	mean antrum	±sd
Control	CR	2.90	0.88	3.40	0.18
	CS	4.00	1.41	17.00	2.47
	CF	13.30	3.24	10.50	2.12
Injury	IR2	3.75	2.73	3.63	2.14
	IR2B2	4.96	0.89	5.76	1.2
	IS2	10.50	3.92	14.60	6.37
	IS2B2	13.50	3.01	12.90	2.49
Challenge	SR2	13.80	8.51	29.50	7.06
	SR2B2	24.80	6.83	46.30	18.60
	SS2	14.00	4.66	26.90	8.86
	SS2B2	7.38	2.40	11.30	3.10
	FR2	6.67	2.50	13.90	4.82
	FR2B2	6.29	3.09	7.25	2.06
	FS2	6.21	2.97	5.08	1.30
	FS2B2	10.02	4.59	8.21	2.01

CR=normal and sham-tubed, CS=unchallenged sucralfate, CF=unchallenged famotidine, prefix F=famotidine, prefix S=sucralfate, I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin.

Table 6.5 Mean white blood cell numbers found in control, pretreated rats and those challenged with injurious agents.



CR=normal & sham-tubed, CS=unchallenged sucralfate, CF=unchallenged famotidine, prefix F=famotidine, prefix S=sucralfate, prefix I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin.

Figure 6.4. Bar graph of the white blood cell infiltrate in control, selected injury groups and those pre-treated with sucralfate and famotidine.

The WBC infiltrate into the antrum of those rats pre-treated with sucralfate and given carprofen combinations was significantly higher than the corporal infiltrate. A matching difference was found between the corpus and antrum of those rats given aspirin after sucralfate. However, this disparity between corpus and antrum was not present in the rats challenged with aspirin/bile. No difference was detectable in the numbers found in the corpus and antrum of those rats pre-treated with famotidine given and challenged with injurious agents.

The infiltrate of WBCs into the corpus and antrum of the rats pretreated with sucralfate and then challenged was significantly higher than the numbers found in those rats that were sham-tubed or those given the sucralfate alone. A similar significant difference was found when the famotidine rats were compared with the sham-tubed rats, with the exception of the antrum of the rats given aspirin. However, this effect was not present when the challenged rats were compared with the rats given famotidine alone (Table 6.6). Indeed, in the corpus there was a drop in the numbers of WBCs found in comparison to the pre-treated famotidine controls, but generally no difference in the antrum.

drug/agent	*vs*	drug	corpus ($p<0.05$)	antrum ($p<0.05$)
SR2		NR	S	S
SR2B2		NR	S	S
SS2		NR	S	S
SS2B2		NR	S	S
SR2		CS	S	S
SR2B2		CS	S	S
SS2		CS	S	S
SS2B2		CS	S	ns
FR2		NR	S	S
FR2B2		NR	S	S
FS2		NR	S	ns
FS2B2		NR	S	S
FR2		CF	@S	ns
FR2B2		CF	@S	ns
FS2		CF	@S	@S
FS2B2		CF	S	ns

NR = normal and sham-tubed, CS = unchallenged sucralfate, CF = unchallenged famotidine, prefix F = famotidine, prefix S = sucralfate, I = injury, R2 = 0.46mM carprofen, B2 = 5mM bile, S2 = 40mM aspirin. S = significantly higher, ns = not significant, @S = significantly less.

Table 6.6. White blood cell infiltrate. Comparison between rats given protective drugs and controls.

Next the results were compared to define if any effect could be attributed either to the addition of bile or the effect of the two drugs (Table 6.7).

	SR2		SR2B2		SS2		SS2B2		FR2		FR2B2		FS2	
	c	a	c	a	c	a	c	a	c	a	c	a	c	a
SR2														
SR2B2	<S	ns												
SS2	ns	ns												
SS2B2			↑S	↑S	↑S	↑S								
FR2	↑S	↑S												
FR2B2			↑S	↑S					ns	↑S				
FS2					↑S	↑S			ns	↑S				
FS2B2							ns	ns			ns	ns	ns	<S

Prefix F=famotidine, prefix S=sucralfate, I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin. c=corpus, a=antrum, < and ↑ point to the combination producing the significantly larger WBC infiltrate..

Table 6.7. White blood cell infiltrate. Effect of injurious agents on protected rats.

Taking the sucralfate pool first, the addition of bile to carprofen had the effect of increasing the WBC infiltrate in the corpus alone. It had no effect on the antral score, nor was there any difference between aspirin and carprofen given alone as injurious agents in the presence of sucralfate. The mixture of aspirin and bile produced significantly lower WBC counts when compared either to the similar carprofen/bile mixture or to aspirin alone. A comparison of the sucralfate pool to the famotidine pool showed that rats pretreated with sucralfate and given any of the agents with the exception of the aspirin/bile combination, had a heavier infiltrate of WBCs than in those receiving famotidine.

As can be seen from Figure 6.4, the famotidine pool had very similar infiltrates in both corpus and antrum regardless of the injurious challenge. Two significant exceptions were i) carprofen in the antrum having a higher infiltrate than those given aspirin alone, and ii) increased numbers found in the antrum of rats given aspirin/bile compared with aspirin alone.

Compared with the mean WBC infiltrate found following the application of injurious agents those rats pre-treated with sucralfate and those given the same injurious agents alone, on the whole, gave rise to significantly greater infiltrates of WBCs (Table 6.8). The exceptions to this generalisation in the sucralfate group were the corpus of those rats given aspirin, the antrum of those given aspirin/bile and the corpus of those rats

given famotidine and aspirin & bile. There was a significant reduction in numbers compared with the similar injurious pool, but only in the antrum of the rats given aspirin/bile and pretreated with famotidine.

drug	*vs*	agent	corpus ($p<0.05$)	antrum
SR2		IR2	S	S
SR2B2		IR2B2	S	S
SS2		IS2	ns	S
SS2B2		IS2B2	S	ns
FR2		IR2	S	S
FR2B2		IR2B2	S	S
FS2		IS2	S	ns
FS2B2		IS2B2	ns	@S

Prefix F=famotidine, prefix S=sucralfate, I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significantly greater, ns=not significant, @S=significantly less.

Table 6.8. White blood cell infiltrate. Comparison between protected rats and those given injurious agents alone.

6.3.2.3 Congestion

The rats given the protective agents were examined for congestion as were those given injurious agents (Table 5.14).

	Time											
	2min				5min				15min			
area total=96	corpus		antrum		corpus		antrum		corpus		antrum	
	n	%	n	%	n	%	n	%	n	%	n	%
Congestion												
0	11	*11.5*	14	*14.6*	6	*6.2*	12	*12.5*	7	*7.3*	11	*11.5*
1	4	*4.2*	2	*2.1*	7	*7.3*	4	*4.2*	7	*7.3*	3	*3.1*
2	1	*1.0*	0	-	3	*3.1*	0	-	2	*2.1*	2	*2.1*

Table 6.9. Protection. Congestion in corpus and antrum with time.

The figures for congestion at the different time intervals failed to reveal evidence of a time effect. An examination of the congestion found in the sections from the rats treated with different drug and injurious agent combinations also failed to show a drug effect (Table 6.10). Sixty-one areas (63.5%) had no congestion noted, which at

time 5 and 15 minutes was more apparent in the antrum. Congestion (scored 1) in 27 areas (28.2%) and was twice as likely to be present in the corpus as the antrum. Intense congestion (scored 2) was uncommon and found in only eight areas. In 6/8 rats this congestion was in the corpus. A time effect was suggested here in that this severity of congestion was found in 4/8 at 15 minutes. There was no difference in the distribution of congestion in the corpus and antrum when sucralfate and famotidine were compared (Table 6.10).

	0 no congestion		1 mild congestion		2 marked congestion	
area	corpus	antrum	corpus	antrum	corpus	antrum
Sucralfate	11	18	11	5	2	1
Famotidine	13	19	7	4	4	1

Table 6.10. Numbers of rats with congestion. Comparison between sucralfate and famotidine.

Expressed as a percentage the number of rats with scores of 1 or 2 in those given protective drugs was much higher than those given the same injurious agents, but not pretreated at 5 and 15 minutes (Table 6.11)

	Time											
	2min				5min				15min			
area	corpus		antrum		corpus		antrum		corpus		antrum	
	I	P	I	P	I	P	I	P	I	P	I	P
Congestion	%				%				%			
0	9.3	11.5	12.1	14.6	8.9	6.2	11.0	12.5	7.9	7.3	12.9	11.5
1	5.0	4.2	2.1	2.1	4.6	7.3	2.1	4.2	5.0	7.3	1.4	3.1
2	0	1	0	0	0.7	3.1	0.7	0	1.4	2.1	0	2.1

I = rats given injurious agents, P = rats given protective drugs and challenged with injurious agents.

Table 6.11. Comparison of congestion in pretreated rats with challenged rats.

6.3.2.4 Apical Damage

The figures for apical damage as far as time and area are concerned are given in Table 6.12, without consideration of the drug and injurious agent.

There appeared to be some anomalous increase in damage with time in that those rats showing the least apical damage were found after 2 minutes, most rats with damage were found in 5 minute rats, but fewer after 15 minutes. This being more

marked in those rats with severe damage (score 2). With specific combinations only those given aspirin and famotidine showed a definite time trend with damage becoming more severe as time progressed.

	Time					
	2min		5min		15min	
area	corpus	antrum	corpus	antrum	corpus	antrum
Damage						
0	9	8	8	3	5	5
1	7	4	5	9	9	3
2	0	4	3	4	0	3
3	0	0	0	0	2	2

Table 6.12. Protection. Apical damage versus time.

In those rats with a damage score of 1 there was a disparity between corpus and antrum. At 5 minutes, compared with the antrum, the corpus appeared to be spared, yet at 15 minutes the position seemed to have been reversed with the antrum being spared. When the damage was scored as 2 there was a more pronounced involvement of the antrum regardless of the time at which the rats were sacrificed.

		Time					
		2min		5min		15min	
area		corpus	antrum	corpus	antrum	corpus	antrum
damage	drug						
0	S	5	2	4	1	2	2
	F	4	6	4	2	3	3
1	S	3	2	2	3	6	3
	F	4	2	3	6	3	0
2	S	0	4	2	4	0	3
	F	0	0	1	0	0	3
3	S	0	0	0	0	0	0
	F	0	0	0	0	2	2

S = sucralfate, F = famotidine

Table 6.13. Protection. Apical damage versus time and drug given.

The results were split on the basis of the drug given (Table 6.13). There was little difference between sucralfate and famotidine where there was no apical damage, except that there were fewer sucralfate rats with no antral damage at 2 minutes. There was more damage (scored as 1) in the antrum of famotidine rats at 5 minutes than in the corpus or the sucralfate rats. In contrast at 15 minutes there more sucralfate rats with this level of damage, with the corpus being twice as affected as the antrum. Damage (scored as 2) was found in rats given sucralfate, predominatly in the antrum. The two rats found to have most severe (scored as 3), had been given famotidine and exposed to aspirin for 15 minutes. This level of damage was not found in those rats given injurious agents alone.

	Damage						
score	0	1		2		3	
Area	both	corpus	antrum	corpus	antrum	corpus	antrum
SR2	3	2	1	2	4	0	0
SR2B2	1	5	0	0	6	0	0
SS2	5	2	4	0	1	0	0
SS2B2	7	2	3	0	0	0	0
FR2	6	3	2	0	1	0	0
FR2B2	10	1	1	0	0	0	0
FS2	6	0	1	1	0	2	2
FS2B2	0	5	4	1	2	0	0
IR2	9	0	1	2	0	0	0
IR2B2	9	1	2	0	0	0	0
IS2	9	1	2	0	0	0	0
IS2B2	6	3	3	0	0	0	0

NC=normal, CT=sham-tubed, CS=unchallenged sucralfate, CF=unchallenged famotidine, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin.

Table 6.14. Protection. Apical damage and injurious agent combinations.

When those rats that had been pre-treated with sucralfate were compared with those that had been given injurious agents alone, only those rats challenged with aspirin and bile had the same range of apical damage as those pre-treated with sucralfate. In the other three combinations pre-treatment increased the level of apical damage.

When the same comparison was made with rats pre-treated with famotidine, there was more damage amongst rats pre-treated and given carprofen alone, whereas there was no difference with those given carprofen and bile. Those pre-treated rats given aspirin combinations had more damage than rats given the same injurious agents

without pre-treatment. This was most apparent in those given aspirin and bile where all pre-treated rats had apical damage recorded.

6.3.2.5 Luminal Contents

The luminal contents of the protected rats was examined, but no time-related pattern was apparent (Table 6.15).

	Time					
	2min		5min		15min	
area	corpus	antrum	corpus	antrum	corpus	antrum
contents						
NF	10	9	10	4	7	4
TF	0	0	0	0	0	0
NM	4	2	4	4	7	6
TM	2	5	2	8	2	6

NF= little mucus, few cells, TF= thick mucus few cells, NM= little mucus, many cells, TM= thick mucus, many cells

Table 6.15. Luminal contents by time and area.

	Luminal Contents							
	corpus				antrum			
agents	NF	TF	NM	TM	NF	TF	NM	TM
Sucralfate	13	0	7	4	6	1	5	12
Famotidine	14	0	8	2	11	0	10	3
Injury	20	0	3	1	17	0	3	4

NF= little mucus, few cells, TF= thick mucus few cells NM= little mucus, many cells, TM= thick mucus, many cells

Table 6.16. Luminal contents. Comparison between rats grouped as protected and injured.

In Table 6.16 the results for the luminal contents were placed in three groups determined by the presence or absence of a protective agent and the particular agent. Those animals pre-treated with protective agents had a different range of intraluminal contents compared with similar rats infused with injurious agents alone. Most of the injured rats had no mucus and few if any cells in the lumen. Over half of the rats given sucralfate had no mucus and few cells on the surface of the corpus, though a

significant number (11/24) had many cells littering the lumen. In the antrum 50% of the rats had an apical surface coated with a thick mucus layer containing large numbers of cells, though in five only the cells were present.

The corpus of those rats given famotidine was rather similar to the corporal region of the sucralfate group, as 50% had a little in the way luminal contents. However, 10/24 rats had many cells lining the lumen. In the antrum, more of the injured rats compared with those of the sucralfate group had no luminal contents. In contrast to the sucralfate group, which had a profusion of both mucus and cells, those in the famotidine group had large numbers of cells in the lumen unaccompanied by mucus.

The three groups were then broken down into their constituent parts in Table 6.17.

	Luminal Contents							
	Corpus				Antrum			
agents	NF	TF	NM	TM	NF	TF	NM	TM
SR2	3	0	1	2	0	1	1	4
SR2B2	0	0	4	2	0	0	0	6
SS2	5	0	1	0	3	0	1	1
SS2B2	5	0	1	0	2	0	3	1
FR2	5	0	1	0	2	0	3	1
FR2B2	6	0	0	0	4	0	2	0
FS2	2	0	3	1	3	0	1	2
FS2B2	1	0	4	1	2	0	4	0
IR2	5	0	0	1	4	0	2	0
IR2B2	6	0	0	0	6	0	0	0
IS2	6	0	0	0	3	0	0	3
IS2B2	3	0	3	0	4	0	1	1

prefix F=famotidine, prefix S=sucralfate, I=injury, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin. NF= little mucus, few cells, TF= thick mucus few cells NM= little mucus, many cells, TM= thick mucus, many cells

Table 6.17. Protection. Luminal contents in rats with and without pre-treatment with sucralfate and famotidine.

In the sucralfate group, the addition of bile to those rats given carprofen appeared to contribute to the proliferation of cells in the lumen of the corpus when compared with the other injurious combinations. Indeed in the corpus those rats given aspirin were found to have little luminal contents. In the antrum, carprofen caused the production of large numbers of cells in a thick layer of mucus, being possibly accentuated by the

addition of bile. Comparing those given sucralfate with those challenged with injurious agents alone, it appeared that only three of the rats challenged with carpofen alone or with bile had any luminal contents. When pre-treated with sucralfate, many of those rats challenged with carprofen combinations had luminal contents containing either cells alone or a layer of mucus with cells embedded. The difference between those rats given aspirin with and without pre-treatment was not as marked.

There was substantially little difference between the corpus of those rats injured with carprofen, with and without bile, and those pre-treated with famotidine. In the antrum, however, there was an increase in the cell content. In the famotidine group the main contributor to the increase in luminal contents compared with the injurious group was in those rats given aspirin, regardless of the inclusion of bile in the injected solution. Similar to the antral region of the rats given carprofen and famotidine, there was an increase in the number of cells in the lumen, but not in the volume of mucus detected.

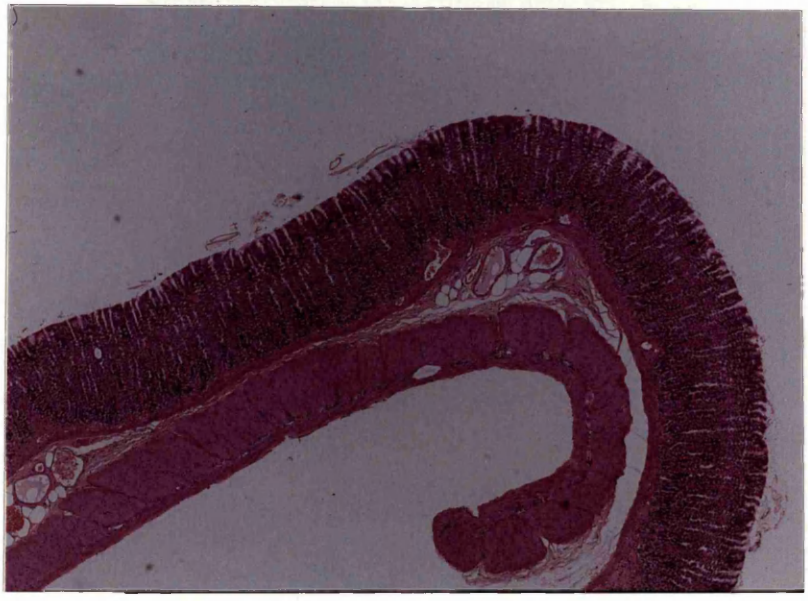


Figure 6.5. Photomicrograph of corpus pre-treated with sucralfate. There is some debris in the lumen. (H&E x30)

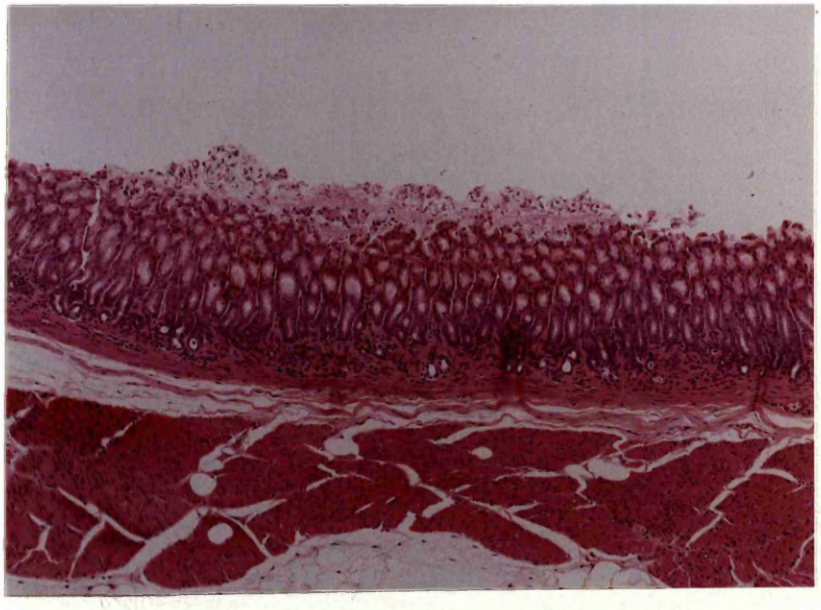


Figure 6.6. Photomicrograph of antrum pre-treated with sucralfate. There is an area of epithelium covered by a layer of mucus/sucralfate with a heavy cellular infiltrate. (H&E, PAS/Alcian Blue x75)

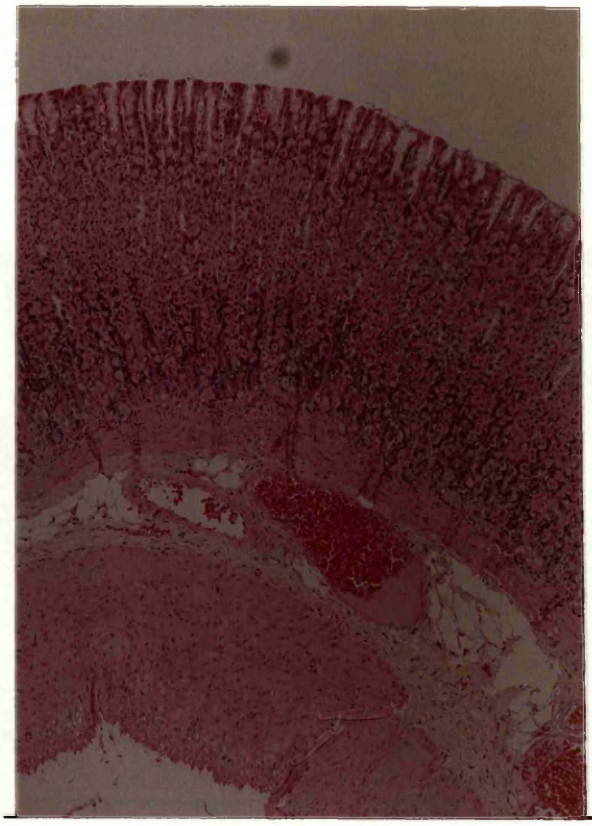


Figure 6.7. Photomicrograph of corpus pre-treated with famotidine. (H&E x75)

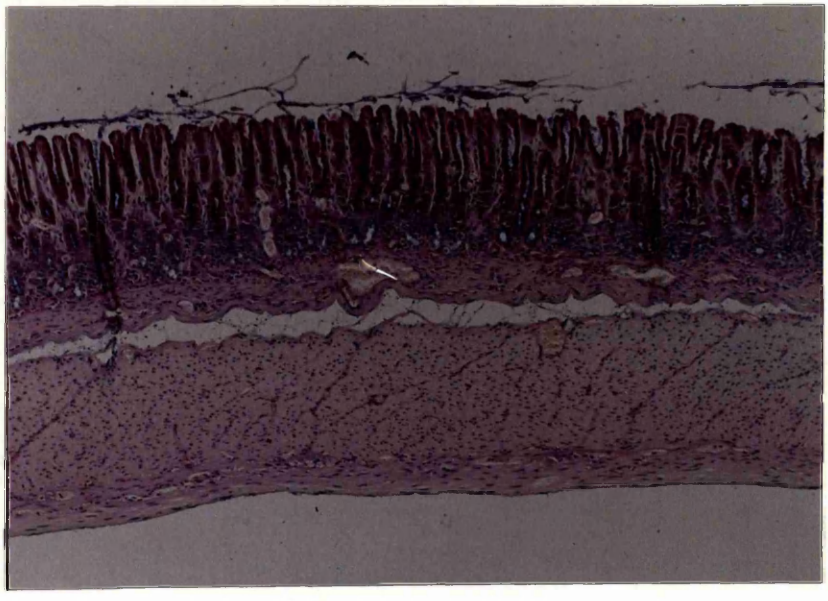


Figure 6.8. Photomicrograph of antrum pre-treated with famotidine. There is some mucus on the surface. (PAS/Alcian Blue x75)

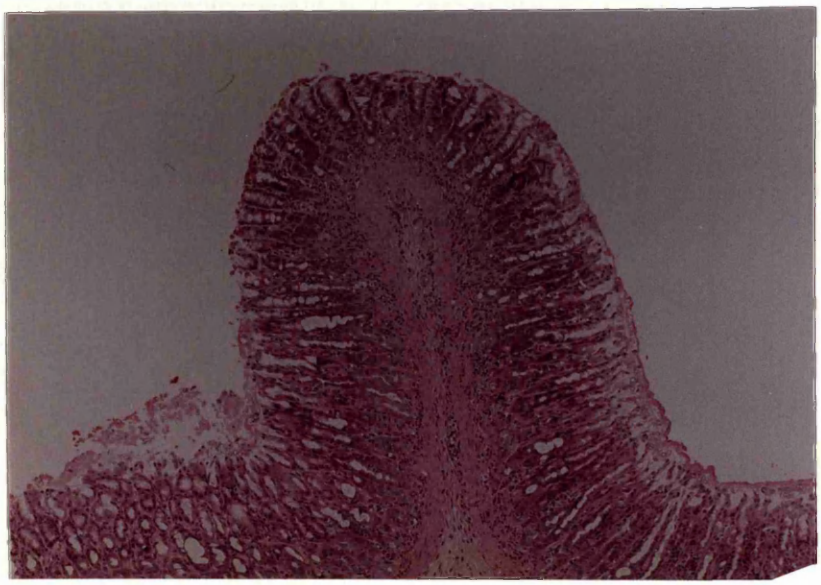


Figure 6.9a & 6.9b. Photomicrographs of antrum following sucralfate// 0.46mM carprofen. Two and 5 minutes exposure to carprofen. There is a thick overlay of mucus/sucralfate and ghost cells. The surface epithelium is disrupted and there is an intense white blood cell infiltrate visible. (H&E x75, x150)

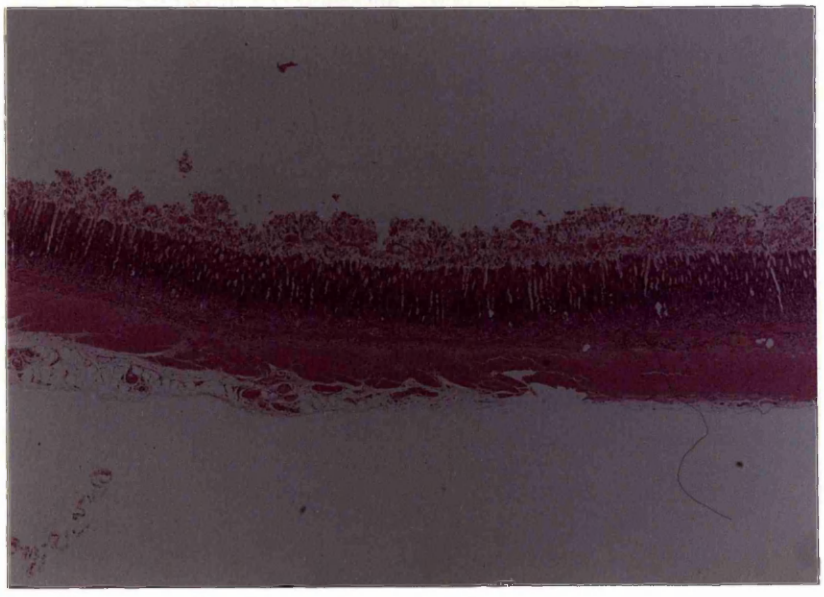


Figure 6.10. Photomicrograph of antrum following sucralfate// 40mM aspirin. Five minutes exposure to aspirin. There is a thick overlay of mucus/sucralfate and ghost cells. (H&E x30)

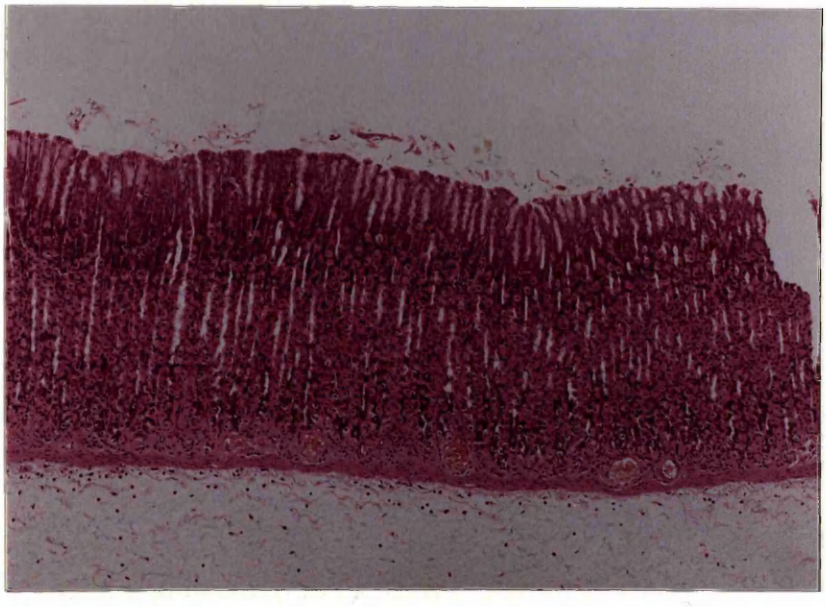


Figure 6.11. Photomicrograph of corpus following sucralfate// 40mM aspirin. Five minutes exposure to aspirin. There is debris in the lumen and there is an underlying white blood cell infiltrate with oedema evident. (H&E x75)

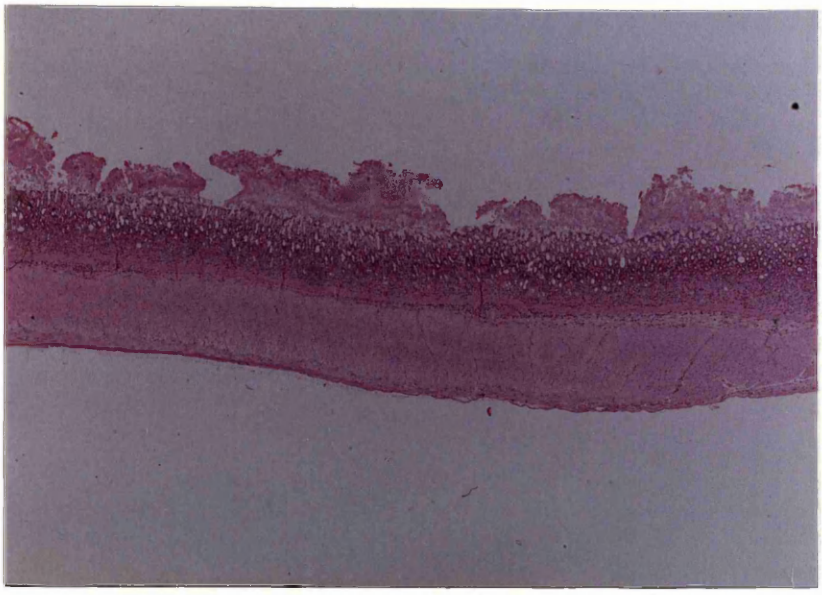


Figure 6.12. Photomicrograph of antrum following sucralfate// 0.46mM carprofen/ 5mM bile. Two minutes exposure to the injurious combination. There is a marked overlay of mucus/ sucralfate containing ghost cells. (H&E x30)

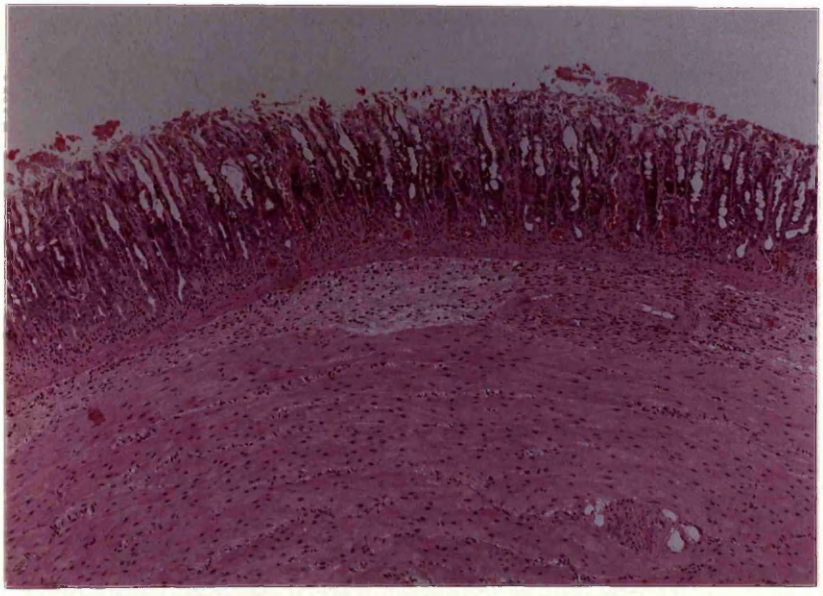


Figure 6.13. Photomicrograph of antrum following sucralfate// 0.46mM carprofen/ 5mM bile. Fifteen minutes exposure to the injurious combination. There is a an overlay of mucus/ sucralfate containing ghost cells, beneath this the surface epithelium is disrupted. There is an obvious cellular infiltrate with some congestion. (H&E x75)

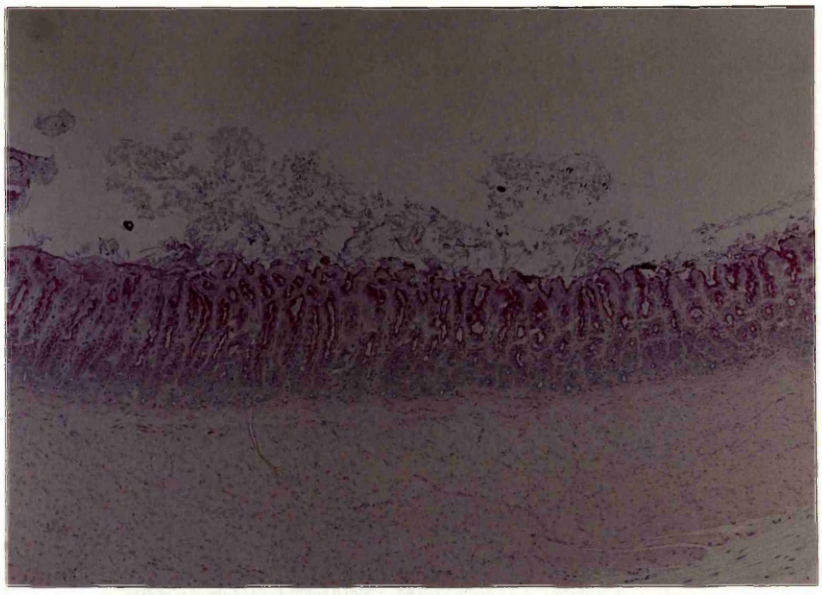


Figure 6.14. Photomicrograph of antrum following sucralfate// 40mM aspirin/ 5mM bile. Two minutes exposure to the injurious combination. There is an overlay of mucus and sucralfate. (PAS/Alcian Blue x75)

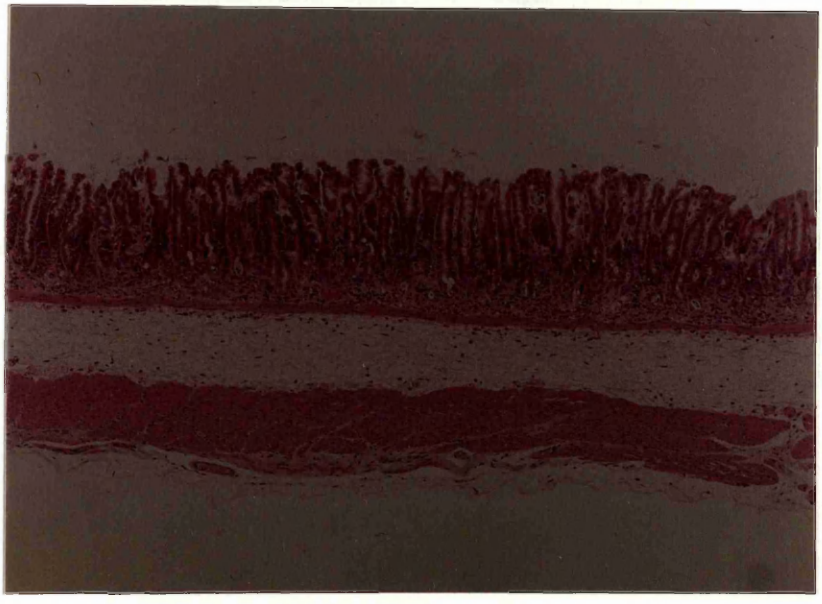


Figure 6.15. Photomicrograph of corpus antrum following sucralfate// 40mM aspirin/ 5mM bile. Two minutes exposure to the injurious combination. Oedema is evident and there is an obvious infiltrate of white blood cells. There is some disruption of the apical epithelial cells. (H&E, x75)

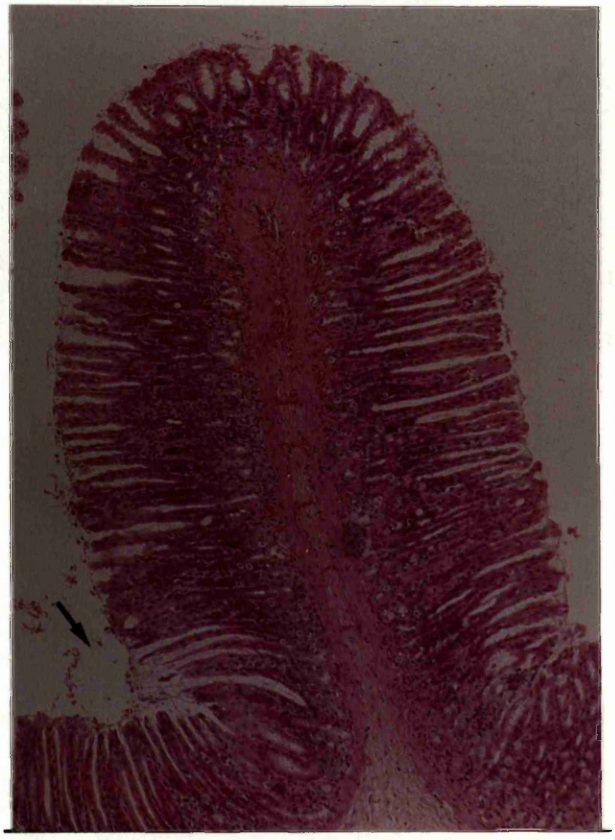
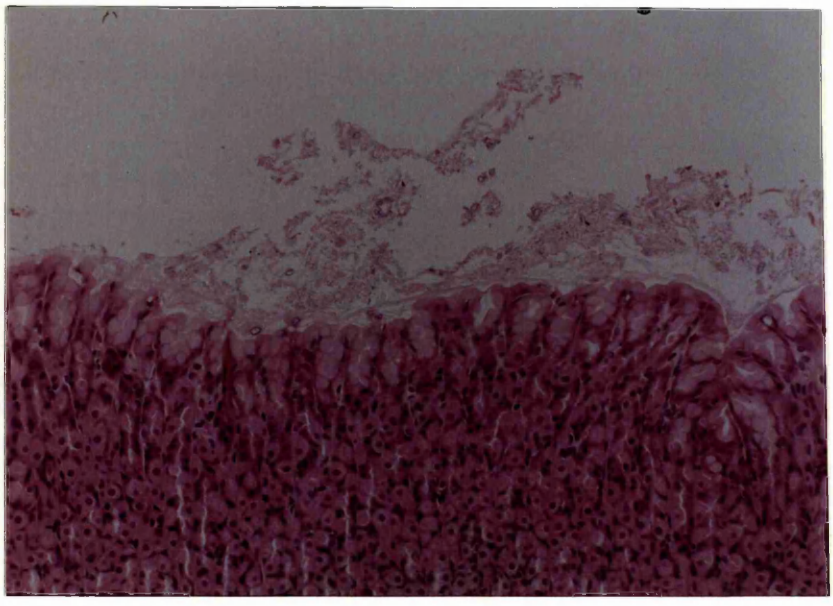


Figure 6.16. Photomicrograph of antrum following famotidine// 0.46mM carprofen. Five minutes exposure to carprofen. There is a marked cellular infiltrate, and although the apical epithelium appears intact there are some free lumenal cells visible, which may be arising from an area at the base of the rugal fold (*arrow*). (H&E x75)



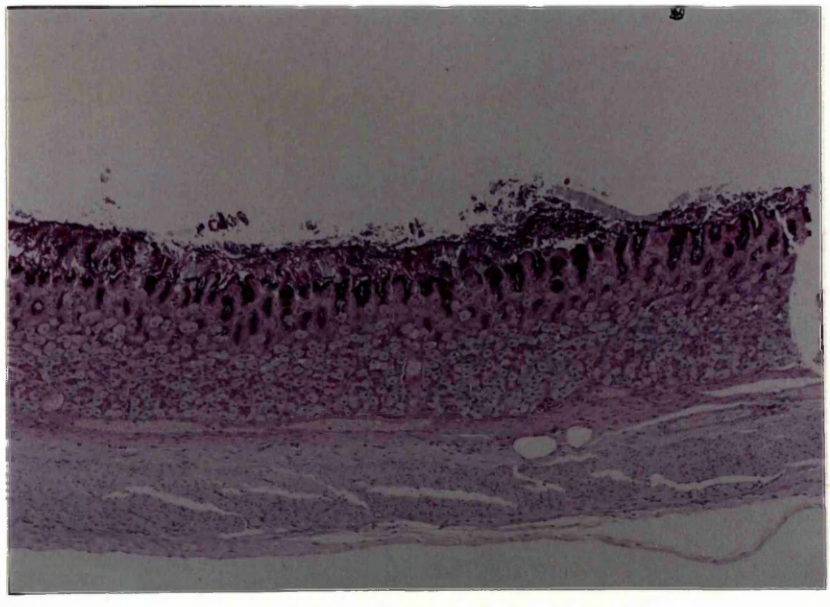


Figure 6.17a & 6.17b. Photomicrographs of corpus following famotidine// 0.46mM carprofen/ 5mM bile. Fifteen minutes exposure to the injurious combination. There is a moderately thick acellular layer of mucus overlying substantially normal surface epithelium. (H&E x150, PAS/Alcian Blue x75)

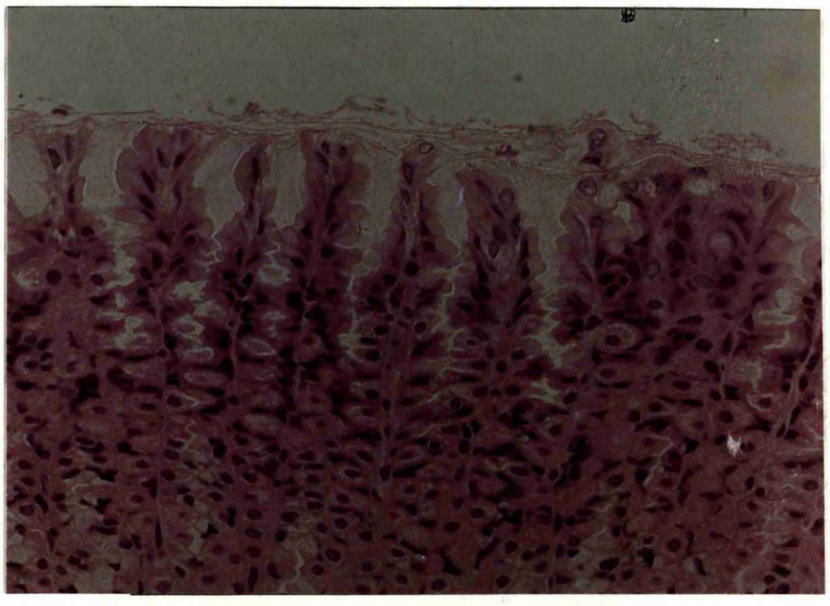


Figure 6.18. Photomicrograph of corpus following famotidine// 40mM aspirin. Five minutes exposure to aspirin. There is disruption of the apical epithelial cells and a thin overlay of mucus. (H&E x300)

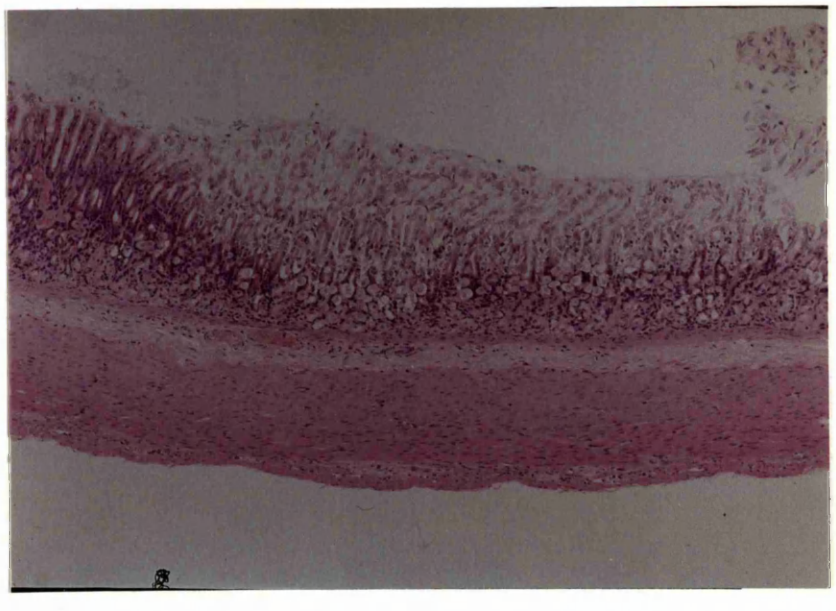


Figure 6.19. Photomicrograph of antrum exposed to following famotidine// 40mM aspirin/ 5mM bile. Five minutes exposure to the injurious combination. There is an erosion of the surface epithelial cells. There is a heavy overlay of mainly ghost cells. (H&E, PAS/Alcian Blue x75)

6.3.3 Scanning Electron Microscopy

The general and individual mean summed scores obtained from the control rats given protective agents for five days were compared with the untreated rats. There was no statistical difference found between these rats and those sham-tubed with saline, or those not tubed (see Tables 6.18-6.22).

There was also no difference found between the mean scores derived from the corpus and antrum of those rats sham-tubed and those given sucralfate or famotidine (see Tables 6.18-6.22, Figures 6.26ab). Though graphically pre-treatment appeared to have caused and increase in both general and individual scores (Figures 6.20-6.25).

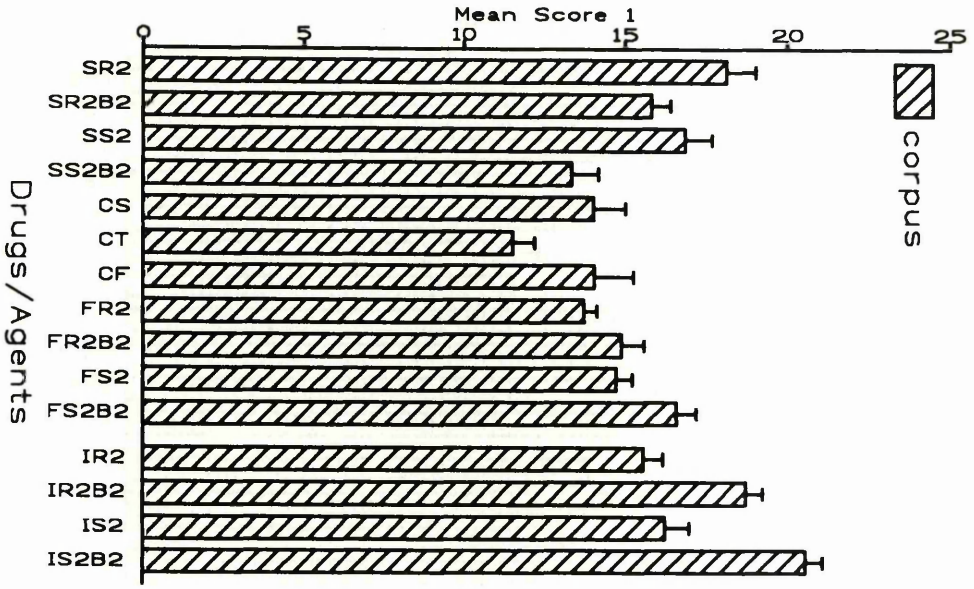
The results of summed scores obtained at the three time intervals from the rats given both drug and injurious agents were evaluated, and there was no demonstrable time effect for either drug or drug/agent combination.

Corpus															
Features	CT	CS	CF	SR2	SR2B2	SS2	SS2B2	FR2	FR2B2	FS2	FS2B2	IR2	IR2B2	IS2	IS2B2
General															
mucus covering	2.50	2.00	4.50	3.83	2.50	3.00	3.50	2.17	3.00	2.83	3.17	2.83	2.17	3.50	2.67
mucus thickness	2.50	4.00	2.50	2.83	2.50	3.50	2.17	2.00	2.83	2.33	2.83	2.17	2.50	2.17	3.00
cell extrusion	1.00	1.50	1.50	2.83	2.33	2.67	1.33	1.67	1.67	1.67	1.83	2.00	1.67	2.00	2.83
rosette formation	1.50	2.00	1.50	2.50	2.33	2.00	1.50	1.83	1.67	1.67	2.17	1.83	1.83	2.00	2.83
gastric pits enlarged	1.00	1.00	1.00	1.00	1.00	1.67	1.50	1.00	1.00	1.67	1.67	1.17	1.83	2.33	3.17
loss of cobblestoning	1.00	1.00	1.00	1.83	1.50	1.50	1.00	1.33	1.50	1.33	1.83	1.33	3.00	1.67	2.33
intracellular junction	1.00	1.00	1.00	1.50	1.50	1.33	1.33	1.50	1.33	1.50	1.50	1.50	2.83	1.50	1.83
cell convexity	1.00	1.50	1.00	1.83	2.17	1.17	1.00	2.17	1.83	1.67	1.50	2.67	2.83	1.00	1.83
[pooled score 1]	**11.50**	**14.00**	**14.00**	**18.15**	**15.83**	**16.84**	**13.33**	**13.67**	**14.83**	**14.67**	**16.50**	**15.50**	**18.66**	**16.17**	**20.49**
Individual															
apical ruffling	1.00	1.00	1.00	1.50	1.00	1.17	2.33	1.33	1.83	1.33	2.17	3.00	2.33	1.33	2.67
apical holes	1.00	1.00	1.00	1.17	1.17	1.00	2.00	1.50	1.50	1.33	1.83	2.50	2.50	2.00	2.33
apical concavity	1.00	1.50	1.00	1.67	1.33	1.67	1.33	1.67	1.33	1.33	1.33	1.67	1.83	2.00	2.00
apical erosions	1.50	1.50	1.00	2.17	2.33	1.33	1.17	2.33	2.17	2.17	2.17	3.17	3.33	1.83	2.50
intracellular contents	1.50	1.00	1.17	1.17	1.00	1.83	1.50	1.00	1.33	1.33	1.83	2.50	2.50	1.50	2.00
empty cells	1.00	1.00	1.00	1.33	1.00	1.00	1.50	1.00	1.00	1.00	2.17	2.00	1.67	1.33	1.67
[pooled score 2]	**7.00**	**7.00**	**6.17**	**9.01**	**7.83**	**8.00**	**9.83**	**8.83**	**9.16**	**8.49**	**11.50**	**14.84**	**14.16**	**9.99**	**13.17**

Table 6.18. Corpus: scanning electron microscopy scores pooled for each agent/drug (6rats/group). CT=sham-tubed, C=Control, F=famotidine, S=sucralfate. I=injury. A1=0.05N HCl, A2=0.1N HCl, B1=2.5mM bile, B2=5mM bile, S1=20mM aspirin, S2=40mM aspirin, R1=0.23mM carprofen, R2=0.46mM carprofen

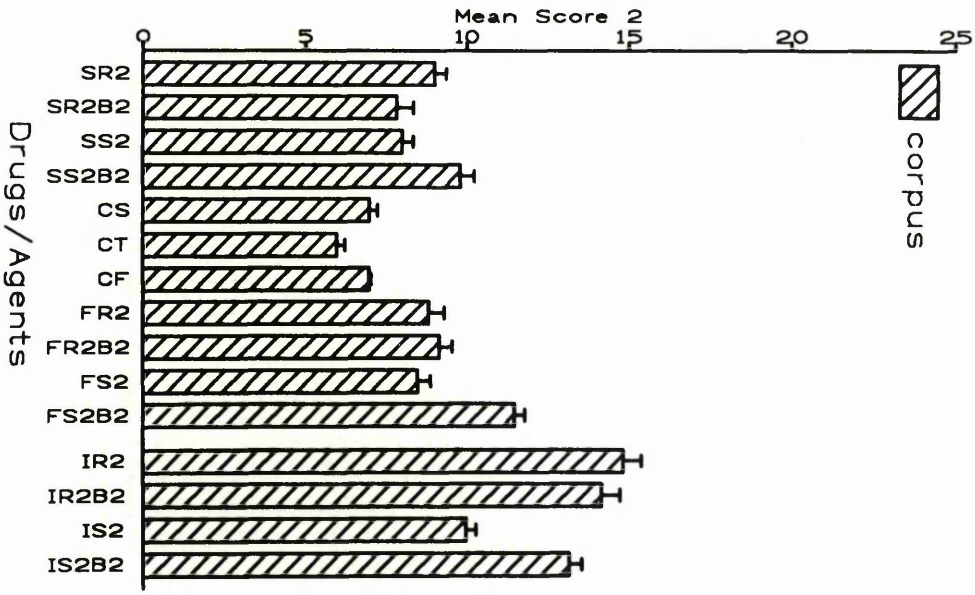
Antrum															
Features	CT	CS	CF	SR2	SR2B2	SS2	SS2B2	FR2	FR2B2	FS2	FS2B2	IR2	IR2B2	IS2	IS2B2
General															
mucus covering	3.00	3.00	3.50	3.67	3.33	3.33	2.67	3.00	2.33	3.33	2.50	3.00	2.83	3.17	2.00
mucus thickness	2.50	4.50	2.50	3.00	2.83	2.83	2.33	2.17	2.17	2.17	2.17	2.67	2.17	2.17	2.83
cell extrusion	1.00	1.50	1.00	2.17	2.67	2.50	2.33	1.50	2.33	1.67	2.67	1.50	1.33	1.67	2.33
rosette formation	1.50	2.00	1.00	2.00	2.50	2.33	2.33	2.00	1.83	2.00	2.50	1.50	1.83	1.17	2.50
gastric pits enlarged	1.00	1.00	1.00	1.00	1.00	1.67	2.00	1.00	1.00	2.17	2.17	1.00	2.00	1.83	2.67
loss of cobblestoning	1.00	1.00	1.00	1.67	1.17	1.67	1.50	1.50	2.50	1.83	2.00	1.17	1.83	1.50	2.17
intracellular junction	1.00	1.00	1.00	1.33	1.17	1.67	1.50	2.00	1.50	2.00	1.33	1.33	2.33	1.00	1.83
cell convexity	1.00	1.50	1.00	1.33	2.50	1.17	1.83	2.33	2.67	1.83	2.50	2.17	3.00	1.00	2.33
[pooled score 1]	**12.00**	**15.50**	**12.00**	**16.17**	**17.17**	**17.17**	**16.49**	**15.50**	**16.33**	**17.00**	**17.84**	**14.34**	**17.32**	**13.51**	**18.66**
Individual															
apical ruffling	1.00	1.00	1.00	1.67	1.17	1.33	2.83	1.17	2.33	1.33	2.33	2.17	2.33	1.00	1.83
apical holes	1.00	1.50	1.00	1.17	1.00	1.67	2.33	1.33	1.83	1.33	2.00	1.50	1.67	1.67	2.17
apical concavity	1.00	1.00	1.00	1.33	1.00	1.33	1.67	2.17	1.67	1.67	1.67	1.17	2.00	1.33	2.83
apical erosions	1.50	1.50	1.00	1.50	2.00	1.83	1.83	2.33	1.83	1.67	2.33	2.17	2.50	1.67	2.83
intracellular contents	1.00	1.00	1.00	1.17	1.00	1.33	1.83	1.00	1.17	1.50	1.50	1.50	1.50	1.17	2.33
empty cells	1.00	1.00	1.17	1.00	1.00	1.17	1.83	1.17	1.17	1.67	1.33	1.33	1.33	1.33	2.17
[pooled score 2]	**6.50**	**7.00**	**6.17**	**7.84**	**7.17**	**8.66**	**12.32**	**9.17**	**10.00**	**9.17**	**11.16**	**9.84**	**11.33**	**8.17**	**14.16**

Table 6.19. Antrum: scanning electron microscopy scores pooled for each drug/agent (6rats/group). CT = sham-tubed, C = control, F = famotifine, S = sucralfate, I = injury. A1 = 0.05N HCl, A2 = 0.1N HCl, B1 = 2.5mM bile, B2 = 5mM bile, S1 = 20mM aspirin, S2 = 40mM aspirin, R1 = 0.23mM carprofen, R2 = 0.46mM carprofen



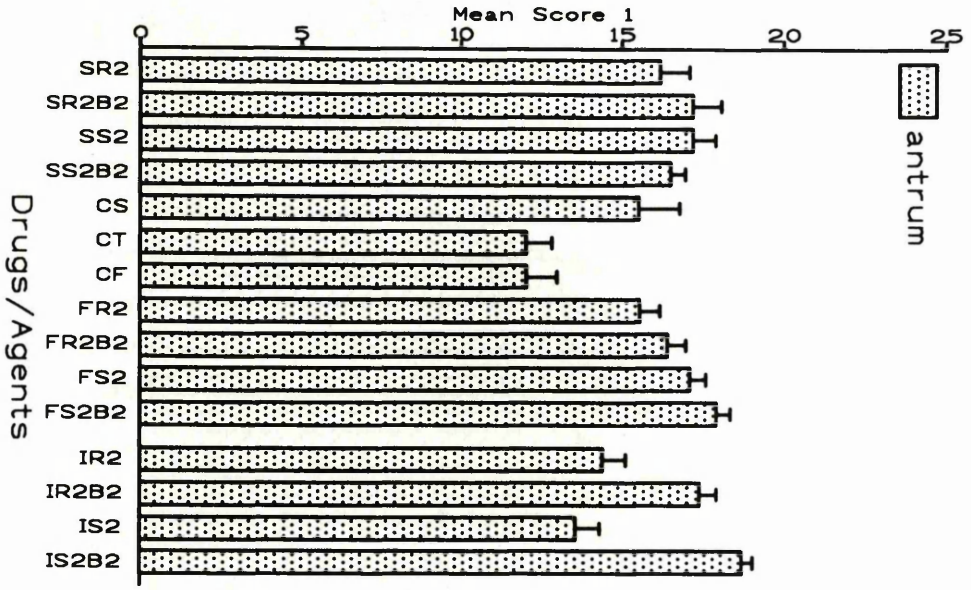
N=Normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.20. Bar graph of corpus scanning electron microscopy general scores obtained from pre-treated rats given injurious agents.



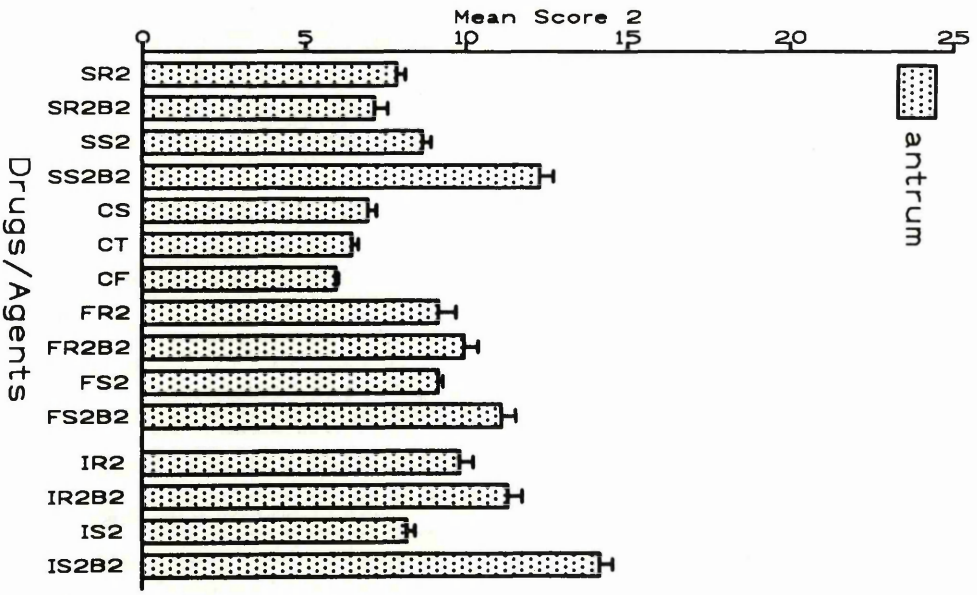
N=normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.21. Bar graph of corpus scanning electron microscopy individual scores obtained from pre-treated rats given injurious agents.



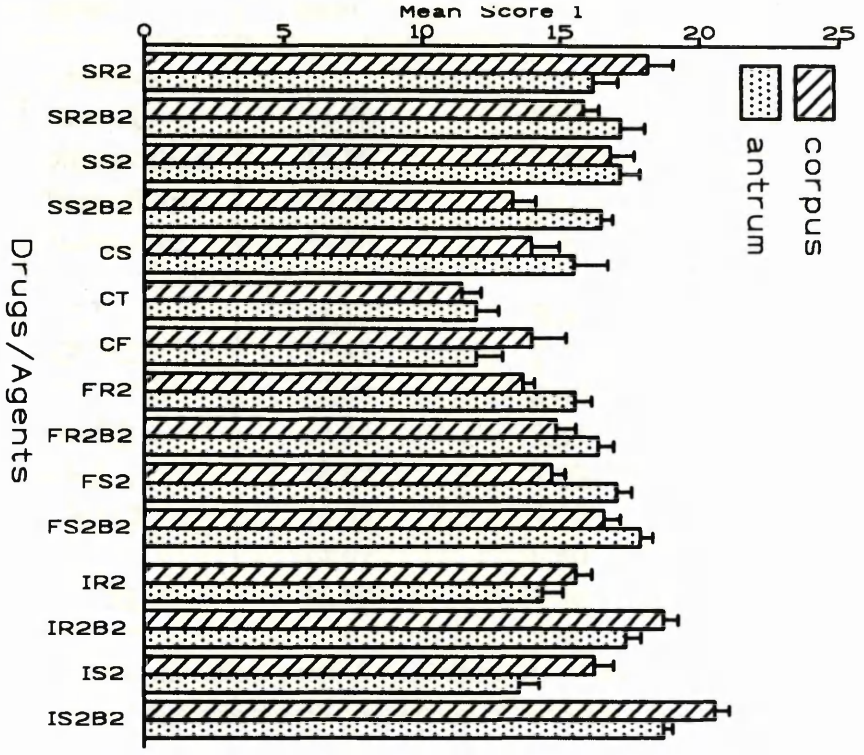
N=normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.22. Bar graph of antrum scanning electron microscopy general scores obtained from pre-treated rats given injurious agents.



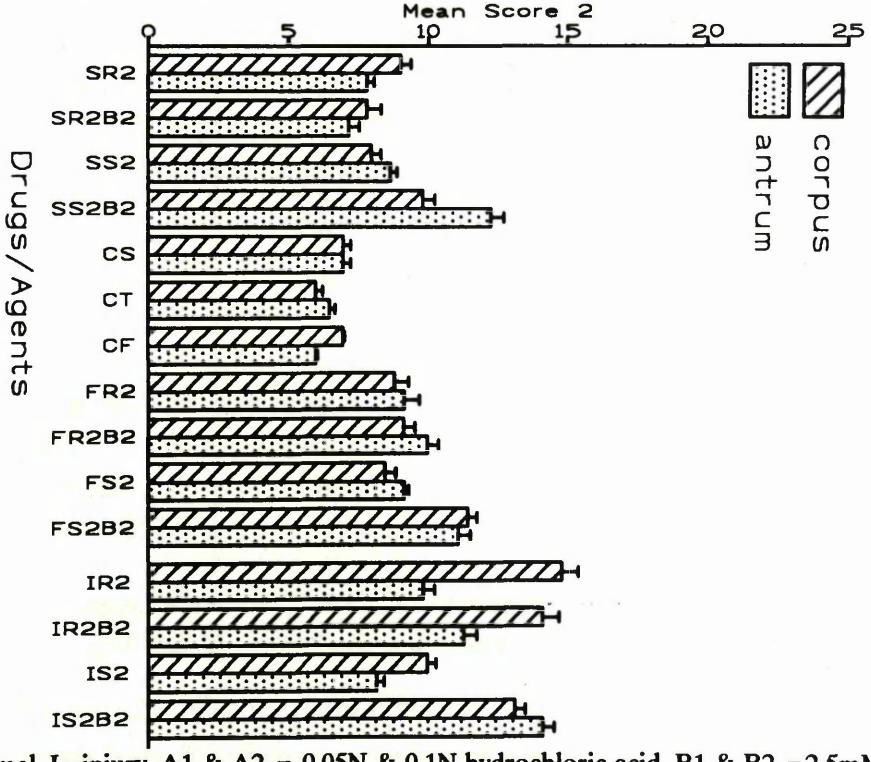
N=normal, I=injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 =2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.23. Bar graph of antrum scanning electron microscopy individual scores obtained from pre-treated rats given injurious agents.



N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.24. Bar graph of scanning electron microscopy general scores obtained from the corpus and antrum of pre-treated rats given injurious agents.



N = normal, I = injury, A1 & A2 = 0.05N & 0.1N hydrochloric acid, B1 & B2 = 2.5mM & 5mM bile, S1 & S2 = 20mM & 40mM aspirin, R1 & R2 = 0.23mM & 0.46mM carprofen

Figure 6.25. Bar graph of scanning electron microscopy individual scores obtained from the corpus and antrum of pre-treated rats given injurious agents.

drug/ agent	Corpus score 1	Corpus score 2	Antrum score 1	Antrum score 2
Controls				
Normal	15.50	7.33	13.90	7.33
Sham-tubed	11.50	7.00	12.00	6.50
Famotidine	14.00	6.00	12.00	6.00
Sucralfate	14.00	7.00	15.50	7.00
SR2	18.17*	9.00	16.17	7.83
SR2B2	15.83	7.83	17.17	7.17
SS2	16.83	8.00	17.17	8.67
SS2B2	13.33	9.83	16.50*	12.33*
FR2	13.67	8.83	15.50*	9.17
FR2B2	14.83	9.17	16.33	10.00
FS2	14.67	8.50	17.00	9.17
FS2B2	16.50	11.50	17.83	11.17

Prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin. * indicates a significant difference between corpus and antrum.

Table 6.20. Mean summed scores of variables for change seen by scanning electron microscopy.

drug/ agent	Controls sham-tubed C	sham-tubed A	sucralfate C	sucralfate A	famotidine C	famotidine A
SR2	S	S	S	ns		
SR2B2	S	S	ns	S		
SS2	S	S	S	S		
SS2B2	ns	S	ns	ns		
FR2	ns	S			ns	S
FR2B2	ns	S			ns	S
FS2	ns	S			ns	S
FS2B2	S	S			S	S
($p<0.05$)						

C=corpus, A=antrum, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significant, ns=not significant.

Table 6.21. Comparison between scanning electron microscopy score 1 for rats given drug & injurious agents and controls.

Score 1 from protected rats then challenged with injurious agents was compared with the control rats and is illustrated Figure 6.21 and in Table 6.21.

With those rats given sucralfate and agent/s, score 1 was significantly higher when compared with the sham-tubed rats in both corpus and antrum, with the exception of the carprofen/bile combination. Differences were less consistent against sucralfate controls. Only those given aspirin alone had significant increases in score 1 in both antrum and carpus. With those given aspirin and bile there was no significant difference in either corpus or antrum.

With those rats given famotidine and agent/s, score 1 was significantly increased in the antrum of all the combinations compared with the sham-tubed rats. Only in the rats given aspirin/bile was there a corporal effect. Against famotidine controls there was similar a significant increase in the antrum, and a corporal effect in the rats given aspirin/bile.

Score 1 from the corpus and antrum were compared in an attempt to establish an area effect (Table 6.20). In only three of the combinations could an area effect be shown. Of these three, sucralfate with carprofen induced a significantly higher score in the corpus. The other two were antral area effect in rats given sucralfate with aspirin/bile, and famotidine with carprofen alone.

Score 2 from those rats then challenged with injurious agents were compared with the control rats and the results are illustrated in Figure 6.22 and in Table 6.20.

drug/ agent	Controls sham-tubed C	A	sucralfate C	A	famotidine C	A
SR2	ns	ns	ns	ns		
SR2B2	ns	ns	ns	ns		
SS2	ns	S	ns	S		
SS2B2	S	S	S	S		
FR2	ns	ns			S	S
FR2B2	S	S			S	S
FS	S	ns			S	S
FS2B2	S	S			S	S
($p < 0.05$)						

C=corpus, A=antrum, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significant, ns=not significant.

Table 6.22. Comparison between scanning electron microscopy score 2 for rats given drug with injurious agents and controls.

In the sucralfate subgroup, there was no significant difference in either corpus or

antrum in those given carprofen alone or in combination. Those given aspirin combinations had a significant increase in all areas except the corpus of rats given aspirin alone. The difference between the corpus of sham-tubed and those given aspirin/bile was most marked. Compared with the sucralfate controls a similar pattern emerged. There was no difference in those given sucralfate alone and those given carprofen combinations. The same aspirin combinations and areas showed significant increase in score 2.

In the famotidine subgroup, there were significant increases in score 2 against both sham-tubed and famotidine control rats. The exception to this was the lack of difference between carprofen challenged rats and sham-tubed rats. A lack of effect was also apparent in the antrum of rats given aspirin alone.

Only in the rats given sucralfate and aspirin/bile was there an area effect, with the antrum having a significantly higher score than the antrum.

The scores from the different injurious agents used were compared where appropriate to each other to see if there was an agent effect. Within the sucralfate subgroup, only the corpus of the rats given aspirin/bile showed any difference (decrease) from the same areas of rats given other combinations (carprofen/bile and aspirin). In the famotidine subgroup there were no intra-group differences detected.

A similar exercise was carried out between sucralfate and famotidine subgroups for score 1 (Table 6.23) and score 2 (Table 6.24).

drug/ agent	SR2		SR2B2		SS2		SS2B2	
	C	A	C	A	C	A	C	A
FR2	S	ns						
FR2B2			ns	ns				
FS2					S	ns		
FS2B2							@S	ns
($p<0.05$)								

C=corpus, A=antrum, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significantly higher, ns=not significant, @S=significantly smaller.

Table 6.23. Comparison of score 1 between sucralfate and famotidine subgroups.

There was no difference in the antrum between those given sucralfate and famotidine. The score 1 for those given carprofen or aspirin alone was higher in the corpus of those rats tubed with sucralfate than for famotidine. Those rats given aspirin/bile had lower scores in corpus when given sucralfate than those given famotidine.

drug/ agent	SR2		SR2B2		SS2		SS2B2	
	C	A	C	A	C	A	C	A
FR2	ns	ns						
FR2B2			@S	ns				
FS2					ns	ns		
FS2B2							ns	ns
($p<0.05$)								

C=corpus, A=antrum, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significantly higher, @S=significantly smaller, ns=not significant.

Table 6.24. Comparison of score 2 between sucralfate and famotidine subgroups.

For score 2, there was little difference between the drugs used. The only significant difference was in the corpus of rats challenged with carprofen/bile, where the individual score was higher for famotidine pre-treated rats.

The effect of pre-treating rats with sucralfate and famotidine was examined by comparing the scores obtained from pre-treated rats and those from the injurious group given the same concentration of agents (Table 6.25).

For score 1 the results of comparison were rather uneven. In the sucralfate subgroup 5/8 areas demonstrated no significant difference. Of the remaining three areas, two showed an increased score with sucralfate compared with the comparable injured rats. In the famotidine subgroup, 4/8 areas showed significant differences. In three of the four, the corpus pre-treated rats had lower scores than corresponding rats from the injurious groups.

With score 2, the individual features indicative of damage were lower in those rats pretreated with sucralfate and challenged with carprofen ±bile compared with rats from the injurious group. With famotidine pre-treatment only the corpus of rats challenged with carprofen ±bile had lower scores. Pre-treatment with sucralfate and famotidine made no significant difference to the scores obtained with asprin ±bile challenge.

Subjecting the data to polychotomous logistic regression (BMDP) suggested that

sucralfate had a more protective effect than famotidine, but this was not substantiated by generalised linear modelling (GLIM).

drug/ agent	score 1 C	score 1 A	score 2 C	score 2 A	agent
SR2	<S	ns	S>	S>	IR2
SR2B2	ns	ns	S>	S>	IR2B2
SS2	ns	<S	ns	ns	S2
SS2B2	S>	ns	ns	ns	S2B2
FR2	S>	ns	S>	ns	IR2
FR2B2	S>	ns	S>	ns	IR2B2
FS2S>	ns	<S	ns	ns	S2
FS2B2 ($p<0.05$)	S>	ns	ns	ns	S2B2

C=corpus, A=antrum, prefix F=famotidine, prefix S=sucralfate, R2=0.46mM carprofen, B2=5mM bile, S2=40mM aspirin, S=significant, ns=not significant. < > points to combination giving significantly higher score.

Table 6.25. Comparison between scores 1 and 2 obtained from injurious groups and protective groups.

6.3.3.1 Individual Control

There was little variability in the individual grades ascribed to the different features noted between those rats not tubed and those sham-tubed (Tables 6.18 and 6.19). There were a few differences between the control rats given sucralfate and famotidine. The rats given famotidine had a much more complete mucus covering in the corpus than either the sucralfate or sham-tubed rats. This finding was not mirrored in the antrum. In both the corpus and antrum the thickness of the mucus covering was much greater in the sucralfate rats than in the famotidine rats. Apart from these changes the only other change worthy of note was an increase in the number of rosettes found in the antrum of the sucralfate rats. The features ascribed to damage were the same in both groups. No time effect emerged from examination of the individual scores from any of the groups of rats, regardless of injurious agent.

6.3.3.2 Sucralfate

When the rats were challenged with aspirin alone the corporal mucus covering was much more complete, but the thickness was reduced in both corpus and antrum (Figures 6.30-6.32). An increased number of extruded cells and enlarged gastric pits were also a feature in both corpus and antrum. In the corpus more cells had

intracellular contents exposed. In the antrum there was loss of cobblestoning and more prominent intracellular junctions, but no increased individual scores.

The addition of bile to aspirin resulted in a further reduction in the thickness of the mucus covering in the corpus and antrum (Figures 6.35 and 6.36). An additional feature evident in both corpus and antrum was an increase in the number of cells showing apical ruffling and apical holes.

Carprofen alone caused an increased mucus covering with a reduction in thickness, and a rise in cell extrusions. In the corpus a loss of cobblestoning and some increase in apical erosions were manifest. In the antrum, only apical ruffling was accentuated amongst the individual scores (Figures 6.28 and 6.29).

With the addition of bile, the mucus covering and thickness in the corpus matched the control rats and therefore a reduction over carprofen alone. In the antrum the mucus covering and thickness were similar to the carprofen alone, showing a decrease in thickness over the control rats. As with carprofen alone there was an increase in the number of extruded cells (Figures 6.33-6.34). The carprofen and bile mixture caused an increased convexity of the cells in both areas.

When the effect of carprofen and aspirin alone on those rats given sucralfate was assessed the differences were mainly in the general scores. A more complete mucus covering and thinner coat was found in those rats given carprofen. Also in the corpus, more apical erosions were found in the carprofen than the aspirin group. Aspirin caused a greater widening of the gastric pits in both corpus and antrum compared with carprofen.

Comparison between those rats given bile in addition to the NSAIDs showed that in aspirin/bile rats there was greater mucus covering, reduction in the thickness of the coat, more apical erosions and holes in the corpus. In the antrum the scores for gastric pits, apical ruffling, holes, concavity and empty shells were all greater in the aspirin/bile than in the carprofen/bile group.

6.3.3.3 Famotidine

The rats given aspirin alone following pre-treatment with famotidine had a more incomplete covering of mucus in the corpus than the control rats (Figures 6.39 and 6.40). The mucus thickness was similar in the antrum as was the mucus covering. There was an accentuation of the gastric pit openings and convexity of the surface cells in the corpus and antrum. The only damage feature in the corpus that differed from the controls was an increased number of cells showing concavity of the apical membrane. In contrast in the antrum, all the general scores; cell extrusion, rosettes,

loss of cobblestoning, prominence intercellular junctions and convexity were higher than controls. Of the individual scores, concavity, apical erosions and empty shells were much more prominent.

Aspirin & Bile

The addition of bile to the injurious mixture served to produce higher general and individual scores. In comparison to the corpus of the rats given aspirin alone, four of the six individual scores were much higher (Figures 6.43 and 6.44).

Carprofen

Compared with the control rats the corporal mucus covering was less complete (Figures 6.37 and 6.38). Apart from this, the only other general score that showed a difference was the number of cells showing increased convexity. Evidence of damage was confined to more cells showing a concave surface or apical erosions. In the antrum, rosette formation, prominence of intracellular junctions and convexity of cells was increased. The only individual score which was greater than controls was apical erosions.

Carprofen/Bile

The addition of bile caused a further diminution in mucus covering in both corpus and antrum (Figures 6.41 and 6.42). No other change was apparent in the corpus compared with those given carprofen alone. In the antrum, other features with increased scores were extruded cells, loss of cobblestoning and apical ruffling.

6.3.3.4 *Injury versus Protection*

When the individual scores obtained from rats pre-treated with sucralfate and challenged with aspirin alone were compared with the injured rats, sucralfate caused increased mucus covering and thickness of the mucus blanket in both corpus and antrum. In the corpus, cell convexity and extruded cells were greater in those given sucralfate. In the antrum, gastric pit enlargement and loss of cobblestoning were reduced with the use of sucralfate. From the score obtained for "*damage*" the scores for sucralfate were higher or unchanged in the corpus and antrum.

In contrast, when the effect of added bile was examined, the general and individual scores from both corpus and antrum were reduced with the pre-treatment of sucralfate in most of the features looked at, with the exception of increased cell extrusion in the antrum,.

When carprofen was substituted for aspirin the general scores for the corpus were higher in those rats given sucralfate, but the individual scores were reduced for all six features. In the antrum there was little difference in the scores obtained for rats with or without sucralfate, apart from increased mucus covering in sucralfate rats.

With the addition of bile to the carprofen challenge solution, the scores obtained from the protected rats were for, almost all features, lower than those obtained from rats injurious agents alone.

The general scores obtained from rats pre-treated with famotidine before challenge with aspirin were little different from those from the injury group with the exception of a poorer mucus covering in the corpus and accentuation of the cell convexity in both the corpus and antrum. In the antrum, the rosette formation and intercellular junctions were more prominent. However, the individual scores in the corpus were all much lower with famotidine, but this was not evident in the antrum.

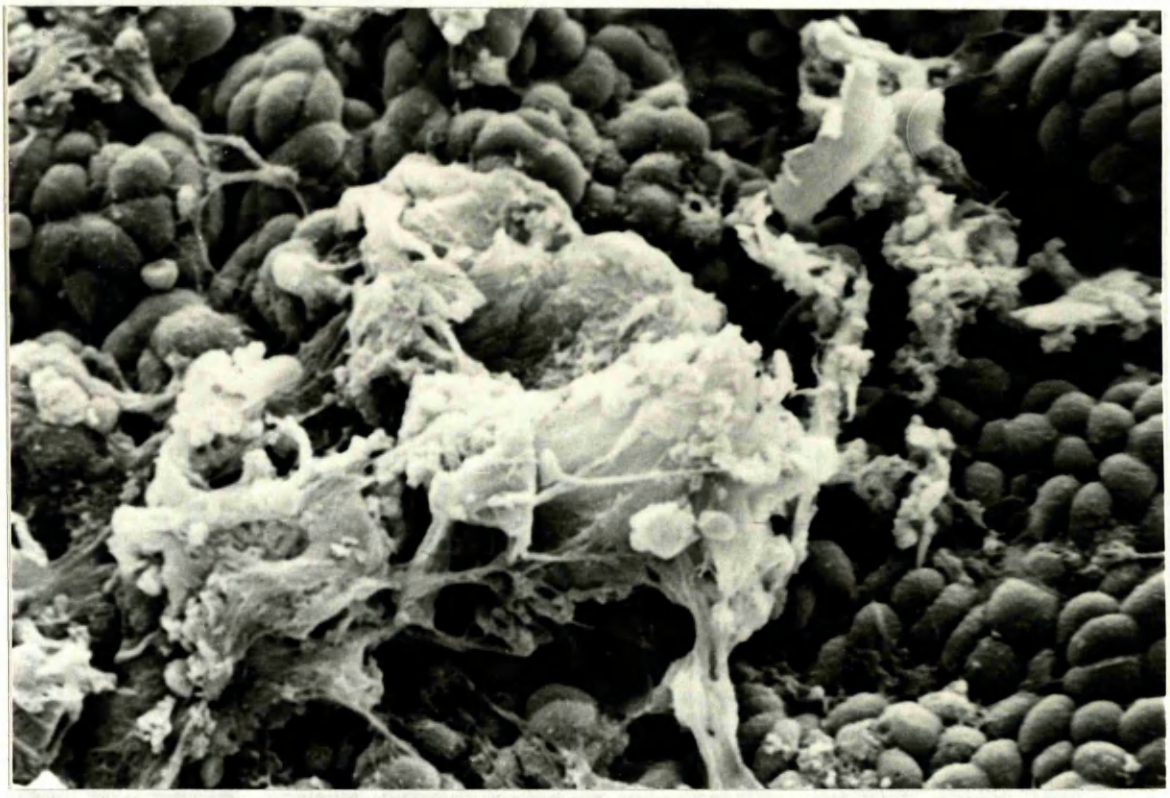
With the addition of bile to the challenge solution of aspirin, famotidine had a similar effect on the general scores in the corpus reducing cell extrusion, rosette formation and enlargement of gastric pits. In the antrum no sparing effect was evident. An amelioration in the individual scores was restricted to reduced apical ruffling and cell concavities in the corpus and antrum, with a reduction in empty shells in the antrum.

Comparison between the rats given famotidine & carprofen and the injured group given carprofen at 0.46mM showed that there was little difference in the scores obtained from the antrum, with the pre-treated rats having higher intercellular junction and concavity scores, but the injured group having higher apical ruffling and hole scores. In the corpus the scores for almost all the features were lower with those rats given famotidine.

The effect of the addition of bile to the equation showed very similar findings as far as the scores were concerned. In the corpus the scores were nearly all lower for those given famotidine, in none were the scores for famotidine higher. In the antrum, the both mucus covering and thickness were reduced with the addition of bile and no sparing effect from famotidine was detected in individual scores.

6.3.3.5 Sucralfate versus Famotidine

The scores from the rats given famotidine and sucralfate and challenged with injurious agents were summed separately and compared. Rats given sucralfate had thicker mucus coats and increased numbers of extruded cells in the corpus. In the antrum the mucus covering famotidine caused an increased loss of cobblestoning.



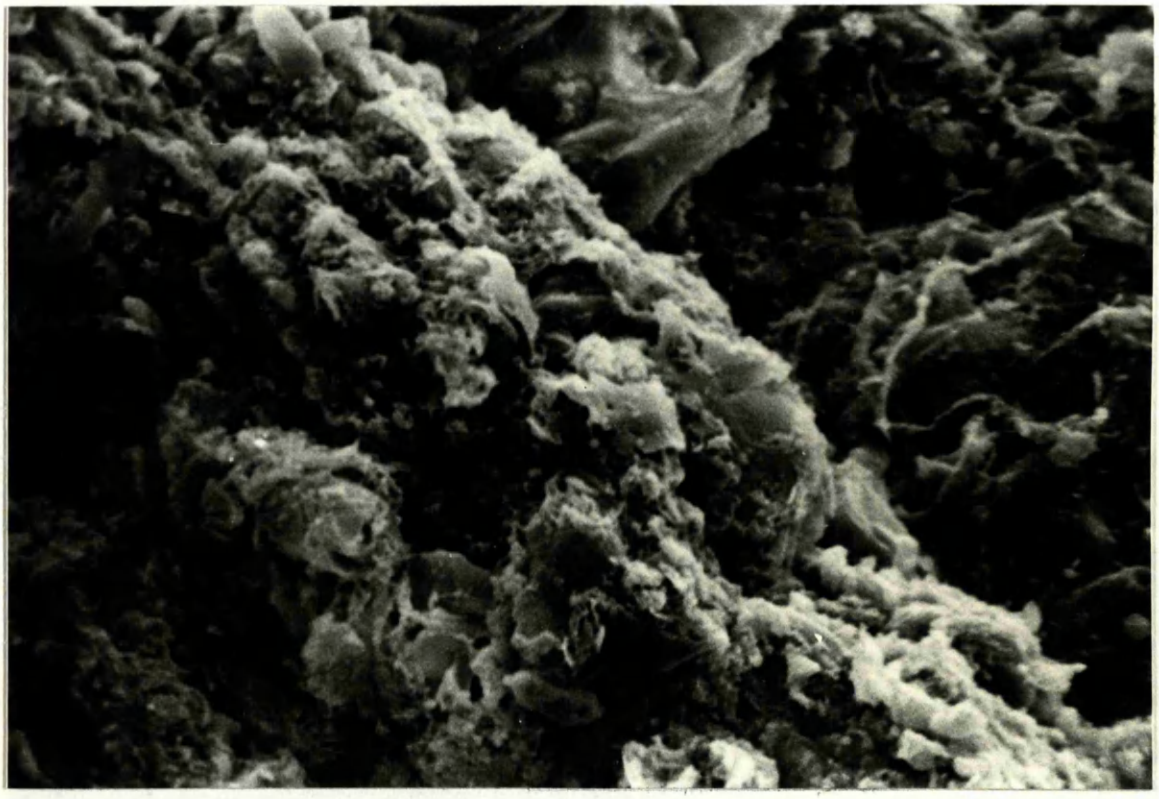


Figure 6.26a & 6.26b. Scanning electron micrographs following pre-treatment with sucralfate. An island of mucus topped with sucralfate is present in the corpus (6.26a). In the antrum a much thicker layer is present and the outlines of embedded cells are visible (6.26b). (x1440)

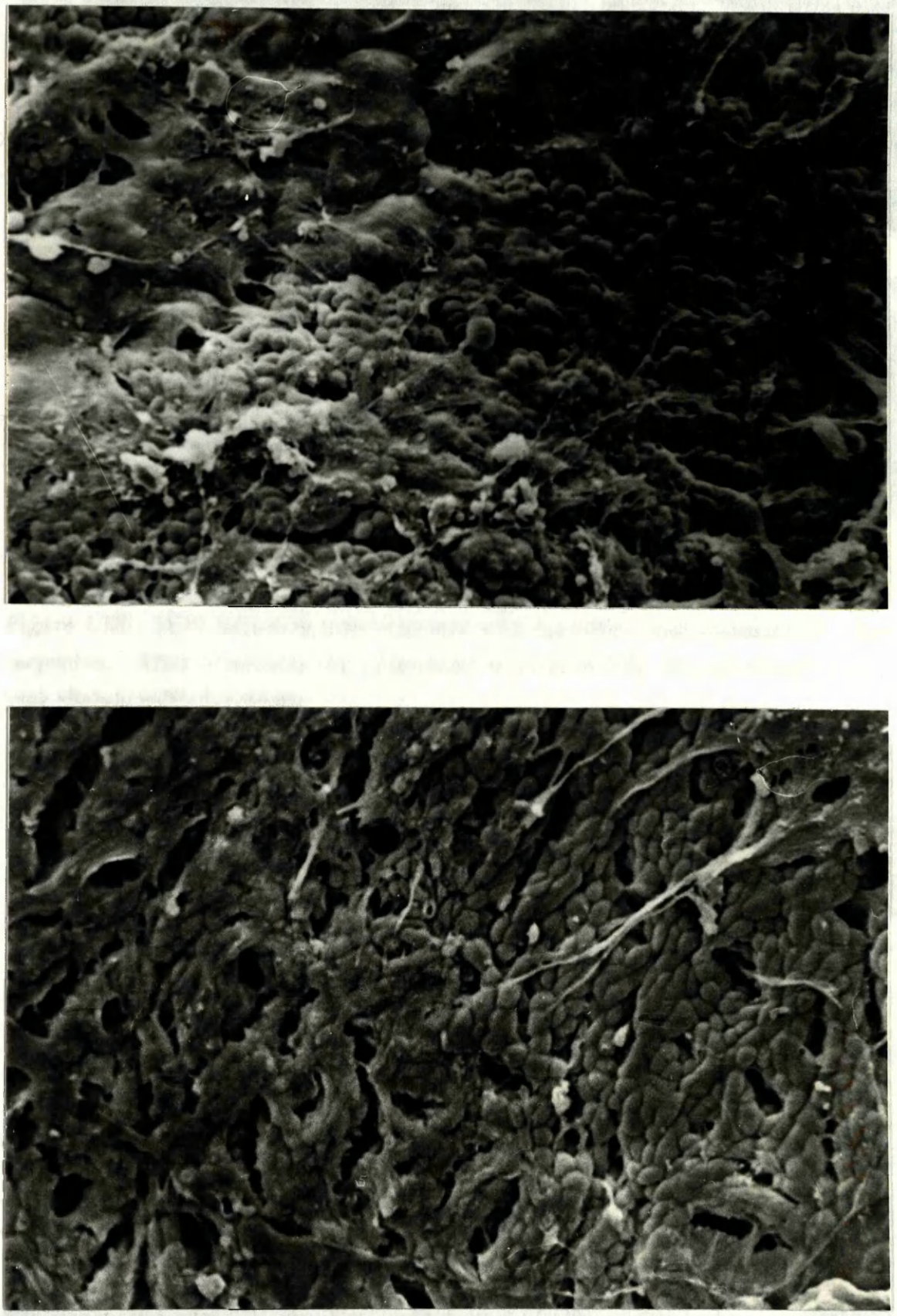


Figure 6.27a & 6.27b. Scanning electron micrographs following pre-treatment with famotidine. This part of the corpus is incompletely covered by a medium layer of mucus (6.27a). The antrum shows some strands of mucus, but the underlying superficial epithelial cells look normal (6.27b). (x1440)

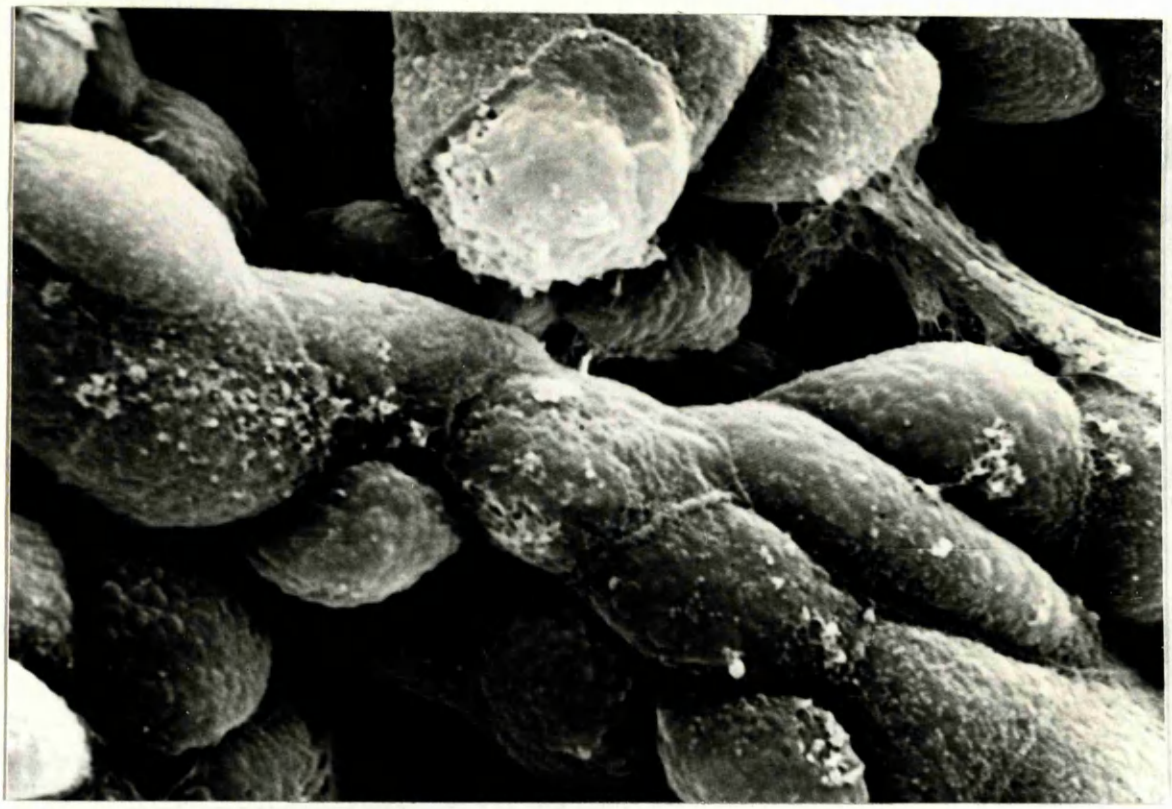


Figure 6.28. SEM following pre-treatment with sucralfate and exposure to 0.46mM carprofen. After 5 minutes the epithelium is substantially normal though the cells look slightly ruffled. (x5600)

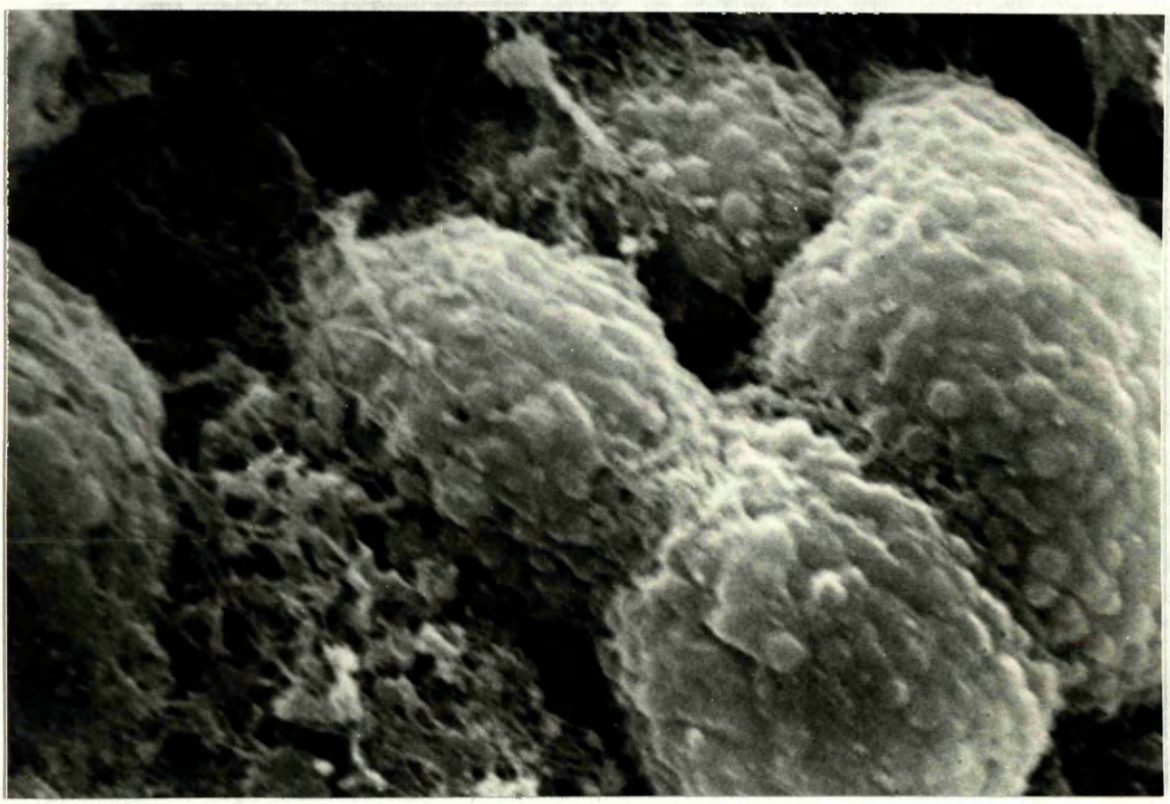


Figure 6.29. SEM following pre-treatment with sucralfate and exposure to 0.46mM carprofen. After 15 minutes the cells are quite swollen and there are small blobs emanating from the surface, possibly mucous granules. (x11250)

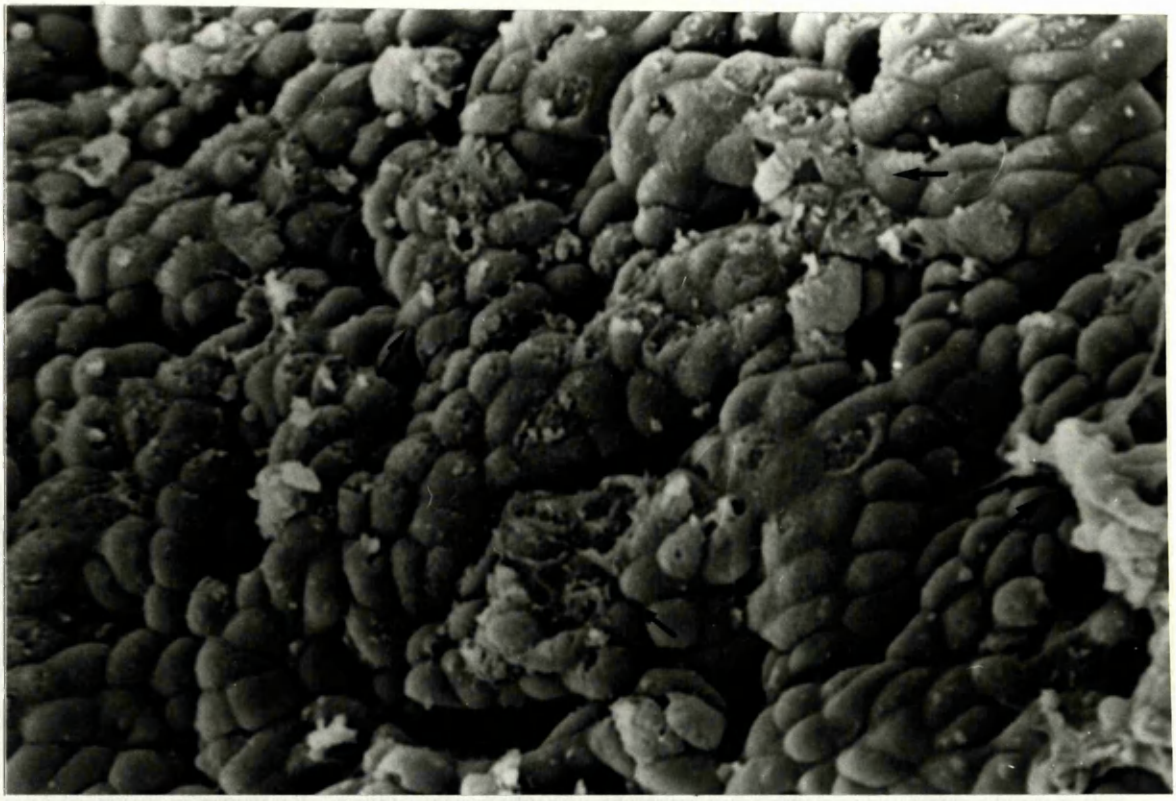


Figure 6.30. SEM following pre-treatment with sucralfate and exposure to 40mM aspirin. After 2 minutes there are areas of damaged cells with extruded cells present (*small arrows*). In places there are small pieces of sucralfate (*large arrow*). (x1440)

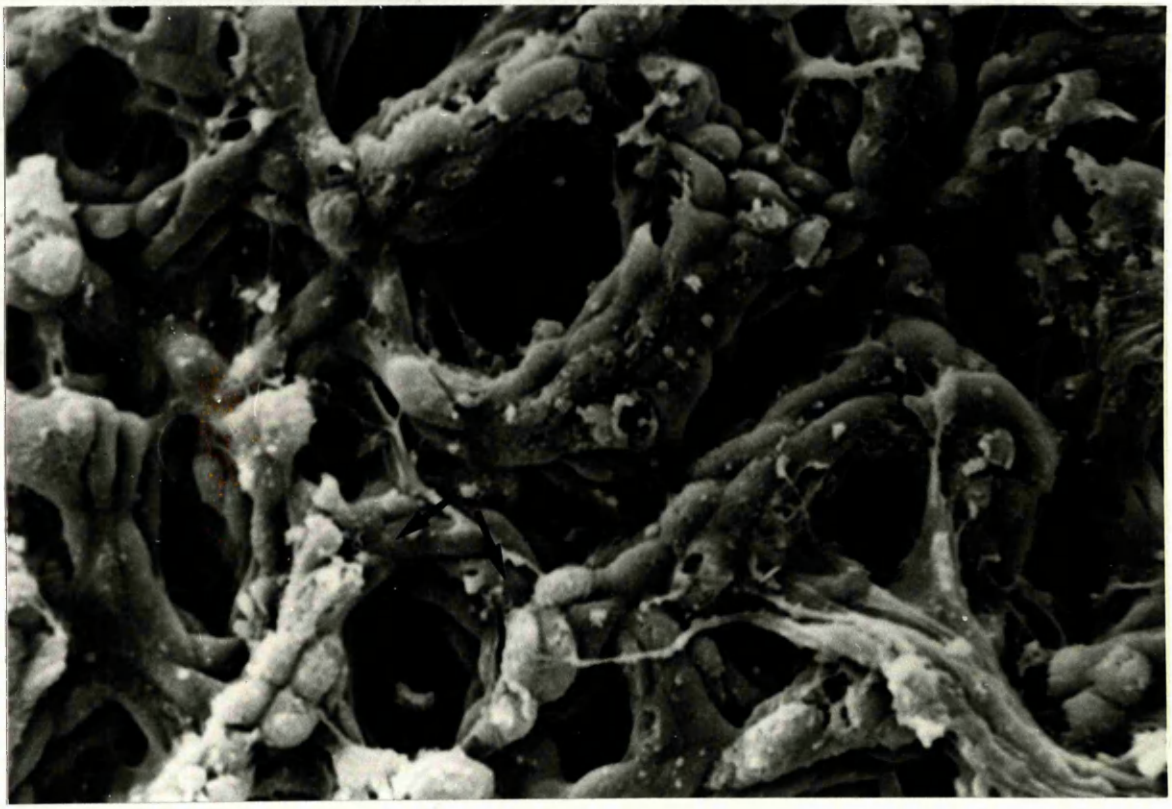


Figure 6.31. SEM following pre-treatment with sucralfate and exposure to 40mM aspirin. Antrum after 15 minutes, the pits are somewhat enlarged and the cells show some apical erosions, and numbers are discarded into the lumen (arrows). (x1440)

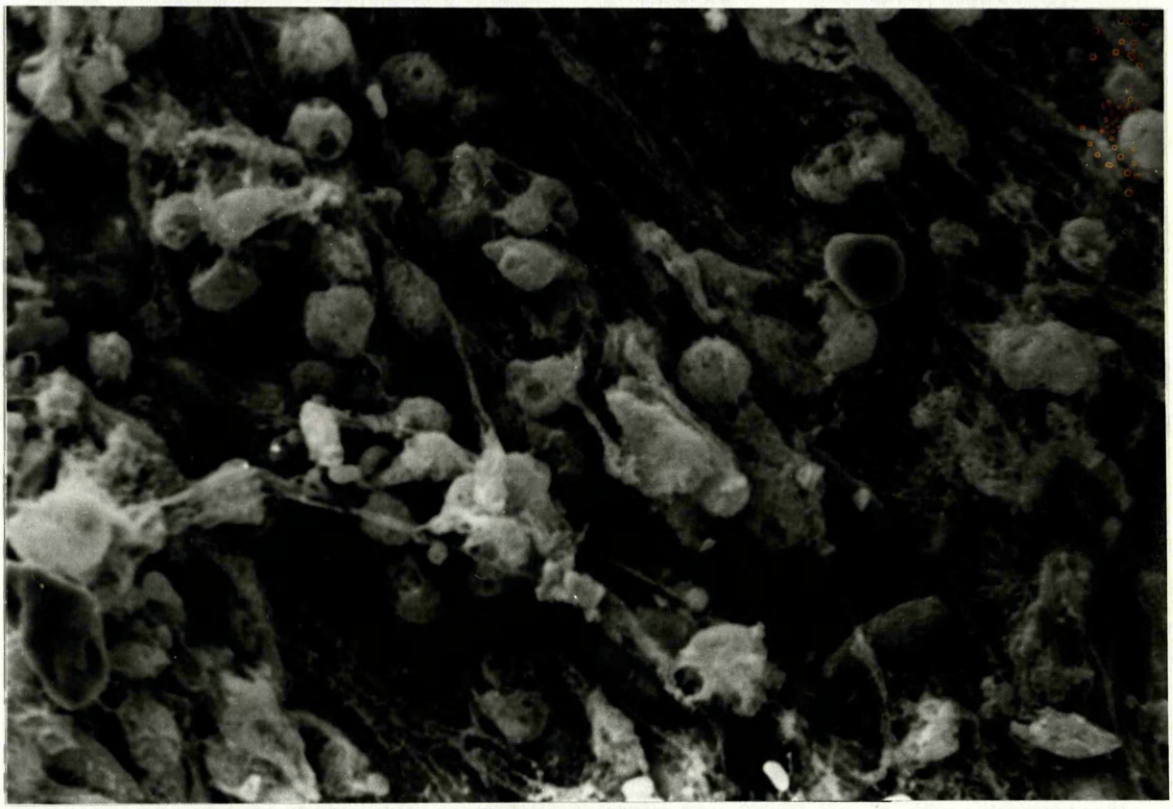


Figure 6.32. SEM following pre-treatment with sucralfate and exposure to 40mM aspirin. Antrum after 15 minutes, the mucus layer has trapped large numbers of cells and strands of sucralfate can be seen. (x2800)

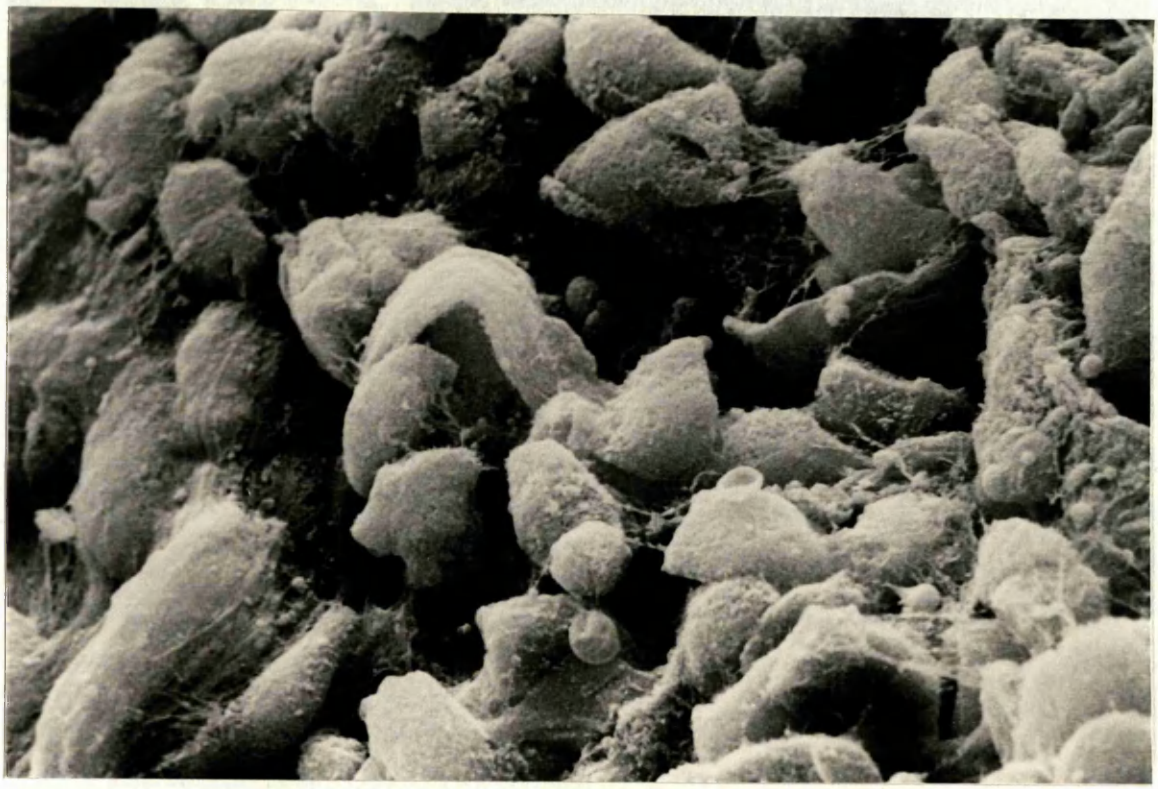


Figure 6.33. SEM following pre-treatment with sucralfate and exposure to 0.46mM carprofen/ 5mM bile. Large numbers of extruded cells are trapped in the mucus/sucralfate layer in the antrum.

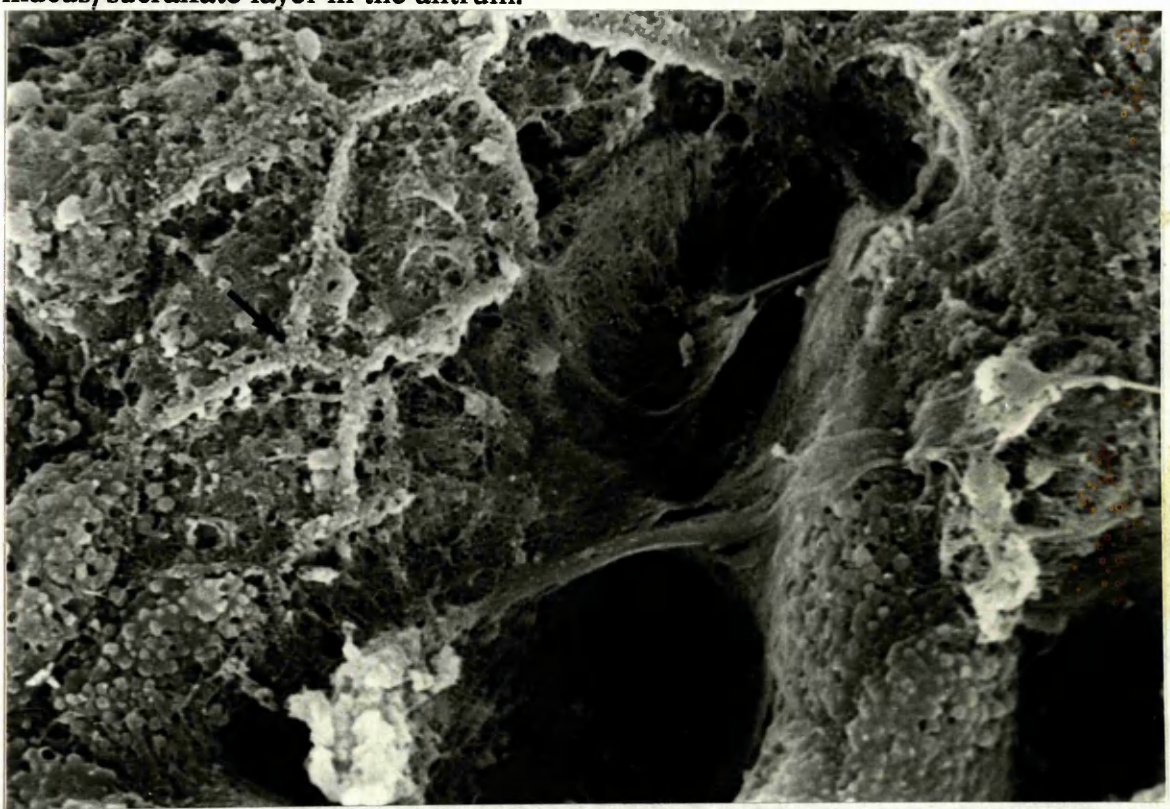


Figure 6.34. SEM following pre-treatment with sucralfate and exposure to 0.46mM carprofen/ 5mM bile. The cells have a marked beaded appearance due massive mucous release. Some of the apical membranes have collapsed highlighting the intercellular junctions (arrow). (x5600)

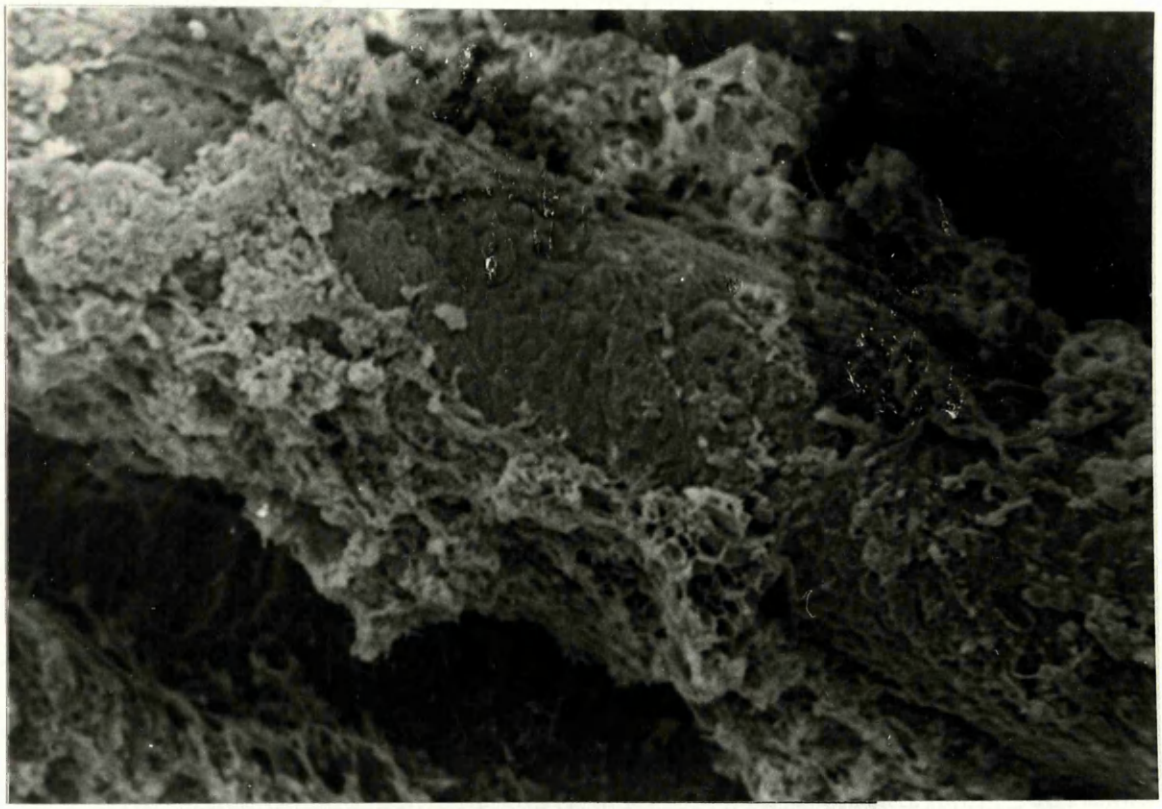


Figure 6.35. SEM following pre-treatment with sucralfate and exposure to 40mM aspirin/ 5mM bile. The extensive coating of mucus/sucralfate almost completely obscures this rugal fold. (x360)

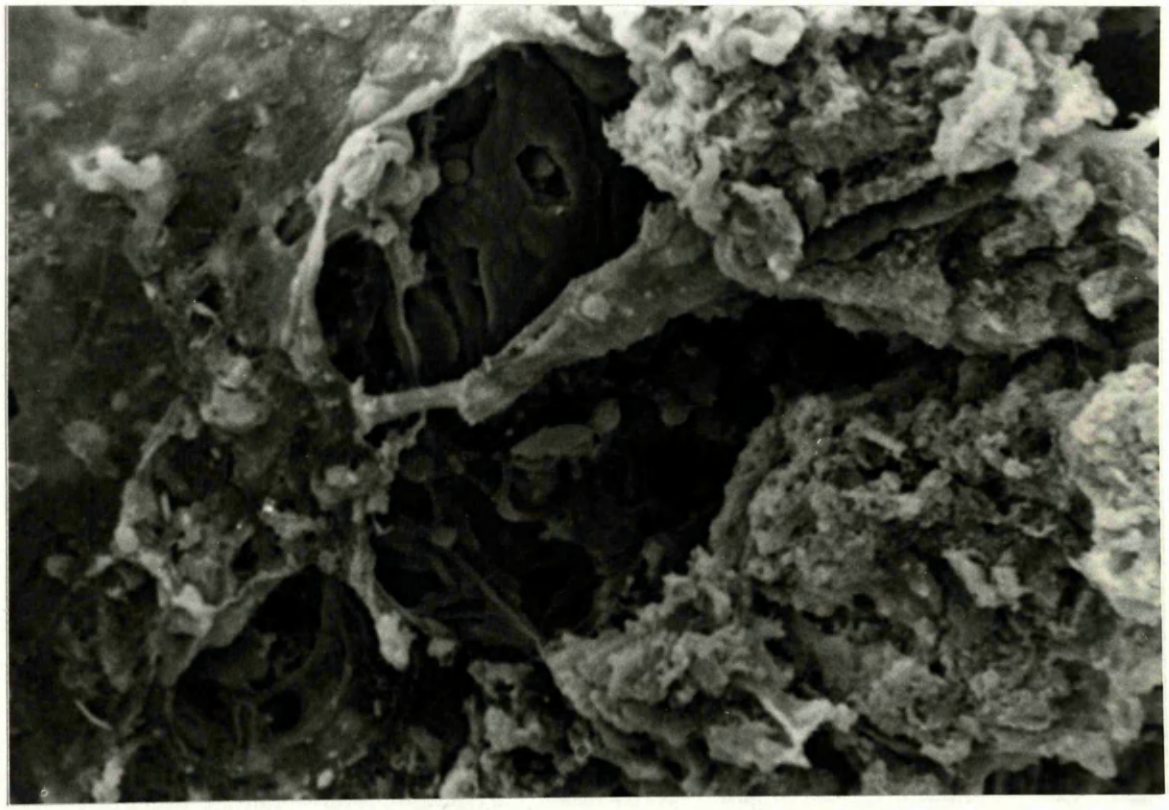


Figure 6.36. SEM following pre-treatment with sucralfate and exposure to 40mM aspirin/ 5mM bile. Extensive coating of sucralfate/ mucus is present and the outlines of trapped cells are visible. Beneath relatively normal pits are present. (x720)

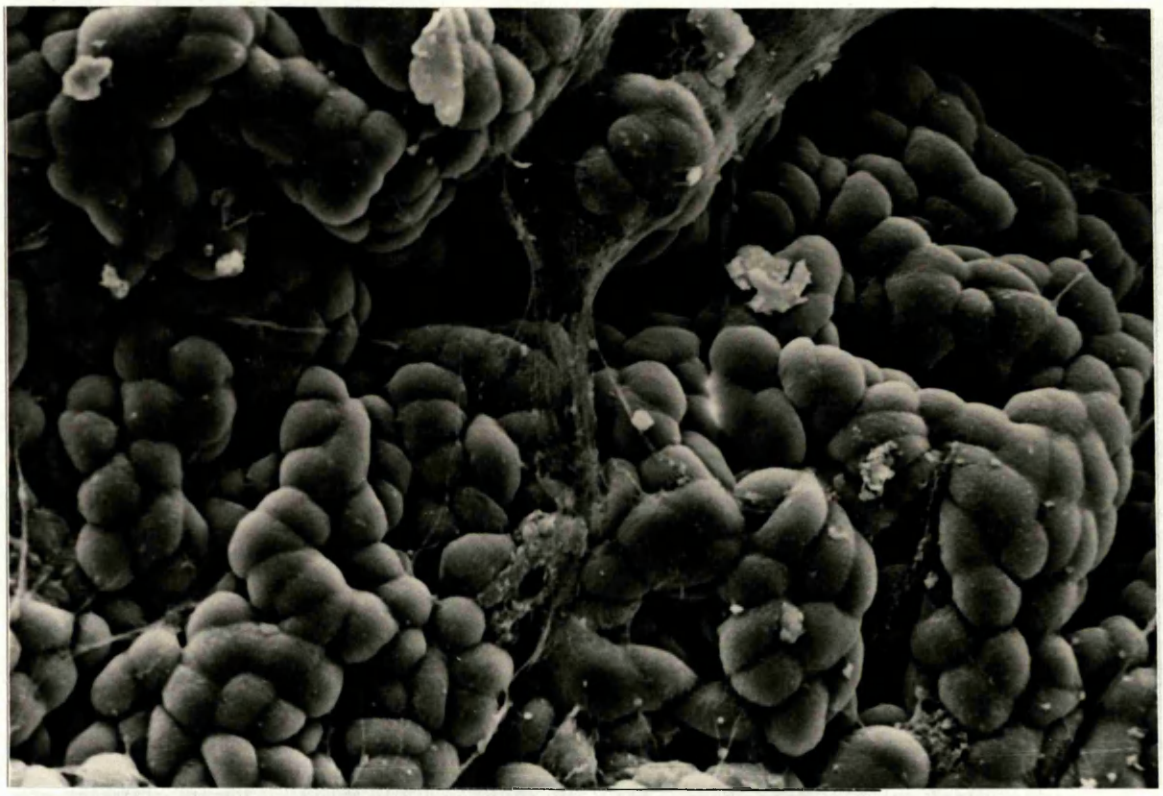


Figure 6.37. SEM following pre-treatment with famotidine and exposure to 0.46mM carprofen. Traces of mucus cover essentially normal epithelium in the corpus. (x1440)

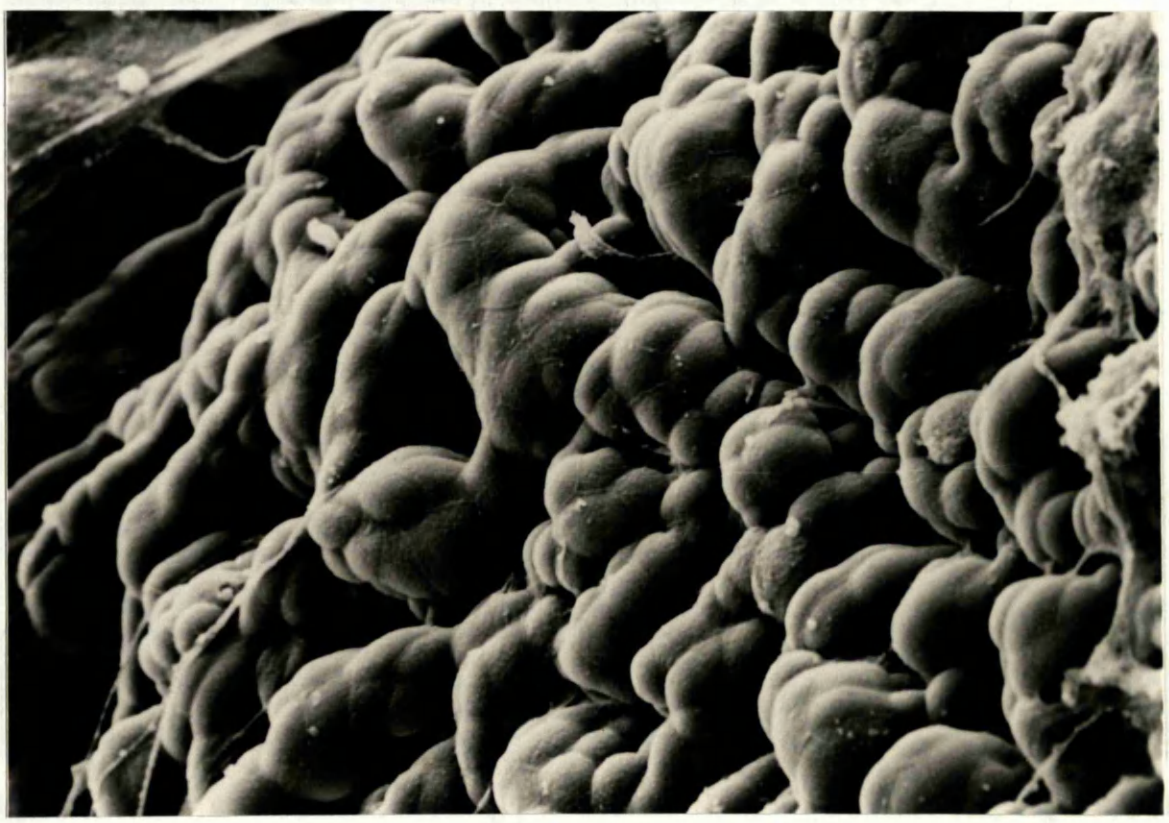


Figure 6.38. SEM following pre-treatment with famotidine and exposure to 0.46mM carprofen. In the antrum the cells are swollen but have an extremely smooth surface, a few cells have apical erosions. (x1440)

Figure 6.39a & 6.39b. SEM following pre-treatment with famotidine and exposure to 40mM aspirin. Traces of mucus cover the epithelium after 2 minutes in the corpus. In the antrum after 2 minutes there are large islands of debris sitting on top of the normal mucus carpet. (x1440, x720)

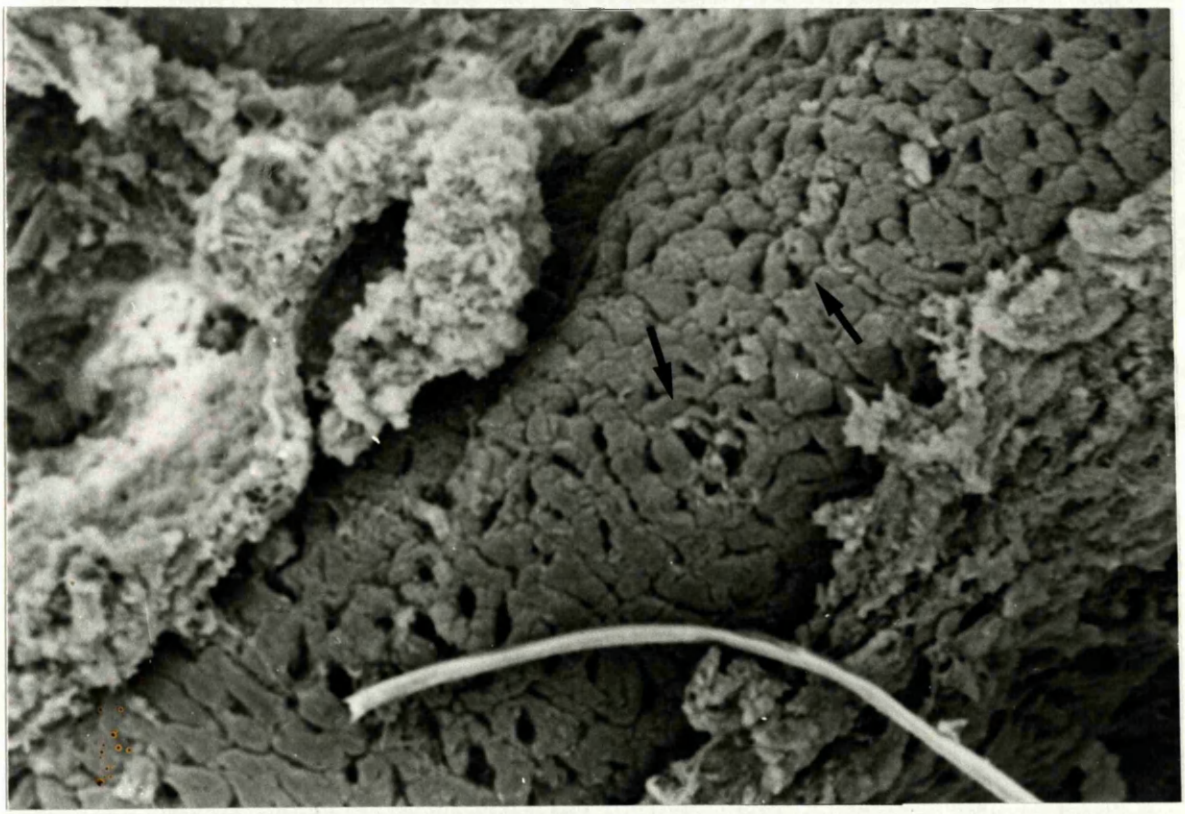


Figure 6.40. SEM following pre-treatment with famotidine and exposure to 40mM aspirin. The walls of this rugal fold are covered in a thick granular mucus coat. On the rugal tip a number of foci of cell damage is apparent (arrows). (x360)

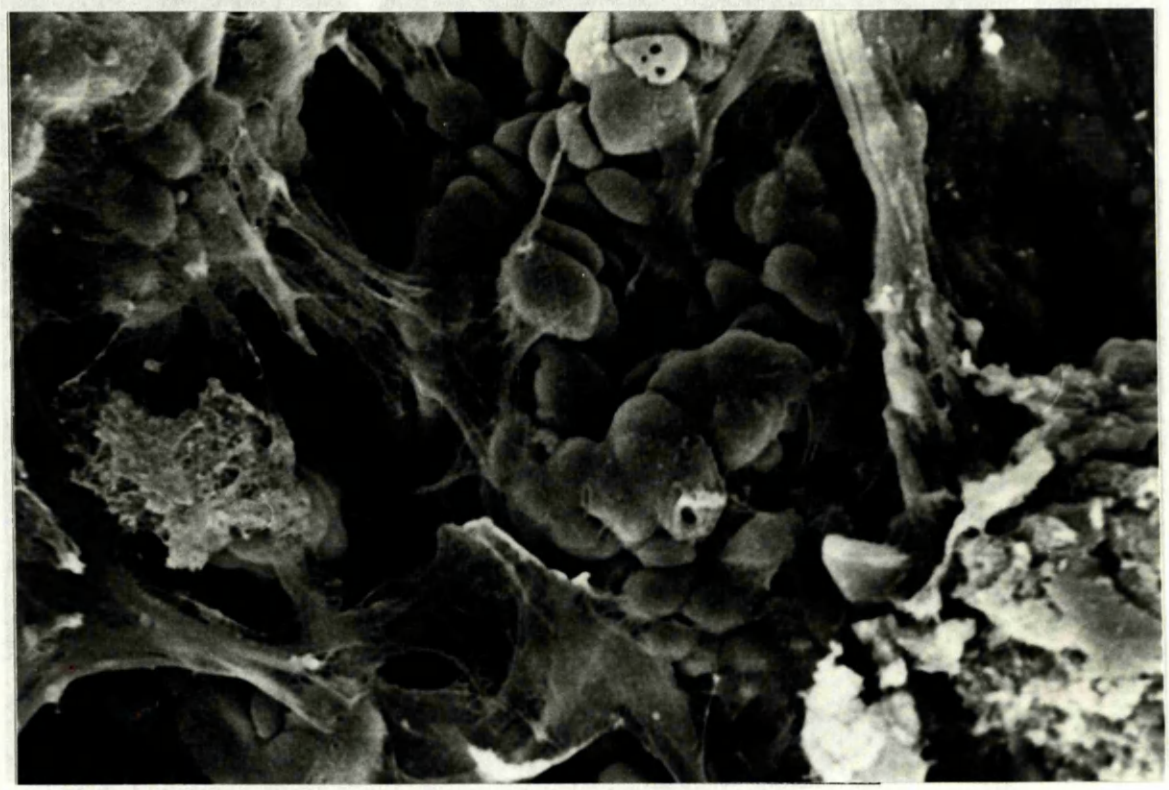


Figure 6.41. SEM following pre-treatment with famotidine and exposure to 0.46mM carprofen/ 5mM bile. The gastric pit cells show some derangement and overlying this the mucus carpet is covered by granular debris. (x1440)

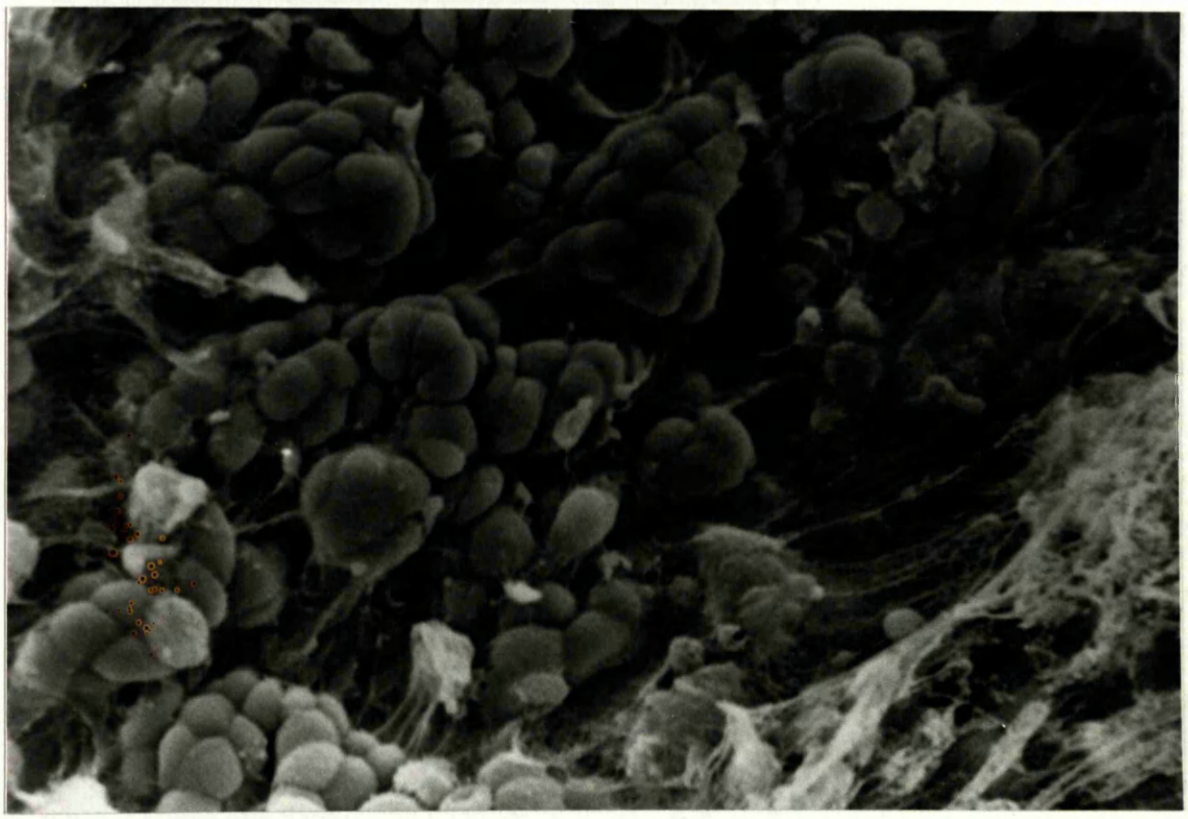


Figure 6.42. SEM following pre-treatment with famotidine and exposure to 0.46mM carprofen/ 5mM bile. After 5 minutes the pits show some derangement and many rosettes and extruded cells are evident. (x1440)

Figure 6.43. SEM following pre-treatment with famotidine and exposure to 40mM aspirin/ 5mM bile. There is thick debris present but the visible cells show extensive apical erosions and exposed intracellular contents. (x2800)

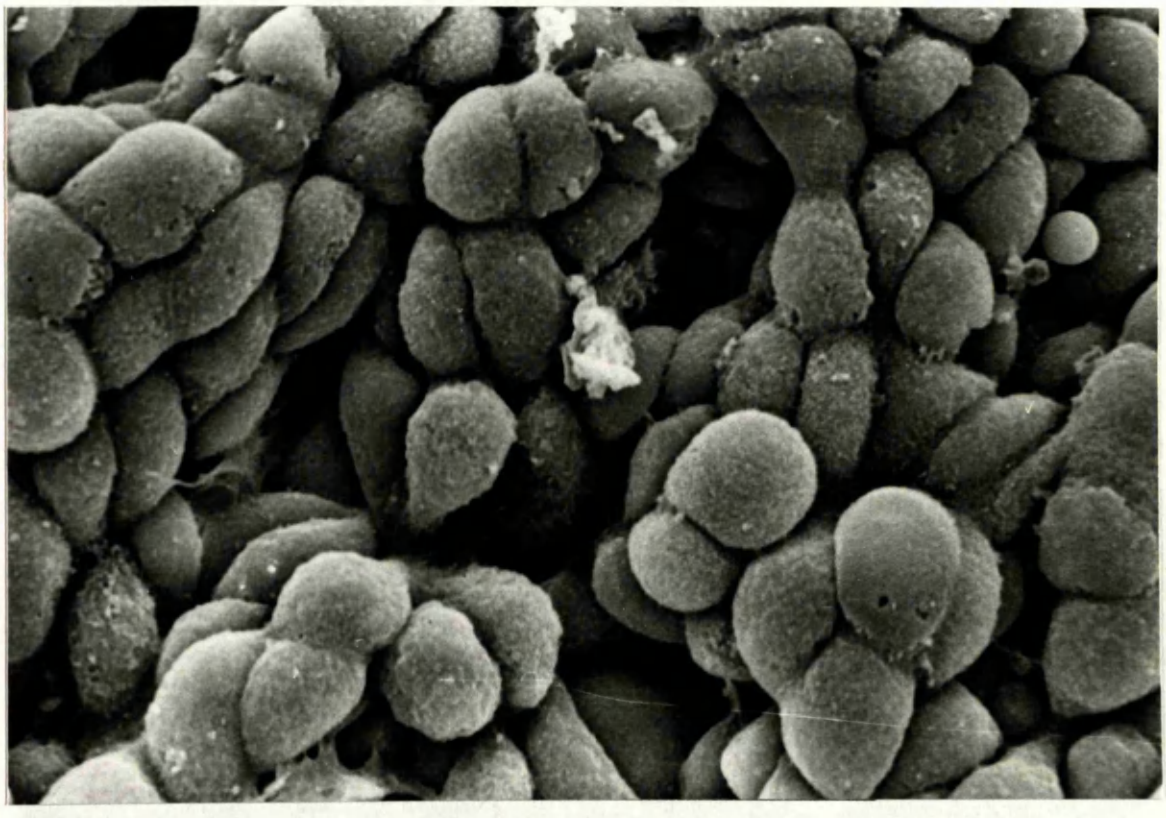


Figure 6.44. SEM following pre-treatment with famotidine and exposure to 40mM aspirin/ 5mM bile. Many of the cells have holes, are swollen, and some are in the process of being extruded. (x2800)

6.3.4 Transmission Electron Microscopy

The surface epithelial structure and integrity of those rats given sucralfate and famotidine alone were not similar to the normal rats or those sham-tubed. In those given sucralfate alone had the apical mucous granule packs, but debris covering the apical membrane (Figure 6.45). In contrast those given famotidine alone the apical membrane was generally intact, but there was a lack of mucous granules and in numbers of cells occasional and patchy areas of vacuolation (Figure 6.46).

In those rats pre-treated with famotidine and challenged with injurious agents the lack of mucous granule packs was again evident. In addition there was a decrease in nuclear and cytoplasmic density with extensive vacuolation, giving the cytoplasm a very lacy appearance (Figures 6.47-6.49).

In those rats pre-treated with sucralfate and challenged with injurious agents there was a characteristic coating of debris above the apical membrane. In addition many of the cells had also a loss of cytoplasmic density with missing nuclei and cell contents exposed or streaming into the lumen. Only after 15 minutes in those rats given aspirin/bile was the type of in situ disruption that was seen in the injured rats seen, with extensive disruption of the cytoplasm and clumping of nuclear chromatin (Figures 6.50-6.52).

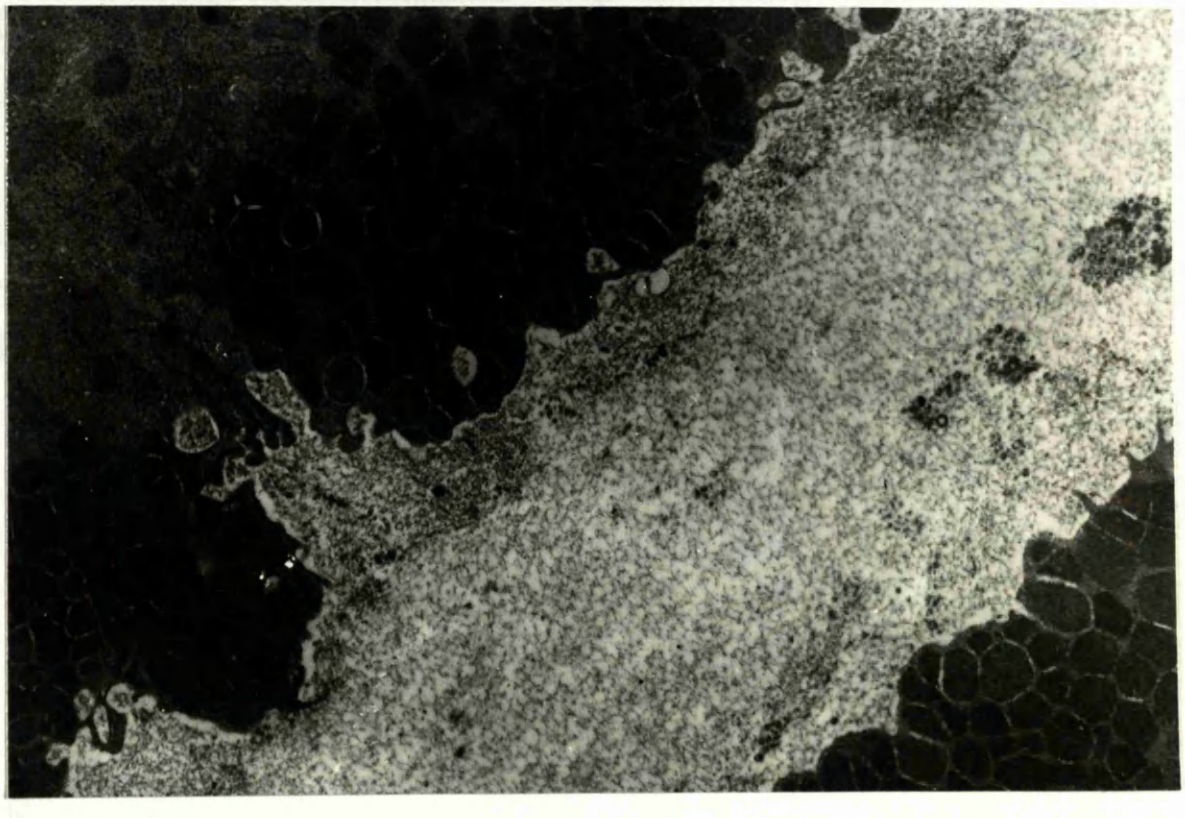


Figure 6.45. TEM of surface epithelium following sucralfate pre-treatment. There is marked lumenal debris present (x10000).

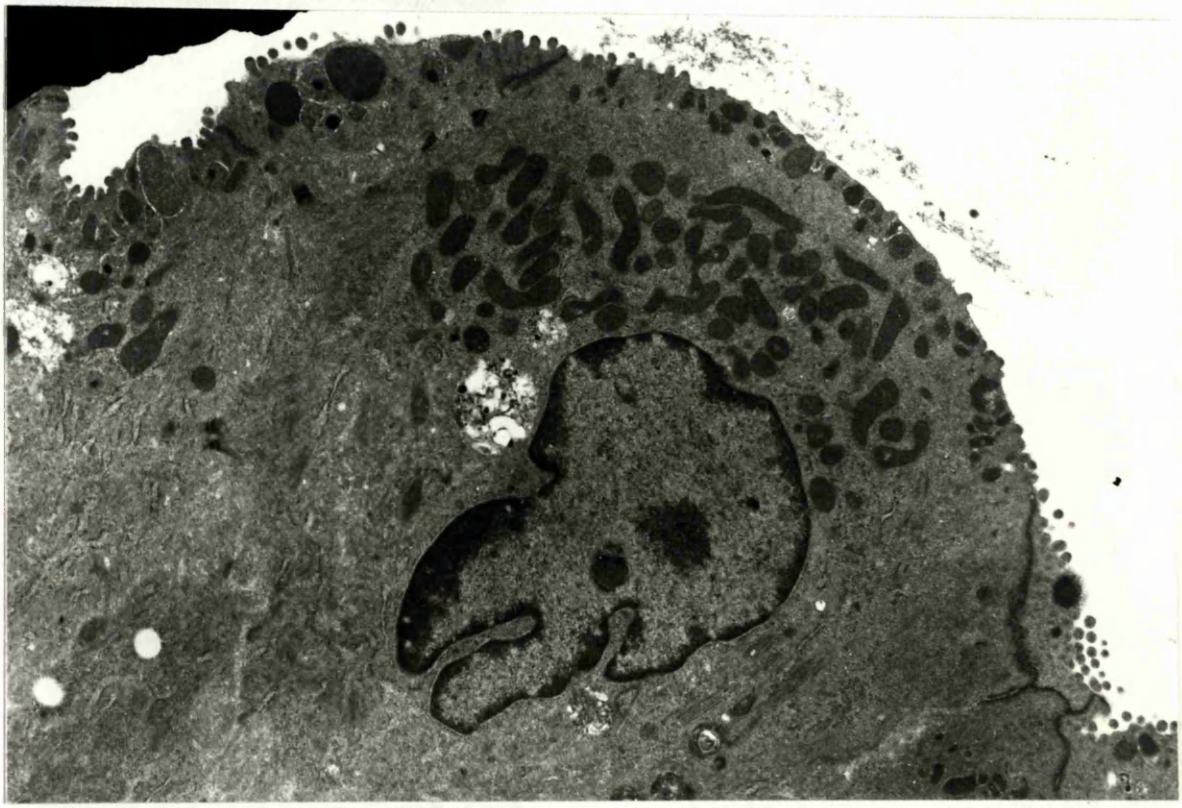
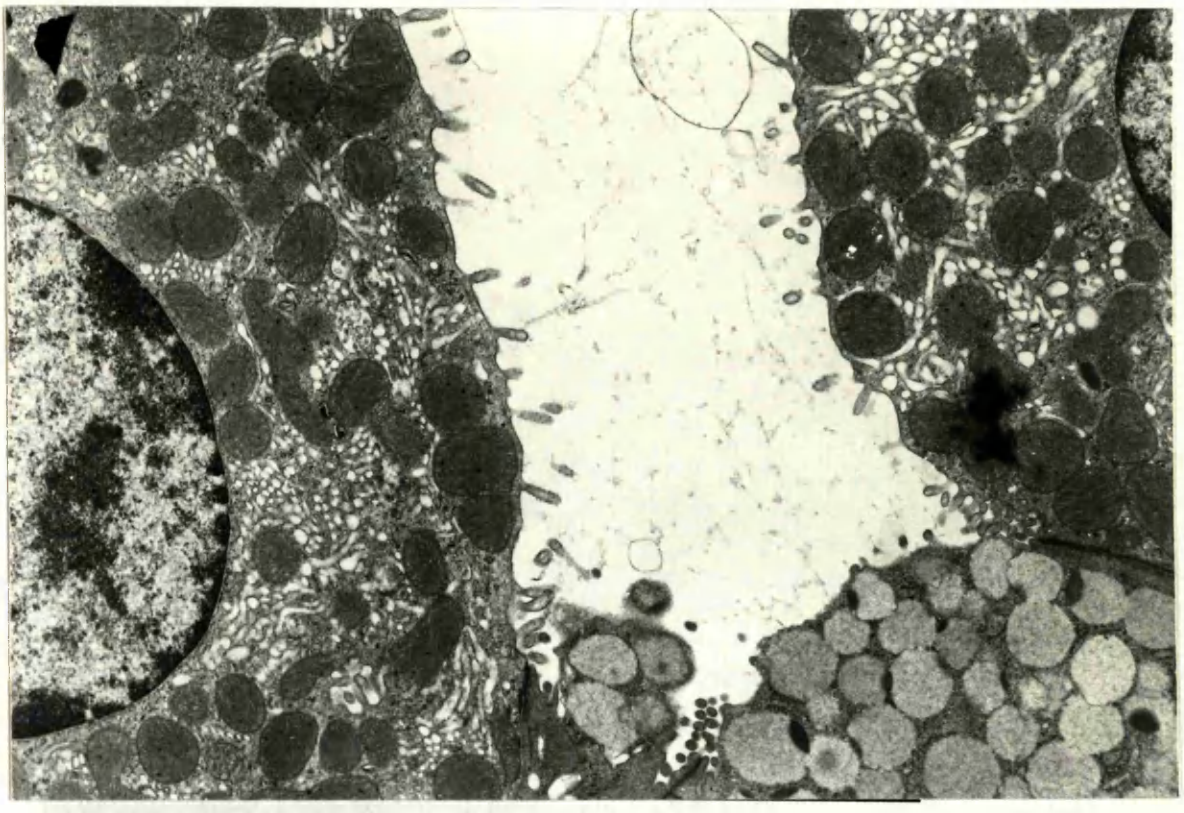
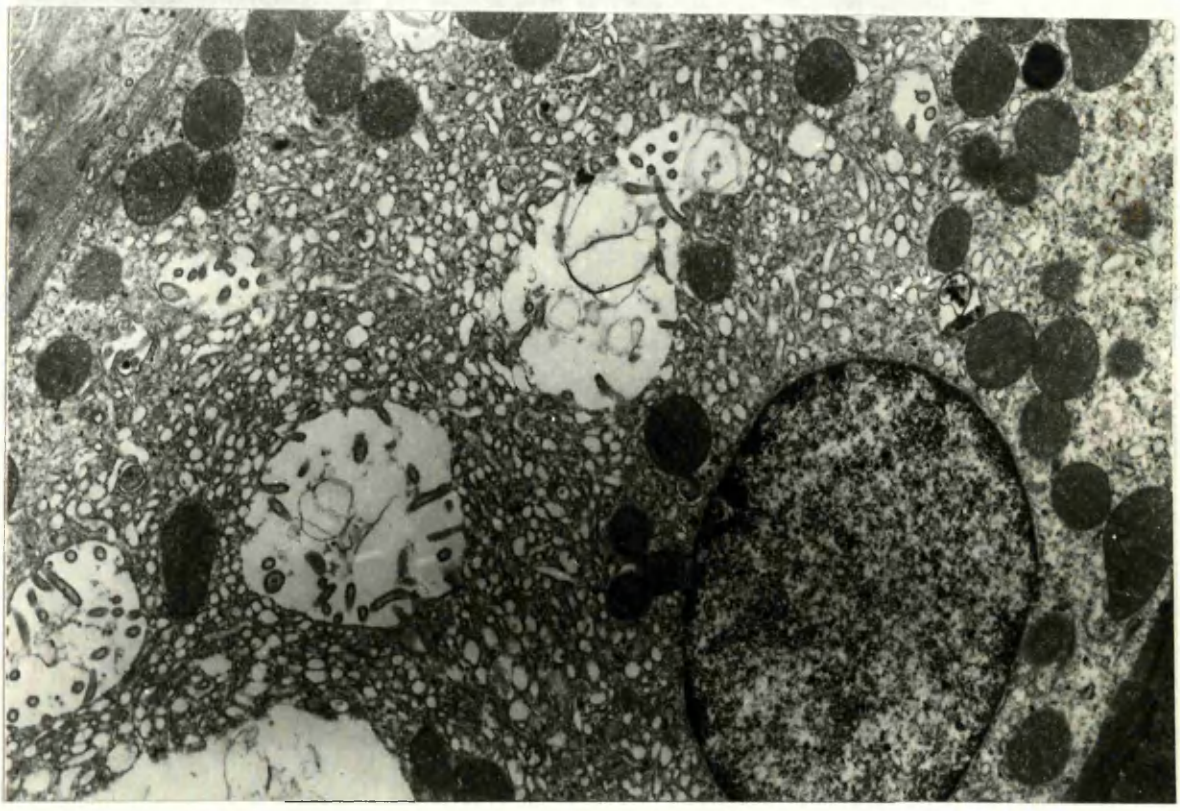


Figure 6.46. TEM of surface epithelium following famotidine pre-treatment. The apical membrane is intact and there is a distinct lack of mucous granules (x8000).





Figures 6.47 & 6.48. TEMs of surface epithelium following famotidine pre-treatment and 5 minutes exposure to 0.46mM carprofen and 15 minutes 0.46mM carprofen/5mM bile. There is a distinct lack of mucous granules, vacuolation of the cytoplasm and nuclear clumping (x10000).

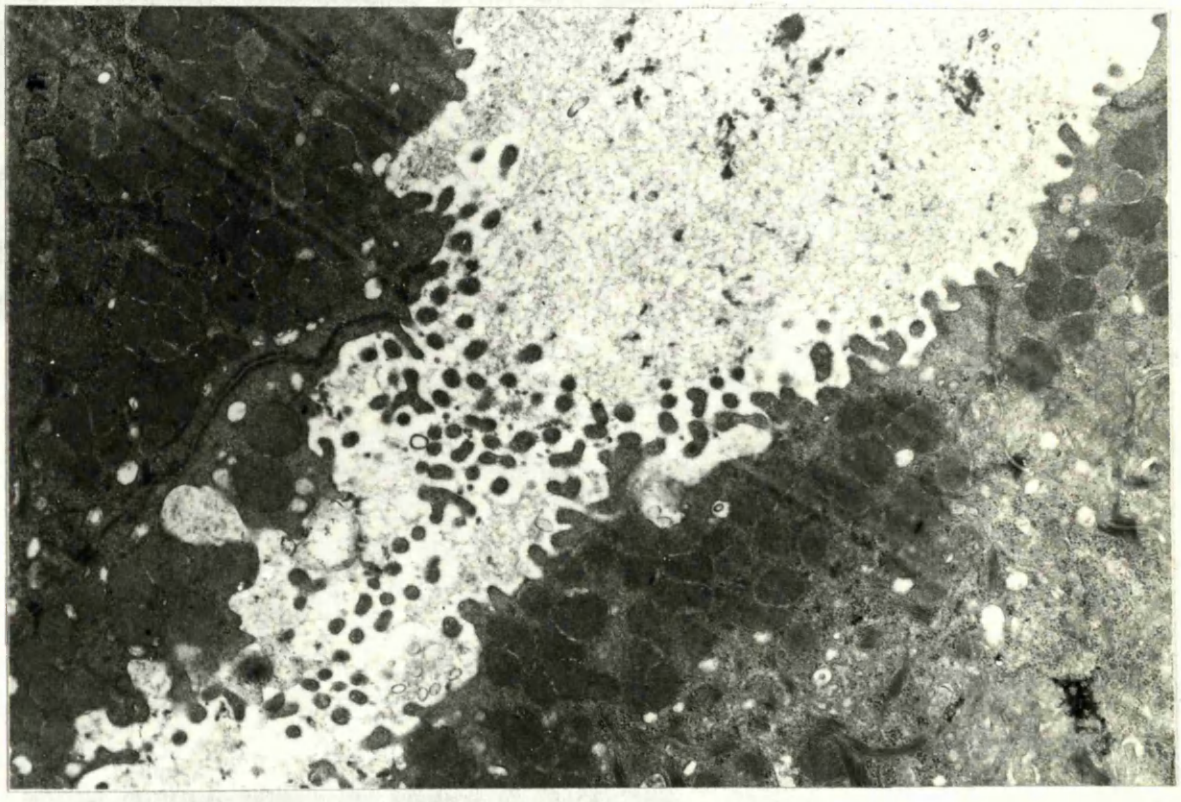
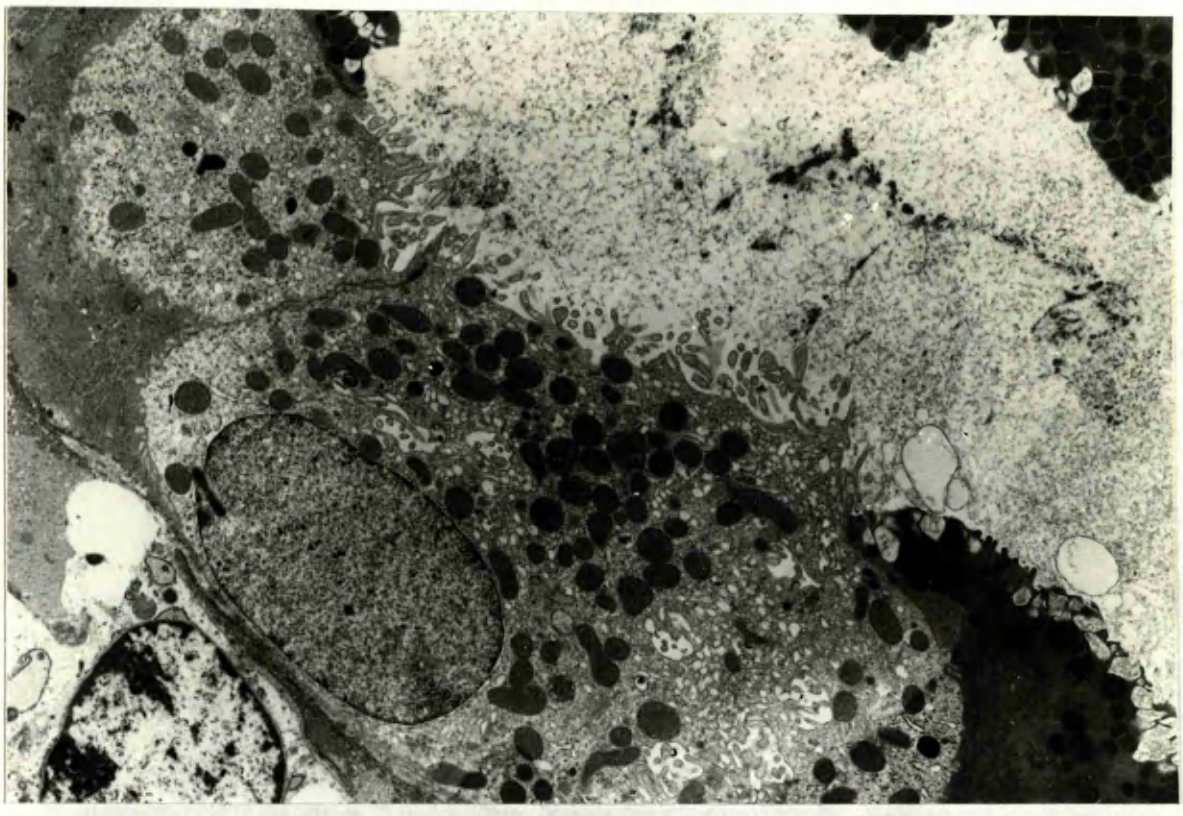
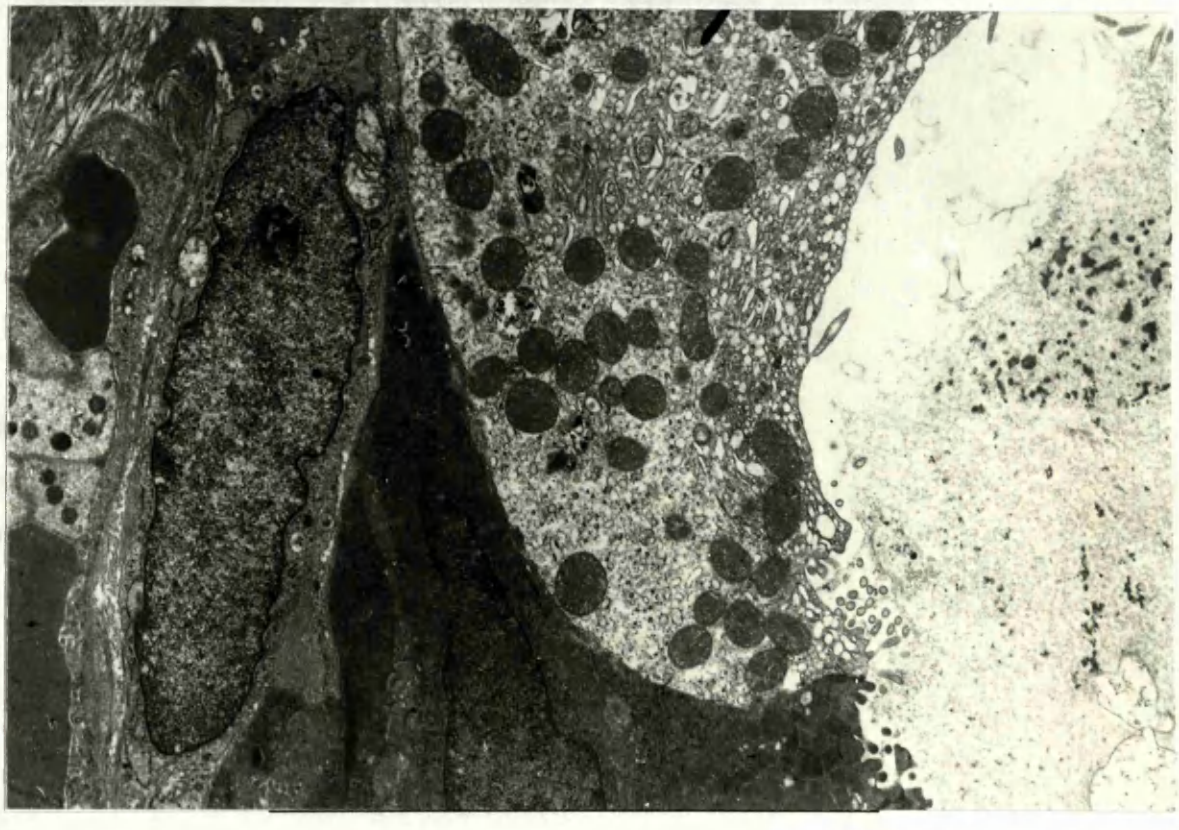


Figure 6.49. TEM of surface epithelium following famotidine pre-treatment and 15 minutes exposure to 40mM aspirin. There is lumenal debris and there appear to be cytoplasmic contents in the lumen (x13400).





Figures 6.50 & 6.51. TEMs of surface epithelium following sucralfate pre-treatment and 5 minutes exposure to 40mM aspirin and 0.46mM carprofen. There is a decrease in cytoplasmic density with vacuolation. The apical membrane is absent and cytoplasm is confluent with the lumen which contains debris (x5400, x8000).

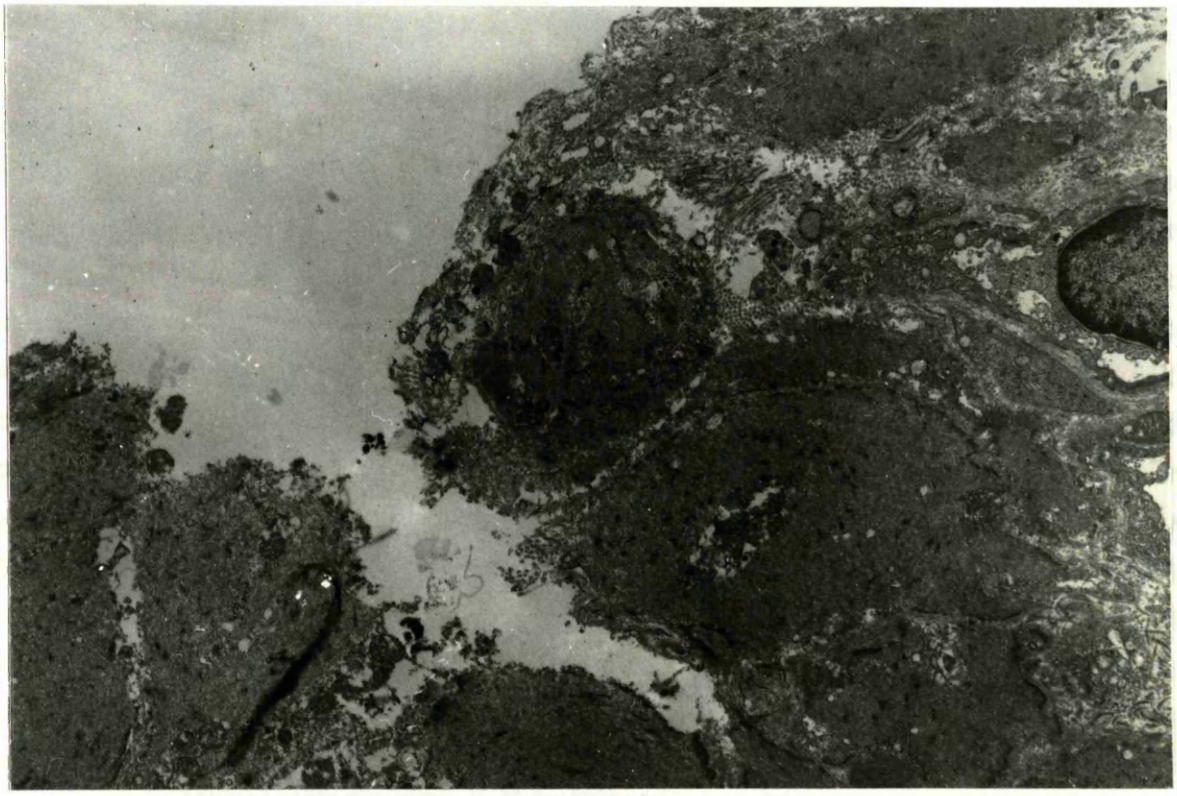


Figure 6.52. TEM of surface epithelium following sucralfate pre-treatment and 15 minutes exposure to 40mM aspirin. The normal cell architecture is lost as is the normal intercellular relationship (x8000).

6.4 Discussion

Initially it was established that there was no difference in any of the parameters investigated between control rats that were not tubed, and sham rats that had been anaesthetised, tubed and had saline introduced, implying that this method of delivery of fluid *per oesophagus* did not result in iatrogenic damage to the gastric mucosa. Thus any changes recorded after the administration of famotidine or sucralfate to rats by this approach were the result of the drugs, not the act of tubing, nor the effect of anaesthesia.

The results of the initial gross and microscopic examinations indicated that sucralfate, caused oedema of the antral mucosa and migration of large numbers of white cells into the antrum, and of a smaller number of cells into the corpus. On the other hand, famotidine produced no evidence of oedema in either antrum or corpus, but did seem to stimulate the appearance of increased numbers of white cells in the mucosa of both regions of the stomach. Thus the administration of both types of protective medication seemed to have marshalled the rats' defense mechanisms, especially in the case of sucralfate where there was increased blood supply and oedema as well as white cell migration. Sucralfate has also been shown to cause disruption and exfoliation of superficial epithelial cells with the release of mucus (Tarnawski and others 1986). Both of which may well create a luminal surface environment that is analogous to the mucoid cap (Wallace and McKnight 1990), but more stable. It has been proposed sucralfate may achieve some of these changes by acting as mild irritant increasing the luminal levels of prostaglandins, and then binding to any albumin and fibrinogen so released (Guth 1987). Hollander and others (1984) have shown that the effect of sucralfate is not completely mediated by prostaglandin release and there is little evidence that sucralfate has any effect on the biosynthesis of prostaglandins (Coleman and others 1987, Quadros, Ramsamooj and Wilson 1987). Further, Shea-Donohue and others (1986) found that sucralfate increased mucus production and protected against aspirin damage, but did not counter the effect of aspirin-induced prostaglandin suppression. Sucralfate produces a protective coat and famotidine suppresses the acid environment thus aiding restitution (Grönbech and others 1987), so that both drugs may protect in differing ways. It would appear on these first investigations that sucralfate overtly causes more response in the mucosa than famotidine. It is of interest to note that none of the rats pre-treated with sucralfate showed evidence of the reactive white coating associated with this form of medication. Although it has been established that sucralfate forms such a coating over damaged epithelium (Sasaki and others 1983) some reports have suggested that it will also adhere to uninjured gastric mucosa (Nagashima 1981b). The results in this thesis indicate that this did not happen. However, as the production of this white

coating depends on the pH of the stomach being less than four, it would have been of interest to have checked the pH of the gastric environment of these particular rats that had been treated with sucralfate for five days prior to being sacrificed. This was not done in these examinations but warrants further investigation.

Following five days pre-treatment with sucralfate when the two injurious agents aspirin and carprofen were administered to rats, different changes in the gastric mucosa occurred. Aspirin produced little evidence of gross change to the mucosa, with only one rat showing some antral coating. This coating, which is the result of electrostatic binding with transudate proteins (Sasaki and others 1983), would tend to suggest that gastric mucosal damage was present in the one rat in the aspirin treated group. In contrast to these findings, all antra of the rats administered carprofen had a white layer on the mucosa indicating sucralfate adherence. Thus at the gross level, carprofen appeared to have caused sufficient mucosal damage in the antrum to lead to sucralfate adherence.

The addition of bile to both aspirin and carprofen produced widespread reddening of the corporal mucosa in rats pre-treated with sucralfate. Although some reddening of the antral region occurred in these rats it was not as consistent as in the corpus. This pattern of reddening of the gastric mucosa was similar to that found when unprotected rats were given carprofen suggesting that the carprofen caused this effect and that sucralfate did little to prevent it. However, as this reddening did not induce a coating of the mucosa with sucralfate in the aspirin plus bile treated rats it suggests that the epithelium was intact. The addition of bile to carprofen led to less binding of sucralfate to the mucosa in the antrum compared to those given carprofen alone implying that this mixture caused less damage to the epithelial layer than carprofen alone, but this may have been due to the sucralfate binding to the bile in preference to the mucosa (Spiro 1982, Steiner and others 1982).

In none of the rats pre-treated with sucralfate and subsequently challenged with the injurious agent, either alone or combined with bile, was mucosal oedema seen grossly. This would tend to suggest that the capability of the sucralfate to induce a degree of oedema as shown in the control rats was overcome by the action of the injurious agent/s. However, another possible explanation for the lack of oedema could be that, where the lacy white pattern of sucralfate was present, it may have been masking underlying oedema in those areas.

The increased migration of white blood cells associated with the administration of sucralfate was maintained when the rats were challenged with either aspirin or carprofen, but neither of these injurious agents caused any further increase in the numbers of white cells infiltrating into either region of the stomach. This may have

been because the initial response initiated by sucralfate was sufficient to meet the further challenge to the integrity of the mucosa from either of these substances.

However, the effect of sucralfate administration in causing a WBC infiltrate that was as great in the sucralfate rats before as after the addition of injurious agents may be related to the fact that the sucralfate had been given over a relatively prolonged period (five days), in contrast to the relatively short period of time (minutes) that the gastric mucosa had been exposed to the injurious agents.

Famotidine pre-treated rats subsequently given either aspirin or carprofen had marked corporal hyperaemia within fifteen minutes. This was rather unexpected especially as no such reddening was found in the pre-treated sucralfate rats in similar circumstances.

Sucralfate does not suppress acid secretion but reduces local acidity by forming a protective barrier and limits the diffusion of hydrogen ions, with the aluminium ion acting as a buffer (Slomiany, Laszewicz and Slomiany 1986). Famotidine, on the other hand, is an H_2 antagonist and thus does suppress acid secretion by the parietal cell of the gastric mucosa One would have expected that the mode of action of the H_2 receptor antagonist would have resulted in marked acid suppression that has been shown to occur in rats (Okabe and Nobuhara 1984), and so reduction in back-diffusion and thus hyperaemia (Ryan and others 1986). However, the capability of sucralfate to reduce reddening may not only have been due to the buffering of hydrogen ions, but also to limiting the action of the injurious agents by coating the surface of the mucosa (Garnett 1982). However, Tesler and Lim (1981) have shown in man that sucralfate does not affect the serum salicylate levels achieved after the oral administration of aspirin. The addition of bile to the famotidine pre-treated rats appeared to make the reddening less severe with both NSAIDs. These findings are in contrast to those obtained from the sucralfate rats and tends to suggest that the bile in the famotidine pre-treated rats interfered with capability of the carprofen and aspirin to produce some of the gross changes associated with injury.

Famotidine alone did not induce oedema in the antrum as sucralfate did. However, like sucralfate, the rats pre-treated with famotidine and challenged with injurious agents showed more evidence of antral oedema. However, there was little evidence of oedema in the corpus - the exception was those rats given carprofen/bile. The induction of oedema can be related to ion movement induced by taurocholate, which O'Brien and Carter (1975) and Lewi and Carter (1983) have shown is not affected by H_2 receptor antagonists. Therefore although famotidine did not in itself induce oedema, it does not suppress oedema, and so when challenged the famotidine treated rats were still capable of producing this reaction. MacKercher and others (1977)

reported that H_2 receptor antagonists, like famotidine, protect the mucosa against aspirin by raising the gastric pH above that of the pK_a value of the NSAID, but in the rat famotidine may not affect basal acid secretion (Bertaccini and others 1986). However, other workers have presented evidence that the limiting of the damage by aspirin may not be solely due to acid suppressive activity of the H_2 receptor antagonists (Kauffman and Grossing 1978, Guth, Aures and Paulsen 1979). Thus just how the damage thought to be caused by aspirin, as well as other NSAIDs, comes about is as yet not fully understood.

Challenging the famotidine rats with injurious agents appeared to have the effect of reducing the numbers of white blood cells found in the corpus of pre-treated famotidine rats - an anomalous finding. The reduction in the numbers of the white blood cells already present in the gastric mucosa in the famotidine treated rats when subsequently challenged by either aspirin or carprofen may have been by leakage into the gastric lumen through areas of damaged gastric mucosa. Preliminary studies on long-term treatment with H_2 receptor antagonists have appeared to show that H_2 receptor antagonists can cause a decrease in gastric mucosal defence mechanisms (Goto, Wakabayashi and Murakami 1985).

Thus in assessing the two forms of protection given to the rats subsequently challenged with injurious agents it was difficult to suggest that one gave more protection than the other, based on the consideration of the initial gross and light microscopic examinations results.

Despite the fact that sucralfate generally induced a more marked WBC infiltrate than famotidine, this was not associated with congestion. Indeed there was no clear cut evidence that either drug caused congestion. If there was little congestion then there would also be little transudation of proteins to which sucralfate could bind electrostatically to (Nagashima and others 1983) and this could explain why in the pre-treated sucralfate rats there was no evidence of sucralfate adhering to the mucosa. Neither sucralfate nor famotidine pre-treatment prevented apical damage in the challenged rats (Hollander and others 1985). In the sucralfate group this was observed in those rats given carprofen or carprofen/bile, whereas it was aspirin alone which gave rise to this in the famotidine pre-treated rats. These results were not unexpected in the rats pre-treated with sucralfate as carprofen was found continually to be the more damaging agent than aspirin, which makes the findings with aspirin in the famotidine rats rather unusual. It is a readily held belief that aspirin is one of the medications most likely to cause gastric mucosal problems and indeed there are plenty of reports in man to substantiate this. However, this usually occurs after longer treatment with this drug and the exposure time in this study was very short.

However, it did not give the overall indications of being nearly as damaging as carprofen.

The administration of famotidine and sucralfate appeared to have a definite effect on the luminal contents. In both corpus and antrum both agents caused the shedding of increased number of cells into the gastric lumen suggesting that both drugs caused some superficial changes to the gastric mucosa, which resulted in a speeding up of the exfoliation of the epithelial cells. The findings with sucralfate are in agreement with those previously reported by Tarnawski and others (1986). Furthermore, sucralfate produced thicker than normal layers of mucus that also contained cells in many of the rats. The mixture of sucralfate, mucus and cells is probably achieved by the sucralfate preventing the normal rate of dissolution of the mucus into gastric luminal contents. Though this thick layer could be construed as damage, it may in fact be evidence of sucralfates cytoprotective action (Hollander and others 1984, Ligumsky, Karmski and Rochmilewitz 1984, Konturek 1985, Stern, Ward and Hartley 1987) and may represent a form of protection in addition to the increased release of prostaglandins that sucralfate is known to produce (Hollander and others 1984, Coleman and others 1987). The presence of a mode of action other than prostaglandin release is supported by work carried out by O'Brien and others (1990) who showed that the administration of indomethacin, which suppresses the release of prostaglandins did not prevent the protective effect of sucralfate. On the other hand, famotidine by its direct effect on the parietal cell inhibiting acid production, leading to a decrease in acid secretion, ought to protect the apical cells to a certain extent. This did not appear to have been the overall position with the rats in these investigations as increased amounts of exfoliated cells were also found in the luminal contents of the famotidine treated rats. One possible explanation for the amount of debris found in the famotidine rats is that the suppression of acid secretion altered the gastric flora in the rat, which in turn increased the epithelial turnover with increased numbers of exfoliated cells. One might have expected in these circumstances to find more obvious retention of mucus on the epithelial surface with famotidine as loss of mucus is caused by abrasion and pepsinolyis (Allen, Bell and McQueen 1984). However, in the famotidine treated rats investigated in this study, this did not appear to be the case. One explanation for the thinner layer of mucus found in the famotidine rats than in either the sucralfate treated or normal rats may be that the sucralfate binding makes removal of the mucus more difficult in contrast to the removal of essentially normal mucus in famotidine rats. Another explanation for the decrease in the layer of mucus present in the famotidine treated rats could be that as mucus secretion is stimulated by antral pH (Menguy 1969), the raised antral pH produced by H_2 receptor antagonists prevents the normal copious secretion of mucus. Menguy (1969) found that fasting in the dog caused a fall in the output of

mucus. In the rats investigated in this study, fasting was carried out as part of the preparation for the study and could explain the reduction in the mucus layer in the famotidine rats (Menguy and Thompson 1967). However, if this was the cause then it would suggest that the sucralfate treated rats, which were also starved, must have been stimulated to produce more mucus by this alternative type of medication (Quadros, Ramsamooj and Wilson 1987).

Scanning electron microscopy demonstrates cellular detail and can either lend support to the findings of light microscopy, but because of the increase in detail can challenge some of the interpretations made by light microscopy. In addition, scanning electron microscopy may identify some changes not seen by the light microscopic examinations. The scanning electron microscopic changes seen in the normal rats and the sham-tubed rats supported the assumption deduced from the previous examinations that the oesophageal route of administration caused no damage to the gastric mucosa. Time of exposure to any of the combinations examined including the protective agents and the injurious substances did not apparently have any real significance in the damage caused. However, one must accept that the length of exposure time of the injurious agents was short. The scanning results of mucus thickness and cover also agreed with those recorded by light microscopy in that sucralfate produced a thicker layer of mucus than did famotidine and again carprofen was as damaging as aspirin. One interesting feature that was noticed in the preliminary studies and further supported by electron microscopy was the effect the addition of bile had on the injurious agents. Apart from the antrum of those rats given sucralfate followed by aspirin/bile, bile in most instances made little difference overall. The reflux of bile is not thought to be a normal situation, but with the increase use of endoscopy as a means of examination of the gastric environment it may be that this assumption is proved to be false and that bile may be present in the stomach much more frequently than supposed. This aspect of gastric protection obviously warrants further examination. The scanning electron microscopy identified some interesting cellular changes that ranged from apical erosion but the cells still containing their contents to shells where only the empty torn membrane of the cells were found. The preliminary stages to these different degrees of damage were also in evidence where some cell had convex protruding upper regions, whilst others showed an imploding of the membrane with a concave appearance with the development of apical holes. This range of changes may be the result of accelerated mucus expulsion by the cells (Forte, Silen and Forte 1976, Fringes, Lorenz and Oehlert 1985). What the actual chain of events leading to these different changes are as yet not fully understood but obviously as our understanding of the mechanisms of medications in use, either harmful or protective to the gastric mucosa become better understood then, these specific changes may play a useful part in identifying just what

the true situation is.

The investigations carried out in rats which were pre-treated with either sucralfate or famotidine as a protective agent against the subsequent effects of aspirin or carprofen indicated that they produced different changes in the gastric mucosa. Both drugs apparently affected the antral region of the stomach more than the corpus. If one accepts that sucralfate only adheres to damaged epithelium as would appear to be the case in the rat, then its action as a mild irritant on its own does not cause any actual damage to the epithelial cells as in the rats so treated, at least at the gross level. This view is reinforced by the findings of Parisio and Clementi (1976) and Schmidt and others (1985), who found that minimally injured cells were difficult to identify at the gross and even light microscopic level. It was only after the administration of injurious agents that the protective white layer that sucralfate produces in the face of damaged epithelium was formed. Both drugs caused an increase in the numbers of epithelial cells shed from the luminal surface of the gastric mucosa. However, in the case of famotidine the mucus coat, that can act as a barrier to injurious agents reaching the mucosa itself, was much thinner than that produced by sucralfate (Hollander and others 1985, Quadros, Ramsamooj and Wilson 1987). One might be tempted to suggest that sucralfate gave greater protection than did famotidine but statistically this was not shown and there was no real evidence from the electron microscopic studies that the degree of damage to gastric mucosa was in any way less by using sucralfate against famotidine. Therefore overall both drugs appeared to show a measure of protection with scanning electron microscopy showing less evidence of degeneration and damage in those rats pre-treated than those which were not. One explanation for the failure to find more demonstrable evidence of protection is that neither sucralfate or famotidine are adequately able to protect the superficial epithelial layer (Lacy 1986), and in the time span used in these experiments the effect of restitution was not adequately observed (Silen and Ito 1985).

7. General Discussion

The stomach is on occasion challenged by injurious agents. Whilst the skin and oesophagus are in a similar situation as regards challenge, they are inured to these influences by the provision of a tough external surface or by permitting short contact time. In contrast, the stomach has no toughened epithelial layer and serves as a temporary repository for a variety of ingesta. To make matters worse the stomach produces two chemicals designed to deal with ingesta, which also have the capability of attacking the gastric mucosa. However, nature has provided the stomach with mechanisms to cope with this anomalous situation under normal circumstances. Gross detrimental agents such as foreign bodies can be retrieved from the stomach. Unfortunately, this is not possible where the inimical agent is a product of the stomach itself as is the case with acid, or as with bile that is produced by an associated area of the alimentary tract and may flow backwards into the stomach. Finally, it is now recognised that certain forms of treatment administered orally in the form of tablet or powder can have harmful effects on the gastric mucosa.

The findings in the vomiting dogs agreed with those of previous workers and with those in the human in that the commonest disorders affecting the canine stomach were gastric carcinoma and the gastritides. Although one may suspect gastric carcinoma from the history and clinical signs it is essential to substantiate the diagnosis by further investigation. The studies described in this thesis identified endoscopy as being the best investigative method for making a positive diagnosis of this condition. Endoscopy allows observation of the entire gastric mucosal surface and identification of the typical volcano-like ulcers associated with gastric carcinoma, whose walls protrude above the surrounding mucosa. With experience the majority of gastric carcinomas can be positively diagnosed by endoscopic examination alone. Thus this technique is one that should be used in cases of vomiting dogs in which such a condition is suspected. Endobiopsies are not essential in the diagnosis of gastric carcinoma if an experienced endoscopist carries out the examination in the dog. Indeed, unless a series of biopsies are taken in cases of gastric carcinoma this method of confirmation can be misleading (Hatfield and others 1975).

The gastritides, the other commonest abnormality in the dog, are so grouped because it is as yet unclear whether these various forms have a common origin, or represent truly different underlying pathophysiological responses to disparate challenges, or reflect errors in the diagnostic evaluation of the case. Here again endoscopy proved to be more rewarding as a means of investigation than radiology. However, unlike carcinoma endoscopic examination of gastritis can produce great variation in the appearance of mucosa from only excessively reddening to multiple areas of erosion.

In some cases of gastritis indeterminable by endoscopy, endobiopsies are often resorted to as a possible means of differential diagnosis. However, one of the underlying problems in this situation is correlating the endoscopic and histological findings. The endoscopic appearance may not be substantiated histologically for a variety of reasons; because inadequate numbers of biopsies are taken, the depth of the biopsy samples is too shallow to penetrate beyond the superficial epithelial layer, what appears to be a normal stomach on endoscopic examination may prove on histological examination to have areas that are abnormal. This last set of circumstances may not indicate a pathological condition of the gastric mucosa but merely the results of the insults that the mucosa faces daily (Whitehead, Truelove and Gear 1972, Taor and others 1975, Karvonen and others 1983, Franzin and others 1984, Roth and others 1990). Thus correlation between endoscopic findings and histological changes need further investigation.

The value of scanning electron microscopy has been demonstrated as a very useful investigative tool as it permits detailed examination of the surface of a much greater area of mucosa than does transmission electron microscopy. Therefore scanning electron microscopic investigations could shed light on some of the unexplained endoscopic findings found in dogs with conditions such as erosive gastritis. This method of examination could explain, the, as yet, poorly understood measles-like appearance that was associated with many of the gastric conditions in the dogs investigated. However, this will only be feasible if the quality of endobiopsies is improved. The opportunity for such improvement should be possible with the development of video-endoscopes that do not need large bundle tracts to carry the visual information back to the eyepiece. Then, after initial studies to establish the significant differences between the appearance of the normal and abnormal stomachs and relating these to changes at electron microscopy level of tissue initially obtained at post-mortem, these findings could be used to determine how to obtain biopsies from the canine stomach of patients, which should achieve similar results. The outcome of such an investigation would hopefully increase our knowledge of the various forms of gastritis and allow a less empirical approach to management of this range of conditions.

Treatments for gastric problems adopted at present include some which minimise the harmful environment, that is acid production, and others like sucralfate that act by producing a protective coating on the gastric mucosa (Harrington, Schegel and Code 1981). Famotidine is the most potent and selective H_2 receptor antagonist currently available. However, there is disagreement over its acid suppression power compared to other H_2 receptor antagonists in the dog (Takagi, Takedo and Maeno 1982, Pendleton and others 1983, Katz, Tobia and Shriver 1987). However, one advantage

famotidine has is that it only requires to be given once daily (Bertaccini and others 1986, Dammann and others 1987, Mann 1987). It has further been shown to stimulate an enhanced mucosal blood flow (Schunack 1989) that might offer a degree of cytoprotection unrelated to the acid suppressive effect. In the investigations reported in this thesis there was no conclusive evidence that famotidine, given as a premedicant, was any more effective than sucralfate at moderating damage to the surface epithelium. Further, in man studies have shown that both H_2 receptor antagonists and sucralfate can be used as co-medicants with NSAIDs (Czarnobilski and others 1985, Ungethum 1991). Therefore its use in the dog in preference to sucralfate needs to be considered and clinical trials in canine patients using these two and other drugs, such as omeprazole, warrants further investigation (Scheiman 1992).

A somewhat unexpected result in the canine investigations was a frequent endoscopic finding of a bile pool and duodenogastric reflux of bile in both the control dogs and the non-neoplastic group. Davenport (1968) reported that bile acting as a detergent could damage the gastric mucosal barrier, and Lawson (1964) that it has an inflammatory effect. Endoscopy has revealed that reflux of bile can be seen in normal animals and humans and such was the case in a number of the control dogs examined in these investigations where a bile pool or bile reflux was noted. It has been suggested that bile acting as a mild irritant can stimulate the mucosal barrier and so protect the stomach against more deleterious challenge (Robert and others 1978). However, in the rats exposed to bile there was an increase in the numbers of surface epithelial cells with altered or damaged membranes. Thus the significance of bile on the gastric mucosa is still not fully resolved, in that it is found in normal patients (Rhodes and others 1969), but more frequently in those with abnormal stomachs (Ritchie 1977). Nonetheless, it has been found to stimulate the cytoprotective mechanisms of the gastric mucosa (Chaudhury and Robert 1980, Takeuchi, Nobuhara and Okabe 1984), yet has been implicated as a factor in the development of gastric carcinoma (Domellöf 1979). This aspect of the gastric environment needs further investigation.

The study of the vomiting dogs supported previous findings in that significant damage was induced by the consumption of NSAIDs, a treatment frequently used in both dogs and humans for degenerative joint disease and other painful conditions (Urquhart 1986). In the dog, the most obvious damage caused by this type of treatment is peptic ulceration or widespread mucosal haemorrhage. Both these clinical entities respond to a certain extent to the use of H_2 receptor antagonists. But some dogs with this damage require prolonged medication that the owners are unable to provide, and surgery has to be resorted to remove the areas of the stomach which are susceptible to peptic ulceration (Bollman, Stalker and Mann 1938) - the pylorus and antrum.

The subsequent investigations carried out in the rats gave some interesting results. However, the question arises as to how many of these findings can be applied directly to the canine, whose gastric structure and function differs from that of the rat. The rat is the species most commonly used in biological research because it is readily available, cheap to maintain, and the recent tightened welfare laws allow more intensive research to be undertaken in this species than in the dog. One specific area that needs further investigation is the question of the increased white blood cell migration in various situations. Does this indicate a role in the defence of the mucosa, or is this merely a consequence of the condition and plays no part in the defense of the mucosal barrier? One further question is whether this infiltrate by WBCs is species specific? All these aspects need clarification.

Therefore it may appear that the investigations reported in this thesis have produced more questions than answers about the very important clinical range of gastric abnormalities in the canine. However, it has demonstrated the advantages of endoscopic examination in this situation, proposed the extension of haematological and biochemical investigations and suggested that medication believed to be ideally suited for certain forms of gastritis might well not be so. Finally, these studies have demonstrated the need for specific clinical trials to be undertaken in the canine itself rather than extrapolate from studies undertaken in either the human or the rat. Much remains to be done.

8. References

Adair, R.K. & Wlodek, G. (1968) Ionic changes in Pavlov pouches after insulin hypoglycaemia, gastrin and pentagastrin. *Archives of Surgery* **97**, 423-439.

Adrian, T., Savage, A., Sagor, G., Allen, J.M., Bacarese-Hamilton, A.J., Tatemoto, K., Polak, J.M. & Bloom, S.R. (1985) Effect of peptide YY on gastric, pancreatic, and biliary function in humans. *Gastroenterology* **89**, 494-499.

Al-Tikriti, M., Henry, R.W., Al-Bagdadi, F.K., Hoskins, J. & Tikeymer, C. (1986) Scanning electron microscopic study of the surface of the feline gastric epithelium: A simple method of removing the coating material. *Scanning Electron Microscopy* **1986/III**, 949-952.

Albinus, M., Blair, E.L., Case, R.M., Cox, D.H., Gomez-Pan, A., Hirst, B.H., Reed, J.D., Schally, A.V., Shaw, B., Smith, P.A. & Smy, J.R. (1977) Comparison of the effect of somatostatin on gastrointestinal function in the conscious and anaesthetized cat and on the isolated cat pancreas. *Journal of Physiology* **269**, 77-91.

Allen, A. & Carroll, N.J.H. (1985) Adherent and soluble mucus in the stomach and duodenum. *Digestive Diseases and Sciences* **30** (Suppl **11**), 55-62.

Allen, A. & Garner, A. (1980) Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut* **21**, 249-262.

Allen, A., Bell, A. & McQueen, S. (1984) Mucus and mucosal protection. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p195.

Alvarez, W.C. (1925) Reverse peristalsis in the bowel, a precursor of vomiting. *Journal of the American Medical Association* **85**, 1051-1054.

Anderson, J.C., Barton, M.A., Gregory, R.A., Hardy, P.M., Kenner, G.W., MacLeod, J.K., Preston, J. & Sheppard, R.C. (1964) Synthesis of gastrin. *Nature* **204**, 933-934.

Andersson, S. & Grossman, M.I. (1966) Effects of histolog and secretin on gastroduodenal profile of pH, potential difference and pressure in man. *Gastroenterology* **51**, 10-17.

Andersson, S., Nilsson, G. & Uvnäs, B. (1967) Effect of acid in proximal and distal duodenal pouches on gastric secretory repsonses to gastrin and histamine. *Acta Physiologica Scandinavica* **71**, 368-378.

Archer, R.K. (1970) Regulatory mechanisms in eosinophil leukocyte production, release, and distribution. In: *Regulation of Hematopoeisis*. Vol II, ed. A.S. Gordon, Appleton-Century Crofts, New York. p917.

Arimura, A., Sato, H., Dupont, A., Nishi, N. & Schally, A.V. (1975) Somatostatin: abundance of immunoreactive hormone in rat stomach and pancreas. *Science* **189**, 1007-1009.

Augur, N.A. (1970) Gastric mucosal blood flow following damage by ethanol, acetic acid, or aspirin. *Gastroenterology* **58**, 311-320.

Baker, B.L. (1964) Cell replacement in the stomach. *Gastroenterology* **46**, 202-203.

Barbour, H.G. & Dickerson, V.C. (1938) Gastric ulceration produced in rats by oral and subcutaneous aspirin. *Archives Internationales de Pharmacodynamie et de Therapie* **58**, 78-87.

Baskin, W.N., Ivey, K.J., Krause, W.J., Jeffrey, G.E. & Gemmell, R.T. (1976) Aspirin-induced ultrastructural changes in human gastric mucosa: Correlation with potential differences. *Annals of Internal Medicine* **85**, 299-303.

Bénichoux, R., Durlik, M. & Mainard, D. (1986) Gastric stress ulcer of the rat: Relative contribution of the pyloric sphincter, HCO_3^- bile reflux and mucosal blood flow. *European Surgical Research* **18**, 159-168.

Bennett, A. & Curwain, B.P. (1977) Effects of aspirin-like drugs on canine gastric mucosal blood flow and acid secretion. *British Journal of Pharmacology* **60**, 499-504.

Bennett, A., Friedman, C.A. & Vane, J.R. (1967) Release of prostaglandin E_1 from the rat stomach. *Nature* **216**, 873-876.

Bensley, R.R. (1899) The structure of the mammalian gastric glands. *Quarterly Journal of Microscopical Science* **41**, 361-389.

Berg, B.N. (1942) Pathological changes in nutritional gastritis in rats. *American Journal of Pathology* **18**, 49-62.

Berg, B.N. & Jobling, J.W. (1930) Biliary and hepatic factors in peptic ulcers. *Archives of Surgery* **20**, 997-1015.

Berger, E.H. (1934) The distribution of parietal cells in the stomach, a histo-topographical study. *American Journal of Anatomy* **54**, 87-114.

Berstad, A. (1982) Antacids and pepsin. *Scandanavian Journal of Gastroenterology* **17** (Suppl **75**), 13-15.

Berstad, A. & Petersen, H. (1970) Dose-response relationship of the effect of secretin on acid and pepsin secretion in man. *Scandanavian Journal of Gastroenterology* **5**, 647-654.

Berstad, K., Vergin, H., Postius, S., Weberg, R., Szelenyi, I. & Berstad, A. (1987) Gastric prostaglandin E_2 release induced by aluminium hydroxide and aluminium hydroxide-containing antacids in rats. *Scandanavian Journal of Gastroenterology* **22**, 884-888.

Bertaccini, G., Coruzzi, G., Poli, E. & Adami, M. (1986) Pharmacology of the novel H_2 anatagonist famotidine: *in vitro* studies. *Agents and Actions* **19**, 180-187.

Bertalanffy, F.D. (1960) Mitotic rates and renewal times of the digestive tract epithelia in the rat. *Acta Anatomica* **40**, 130-140.

Bertoni, G., Gumina, C., Conigliaro, R., Ricci, E., Staffetti, J., Mortilla, M.G. & Pacchione, D. (1992) Randomized placebo-controlled trial of oral liquid simethicone prior to upper gastrointestinal endoscopy. *Endoscopy* **24**, 268-270.

Black, R.B., Hole, O. & Rhodes, J. (1971) Bile damage to the gastric mucosal barrier: the influence of pH and bile acid concentration. *Gastroenterology* **61**, 178-184.

Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R. & Parsons, E.M. (1972) Definition and antagonism of histamine H_2 receptors. *Nature* **236**, 385-390.

Bloom, W. & Fawcett, D.W. (1970) The Esophagus and Stomach. In: *A Textbook of Histology*. 9th edition, W.B. Saunders, Philadelphia. p554.

Bohman, T., Myren, J. & Larson, S. (1980) Inhibition of the histamine-stimulated gastric secretion in healthy subjects by the H_2-receptor antagonists ranitidine. *Scandanavian Journal of Gastroenterology* **15**, 183-189.

Bollard, J.E., Vanderwee, M.A., Smith, G.W., Tasman-Jones, C., Gavin, J.B. & Lee, S.P. (1986) Preservation of mucus *in situ* in rat colon. *Digestive Diseases and Sciences* **31**, 1338-1344.

Bollman, J.L. Stalker, L.K. & Mann, F.C. (1938) Experimental peptic ulcer produced by cinchophen. *Archives of Internal Medicine* **61**, 119-127.

Bolton, J.P. & Cohen, M.M. (1978) Stimulation of non-parietal cell secretion in canine Heidenhain pouches by 16,16-methyl prostaglandin E_2. *Digestion* **17**, 291-299.

Bolton, J.P., Palmer, D. & Cohen, M.M. (1978) Stimulation of mucus and non-parietal cell secretion by the E_2 prostaglandins. *American Journal of Digestive Diseases* **23**, 359-364.

Bolton, J.P. & Cohen, M.M. (1979a) Effect of 16, 16-dimethyl prostaglandin E_2 on the gastric mucosal barrier. *Gut* **20**, 513-517.

Bolton, J.P. & Cohen, M.M. (1979b) The effect of prostaglandin E_2, 15-methyl prostaglandin E_2 and metiamide on established canine gastric mucosal barrier damage. *Surgery* **85**, 333-338.

Bommelaer, G. & Guth, P.H. (1979) Protection by histamine receptor antagonists and prostaglandin against gastric mucosal barrier disruption in the rat. *Gastroenterology* **77**, 303-308.

Bonneau, N.H., Reed, J.H., Pennock, P.W. & Little, P.B. (1972) Comparison of gastrography and contrast radiography for diagnosis of aspirin-induced gastritis in the dog. *Journal of the American Veterinary Medical Association* **161**, 190- 198.

Borg, I. (1959) Bile admixture in gastric juice in health and in peptic ulcer before and after operation

according to Billroth II and Billroth I. *Acta Chirurgica Scandinavica* (Suppl 251), 97-112.

Brawer, W.R. & Bartels, J.E. (1990) Contrast radiography of the digestive tract. Indications, techniques, and complications. *Veterinary Clinics of North America* 13, 599-626.

Brearley, S., Morris, D.L., Hawker, P.C., Dykes, P.W. & Keighley, M.R.B. (1985) Prediction of mortality at endoscopy in bleeding peptic ulcer disease. *Endoscopy* 17, 173-174.

Breitschwerdt, E.B., Turk, J.R., Turwald, G.H., Davenport, D.J., Hedlund, C.S. & Carakostas, M.C. (1986) Hypergastrinemia in canine gastrointestinal disease. *Journal of the American Animal Hospital Association* 22, 585-592.

Brimblecombe, R.W., Duncan, W.A.M., Durant, G.J., Emmett, J.C., Ganellin, C.R. & Parsons, M.E. (1975) Cimetidine - a non-thiourea H_2-receptor antagonist. *Journal of International Medical Research* 3, 86-92.

Brink, B.M., Schlegel, J.F. & Code, C.F. (1965) The pressure profile of the gastrodoudenal junction zone in dogs. *Gut* 6, 163-171.

Brodie, D.A. & Chase, B.J. (1967) Role of gastric acid in aspirin-induced gastric irritation in the rat. *Gastroenterology* 53, 604-610.

Brodie, D.A. & Chase, B.J. (1969) Evaluation of gastric acid as a factor in drug-induced gastric hemorrhage in the rat. *Gastroenterology* 56, 206-213.

Brogden, R.N. (1986) Non-steroidal anti-inflammatory analgesics other than salicylates. *Drugs* 32 (Suppl 4), 27-45.

Brogden, R.N., Heel, R.C., Speight, T.M. & Avery, G.S. (1984) Sucralfate. A review of its pharmacodynamic properties and therapeutic use in peptic ulcer disease. *Drugs* 27, 194-209.

Brough, W.A., Taylor, T.V. & Torrance, H.B. (1984) The effect of cholecystectomy on duodenogastric reflux in dogs and humans. *Scandanavian Journal of Gastroenterology* 19 (Suppl 92), 242-244.

Brownlie, S.E. (1990) A retrospective study of diagnosis in 109 cases of canine lower respiratory disease. *Journal of Small Animal Practice* 31, 371-376.

Brunton, L.L. (1990) Drugs affecting gastrointestinal function. In: *The Pharmacological Basis of Therapeutics*. Eds. A.G. Gilman, T.W. Rall, A.S. Nies & P. Taylor. Pergamon Press, New York. p897.

Buck, G.E., Gourlay, W.K., Lee, W.K, Subramanyam, K., Latimer, J.M. & DiNuzzo, A.R. (1986) Relation of *Campylobacter pyloridis* to gastritis and peptic ulcer. *Journal of Infectious Diseases* 153, 664-669.

Bugat, R., Thompson, M.R., Aures, D. & Grossman, M.I. (1976) Gastric mucosal lesions produced by intravenous infusion of aspirin in cats. *Gastroenterology* 71, 754-759.

Burland, W.L., Duncan, W.A.M., Hesselbo, T., Mills, J.G., Sharpe, P.C., Haggie, S.J. & Wyllie, J.H. (1975) Pharmacological evaluation of cimetidine, a new histamine H_2-receptor antagonist in healthy man. *British Journal of Clinical Pharmacology* 2, 481-486.

Burns, J. & Fox, S.M. (1990) The use of a barium meal to evaluate total gastric emptying time in the dog. *Veterinary Radiology* 27, 169-172.

Butler, B.D., Lichentenberger, L.M. & Hills, B.A. (1983) Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate. *American Journal of Physiology* 244, G645-G651.

Byers, F.M. & Jordan, P.H. (1962) Effect of bile upon gastric mucosa. *Proceedings of the Society of Experimental Biology and Medicine* 110, 864-866.

Bynum, T.E. & Jacobson, E.D. (1971) Blood flow and gastrointestinal function. *Gastroenterology* 60, 325-335.

Caille, G., du Souich, D., Gervais, P., Besner, J.G. & Vezina, M. (1987) Effects of concurrent sucralfate administration on pharmocokinetics of naproxen. *American Journal of Medicine* 83 (Suppl 3B), 67-73.

Calcraft, B., Rhodes, J., Cross, S. & Hole, D. (1973) Strengthening the gastric mucosa. *Gut* 14, 423.

Caldwell, J.R., Roth, S.H., Wu, W.C., Semble, E.L., Castell, D.O., Heller, M.D. & Marsh, W.H. (1987) Sucralfate treatment of non-steroidal anti-inflammatory drug-induced gastrointestinal symptoms and

mucosal damage. *American Journal of Medicine* **83** (Suppl 3B), 74-82.

Calvert, C.A., Mahaffey, M.B., Lappin, M.R. & Farrell, R.L. (1988) Pulmonary and disseminated eosinophilic granulomatosis in dogs. *Journal of the American Animal Hospital Association* **24**, 311-320.

Cannon, W.B. (1898) The movement of the stomach studied by means of roentgen rays. *American Journal of Physiology* **1**, 359-382.

Capper, W.M., Butler, T.J. & Buckler. F.G. (1966) Alkaline areas in gastric mucosa after surgery. *Gut* **7**, 220-222.

Carlson, H.C., Code, C.F. & Nelson, R.A. (1966) Motor activity of the canine gastroduodenal junction: a cineradiographic, pressure and electric study. *American Journal of Digestive Diseases* **11**, 155-172.

Carmichael, H.A., Nelson, L.M. & Russell, R.I. (1978) Cimetidine and prostaglandins: Evidence for different modes of action on the gastric mucosa. *Gastroenterology* **74**, 1229-1232.

Caspary, W.F. (1982) Measurement of intragastric potential difference. In: *Antacids in the Eighties*. Ed. F. Halter. Urban & Schwarzenberg, Munich. p64.

Chaudhury, T.K. & Robert, A. (1980) Prevention by mild irritants of gastric necrosis produced in rats by sodium taurocholate. *Digestive Diseases and Sciences* **25**, 830-836.

Cheung, L.Y. & Chang, N. (1977) The role of gastric mucosal blood flow and H^+ back-diffusion in the pathogenesis of acute gastric erosions. *Journal of Surgical Research* **22**, 357-361.

Cheung, L.Y., Moody, F.G., Larson, K. & Lowry, S.F. (1978) Oxygen consumption during cimetidine and prostaglandin E_2 inhibition of acid secretion. *American Journal of Physiology* **234**, E445-E450.

Cheung, L.Y. (1980) Topical effects of 16, 16-dimethyl prostaglandin E_2 on gastric blood flow in dogs. *American Journal of Physiology* **238**, G514-G519.

Church, E.M., Melhaff, C.J. & Patnaik, A.K. (1987) Colorectal adenocarcinoma in dogs: 78 cases (1973-1984). *Journal of the American Veterinary Medical Association* **191**, 727-730.

Chvasta, T.E. & Cooke, A.R. (1972) The effect of several ulcerogenic drugs on the canine gastric mucosal barrier. *Journal of Laboratory Clinical Medicine* **79**, 302-315.

Clain, J.E., Malagelada, J.-R., Chadwick, V.S. & Hofmann, A.F. (1977) Binding properties *in vitro* on antacids for conjugated bile acids. *Gastroenterology* **73**, 556-559.

Clamp, J.R. (1980) Gastrointestinal mucus. In: *Recent Advances in Gastrointestinal Pathology*. Ed. R. Wright. W.B. Saunders, Philadelphia. p47.

Clark, R.H. & Baker, B.L. (1963) Effect of hypophysectomy on mitotic proliferation in gastric epithelium. *American Journal of Physiology* **204**, 1018-1022.

Clark, C.G., Chowcat, N.L., Lewin, M.R., Gilbert, J.M., Gelister, J.S.K. & Boulos, P.B. (1986) Surgery for peptic ulceration associated with hypergastrinaemia. *British Journal of Surgery* 73, 248-252.

Clark, S., Katz, P.O., Wu, W.C., Geisinger, K.R. & Castell, D.O. (1987) Comparison of potential cytoprotective action of sucralfate and cimetidine: Studies with experimental feline esophagitis. *American Journal of Medicine* **83** (Suppl 3B), 56-60.

Clissold, S.P. & Campoli-Richards, D.M. (1986) Omeprazole: a preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in peptic ulcer disease and Zollinger-Ellison syndrome. *Drugs* **32**, 15-47.

Cloud, W.G. & Ritchie, W.P. (1982) Evidence for cytoprotection by endogenous prostaglandins in gastric mucosa treated with bile acid. *Surgical Forum* **33**, 150-152.

Coceani, F., Pace-Asciak, C., Volta, F. & Wolfe, L.S. (1967) Effect of nerve stimulation on prostaglandin function and release from the rat stomach. *American Journal of Physiology* **213**, 1056-1064.

Cochran, K.M., Cockel, R., Crowe, J., Dickinson, R.J., Gent, A.E., Kennedy, N.P., Royston, C.M., Cedar, E. & Mann, S.G. (1989) Comparison of 40mg famotidine nightly and 150mg ranitidine b.d.: ulcer healing and symptom relief in benign gastric ulcer. *Alimentary Pharmacology and Therapeutics* **3**, 461-470.

Code, C.F. (1977) Reflections of histamine, gastric secretion, and the H_2 receptor. *New England*

Journal of Medicine **296**, 1459-1462.

Code, C.F. (1981) Defence mechanisms of the gastric mucosa. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 201-204.

Code, C.F. & Martlett, J.A. (1975) The interdigestive myo-electric complex of the stomach and small bowel of dogs. *Journal of Physiology* **246**, 289-309.

Code, C.F., Scholer, J.F., Orvis, A.L. & Higgins, J.A. (1955) Barrier offered by gastric mucosa to absorption of sodium. *American Journal of Physiology* **183**, 604.

Code, C.F., Higgins, J.A., Moll, J.C., Orvis, A.L. & Scholer, J.F. (1963) The influence of acid on the gastric absorption of water, sodium and potassium. *Journal of Physiology* **166**, 110-119.

Cohen, M.M. & Pollett, J.M. (1976) Prostaglandin E_2 prevents aspirin and indomethacin damage to human gastric mucosa. *Surgical Forum* **27**, 400-401.

Cohen, S., Morris, D.W., Schoen, H.J. & DiMarino, A.J. (1976) The effect of oral and intra-venous metoclopramide on human lower esophageal sphincter pressure. *Gastroenterology* **70**, 484-487.

Cohen, M.M. & MacDonald, W.C. (1982) Mechanism of aspirin injury to human gastroduodenal mucosa. *Prostaglandins, Leukotrienes and Medicine* **9**, 241-255.

Coleman, J.C., Lacz, J.P., Browne, R.K. & Dress, D.T. (1987) Effects of sucralfate or mild irritants on experimental gastritis and prostaglandin production. *American Journal of Medicine* **83** (Suppl **3B**), 24-30.

Collier, D.S. & Pain, J.A. (1985) Non-steroidal anti-inflammatory drugs and peptic ulcer perforation. *Gut* **26**, 359-363.

Collins, B.J., Watt, P.C.H., O'Reilly, T., McFarland, R.J. & Love, A.H.G. (1984) Measurement of total bile acids in gastric juice. *Journal of Clinical Pathology* **37**, 313-316.

Cooke, A.R. & Kienzle, M.G. (1974) Studies of anti-inflammatory drugs and aliphatic alcohols on the antral mucosa. *Gastroenterology* **66**, 56-62.

Cooke, A.R. (1976) The role of the mucosal barrier in drug-induced gastric ulceration and erosion. *Digestive Diseases and Science* **21**, 154-164.

Coracoran, B.M., Thoday, K.L., Henfrey, J.I., Simpson, J.W., Burnie, A.G. & Mooney, C.T. (1991) Pulmonary infiltration with eosinophils in 14 dogs. *Journal of Small Animal Practice* **32**, 494-502.

Corazziari, E., Pozzessere, C., Dani, S., Anzini, F. & Torsoli, A. (1978) Lower oesophageal pressure sphincter response to intravenous infusions of pentagastrin in normal subjects, antrectomised and achalasic patients. *Gut* **19**, 1121-1124.

Correa, P., Cuello, C. & Duque, E. (1970) Carcinoma and intestinal metaplasia of the stomach of Colombian migrants. *Journal of the National Cancer Institute* **44**, 297-306.

Correa, P. (1984) Chronic gastritis as a cancer precursor. *Scandanavian Journal of Gastroenterology* **19** (Suppl **104**), 131-136.

Corsini, G., Bresci, G., Capria, A., Geloni, M. & Rindi, G. (1984) Sucralfate and carbenoxolone in the treatment of functional disturbances following partial gastrectomy. *International Journal of Tissue Reactions* **6**, 185-188.

Cosenza, S.F. (1984) Drug-induced gastroduodenal ulceration in dogs. *Modern Veterinary Practice* **65**, 923-925.

Couto, C.G., Rutgers, H.C., Sherding, R.G. & Rojko, J. (1989) Gastrointestinal lymphoma in 20 dogs. A retrospective study. *Journal of Internal Veterinary Medicine* **3**, 73-78.

Creamer, B., Shorter, R.G. & Bamforth, J. (1961) The turnover and shedding of epithelial cells. I. The turnover in the gastro-intestinal tract. *Gut* **2**, 110-116.

Crider, J.O. & Thomas, J.E. (1937) A study of gastric emptying with the pylorus open. *American Journal of Digestive Diseases* **4**, 295-300.

Critchlow, J., Takeuchi, K., Ito, S., Magee, D. & Silen, W. (1982) Effect of calcium depletion on the restitution of gastric mucosal injury. *Gastroenterology* **82**, 1038.

Croft, D.N. (1963) Aspirin and the exfoliation of gastric epithelial cells: Cytological and biochemical

observations. *British Medical Journal* **2**, 897-901.

Curwain, B.P. & Holton, P. (1972) The effects of isoprenaline and noradrenaline on pentagastrin-stimulated gastric acid secretion and mucosal blood flow in the dog. *British Journal of Pharmacology* **46**, 225-233.

Czarnobilski, Z., Bem, S., Czarnobilski, K. & Konturek, S.J. (1985) Carprofen and the therapy of gastroduodenal ulceration by ranitidine. *Hepato-Gastroenterology* **32**, 20-23.

Daehler, M. (1986) Transmural pyloric perforation associated with naproxen administration in a dog. *Journal of the American Veterinary Medical Association* **189**, 694-695.

Dammann, H.G., Walter, T.A., Hentschel, E., Muller, P. & Simon, B. (1987) Famotidine: Proven once-a-day treatment for gastric ulcer. *Scandanavian Journal of Gastroenterology* **22** (Suppl **134**), 29-33.

Danesh, J.Z.B., Duncan, A. & Russell, R.I. (1987) Is an acid medium required for the protective effect of sucralfate against mucosal injury. *American Journal of Medicine* **83** (Suppl **3B**), 11-18.

Danesh, B.J.Z., Burke, M., Newman, J., Aylott, A., Whitefield, P. & Cotton, P.B. (1985) Comparison of weight, depth, and diagnostic accuracy of specimens obtained with 16 different biopsy forceps for upper gastrointestinal endoscopy. *Gut* **26**, 227-231.

Davenport, H.W. (1964) Gastric mucosal injury by fatty and acetylsalicylic acids. *Gastroenterology* **46**, 245-253.

Davenport, H.W. (1966) Fluid produced by gastric mucosa during damage by acetic acid and salicylic acids. *Gastroenterology* **50**, 487-499.

Davenport, H.W. (1967a) Salicylate damage to the gastric mucosal barrier. *New England Journal of Medicine* **276**, 1307-1312.

Davenport, H.W. (1967b) Absorption of taurocholate-24-^{14}C through the canine gastric mucosa. *Proceedings of the Society for Experimental Biology and Medicine* **125**, 670-673.

Davenport, H.W. (1968) Destruction of the gastric mucosal barrier by detergents and urea. *Gastroenterology* **54**, 175-181.

Davenport, H.W. (1969) Gastric mucosal hemorrhage in dogs. Effects of acid, aspirin and alcohol. *Gastroenterology* **56**, 439-449.

Davenport, H.W. (1970) Effect of lysolecithin, digitoxin, and phospholipase A upon the dog's gastric mucosal barrier. *Gastroenterology* **59**, 505-509.

Davenport, H.W. (1975) The gastric mucosal barrier. *Mayo Clinic Proceedings* **50**, 507-514.

Davenport, H.W. (1977) Gastric Secretion. In: *Physiology of the Digestive Tract*. 4th edition, Year Book Medical Publishers, Chicago. p102.

Davenport, H.W., Warner, H.A. & Code, C.F. (1964) Functional significance of gastric mucosal barrier to sodium. *Gastroenterology* **47**, 142-152.

Dawson, J. & Cockel, R. (1982) Ranitidine in acute upper gastrointestinal haemorrhage. *British Medical Journal* **285**, 476-477.

Dekker, W. & Tytgat, G.N. (1977) Diagnostic accuracy of fiberendoscopy in the detection of upper gastrointestinal malignancy: a follow-up analysis. *Gastroenterology* **73**, 710-714

Delaney, J.P., Broadie, T.A. & Robbins, P.L. (1975) Pyloric reflux gastritis: The offending agent. *Surgery* **77**, 764-772.

Delaney, J.P., Michel, H.M., Eisenberg, M. & Bonsack, M. (1978) Quantification of antral gastrin cell populations in the dog. *Gastroenterology* **74**, 708-712.

Delle Fave, G.F., Tamburrano, G., De Magistris, L., Natoli, C., Santoro, M.L., Carratu, R. & Torsoli, A. (1977) Gynecomastia with cimetidine. *Lancet* **i**, 1319.

Demling, L., Elster, K., Koch, H. & Rosch, W. (1982) In: *Endoscopy and Biopsy of the Oesophagus, Stomach and Duodenum. A Colour Atlas*. translated by K.H. Soergel, 2nd edn. Philadelphia, W.B. Saunders. p132.

DenBesten, L. & Hamza, K.N. (1972) Effect of bile salts on ionic permeability of canine gastric mucosa

during experimental shock. *Gastroenterology* **62**, 417-424.

Diserens, H., Krstic, R., Burri, B., Mosimann, F. & Mosimann, R. (1984) The gastric mucosa in experimental duodenogastric reflux: A scanning electron microscope study. *Scandanavian Journal of Gastroenterology* **19** (Suppl **92**), 133-135.

Djahanguiri, B., Abtahi, F.S. & Hemmati, M. (1973) Prevention of aspirin-induced gastric ulceration by bile duct or pylorus ligation in the rat. *Gastroenterology* **65**, 630-633.

Dodd, K., Minot, A.S. & Arena, J.M. (1937) Salicylate poisoning; an explanation of the more serious manifestations. *American Journal of Diseases of Children* **53**, 1435-1446.

Domellöf, L. (1979) Gastric carcinoma promoted by alkaline reflux gastritis - with special reference to bile and other surfactants as promoters of post-operative gastric cancer. *Medical Hypothesis* **5**, 463-476.

Domellöf, L., Reddy, B.S. & Weisburger, J.H. (1980) Microflora and deconjugation of bile acids in alkaline reflux after partial gastrectomy. *American Journal of Surgery* **140**, 291-295.

Donovan, I.A., Sorgi, M., Mosimann, F., Wolverson, R.L., Harding, L.K. & Alexander-Williams, J. (1984) Does duodenogastric reflux affect the rate of gastric emptying? *Scandanavian Journal of Gastroenterology* **19** (Suppl **92**), 25-26.

Dooley, C.P., Larson, A.W., Stace, N.H., Renner, I.G., Valenzuela, J.E., Eliasoph, J., Colletti, P.M., Halls, J.M. & Weiner, J.M. (1984) Double-contrast barium meal and upper gastrointestinal endoscopy: a comparative study. *Annals of Internal Medicine* **101**, 538-545.

Douglas, S.W. & Williamson, H.D. (1970) The Alimentary Tract. In: *Veterinary Radiological Interpretation*. Heinemann Medical Books, London. p214.

Douthwaite, A.G. & Linott, G.A.M. (1938) Gastroscopic observations of the effect of aspirin and certain other substances on the stomach. *Lancet* **ii**, 1222-1225.

Dragstedt, L.R., Woodward, E.R., Seito, T., Isaza, J., Rodriquez, J.R. & Samahan, R. (1971) The question of bile regurgitation as a cause of gastric ulcer. *Annals of Surgery* **174**, 548-559.

Duane, C.W. & Weigand, D.M. (1980) Mechanism by which bile salt disrupts the gastric mucosal barrier in the dog. *Journal of Clinical Investigation* **66**, 1044-1049.

Duane, W.C., Weigand, D.M. & Sievert, C.E. (1982) Bile acid and bile salt disrupt gastric mucosal barrier in the dog by different mechanisms. *American Journal of Physiology* **242**, G95-G99.

Dubois, A., Dorval, E.D., Wood, L.R., Rogers, J.E., O'Connell, L., Durakovic, A. & Conklin, J.J. (1985) Effect of *Y*-irradiation on the healing of gastric biopsy sites in monkey: An experimental model for peptic ulcer disease and gastric protection. *Gastroenterology* **88**, 375-381.

Dyck, W.P., Werther, J.L., Rudick, J. & Janowitz, H.D. (1969) Electrolyte movement across canine antral and fundic mucosa. *Gastroenterology* **56**, 488-495.

Easmann, R.P., Steflik, D.E., Pashley, D.H., McKinney, R.V. & Whitford, G.M. (1984) Surface changes in rat gastric mucosa induced by sodium fluoride: a scanning electron microscopic study. *Journal of Oral Pathology* **13**, 255-264.

Eastwood, G.L. (1974) Effect of cholestyramine on taurocholate induced injury to mouse gastric mucosa. *Gastroenterology* **66**, 687.

Eastwood, G.L. (1975) Effect of pH on bile salt injury to mouse gastric mucosa. *Gastroenterology* **68**, 1456-1465.

Eastwood, G.L. (1984) Effects of ulcerogens on gastroduodenal epithelial proliferation. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p27.

Eastwood, G.L. (1985) Ultrastructural effects of ulcerogens. *Digestive Diseases and Sciences* **30** (Suppl **11**), 95S-104S.

Eastwood, G.L. & Kirchner, J.P. (1974) Changes in the fine structure of mouse gastric epithelium produced by ethanol and urea. *Gastroenterology* **64**, 71-84.

Eastwood, G.L. & Quimby, G.F. (1982) Effect of chronic aspirin ingestion on epithelial proliferation in rat fundus, antrum, and duodenum. *Gastroenterology* **82**, 852-856.

Edkins, J.S. (1905) On the chemical mechanism of gastric secretion. *Proceedings of the Royal Society* **B76**, 376.

Ehrlein, H.-J. (1981) Gastric and duodenal motility in relation to duodenogastric reflux in healthy dogs. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 23-27.

Ehrlein, H.-J., Pröve, J. & Schweiker, W. (1980) The function of the pyloric sphincter for regulating gastric and for preventing reflux in dogs. In: *Gastrointestinal Motility*. Ed. J. Christensen. Raven Press, New York, p177.

Ekman, L., Hansson, E., Havu, N., Carlsson, E. & Lundberg, C. (1985) Toxicology studies on omeprazole. *Scandanavian Journal of Gastroenterology* **20** (Suppl **108**), 53-59.

Ellenport, C.R. (1975) The Digestive System. In: *Sissons & Grossman's -The Anatomy of the Domestic Animals*. 5th edition, ed. R. Getty. W.B. Saunders, Philadelphia. p1538.

Else, R.W. & Head, K.W. (1980) Some pathological conditions of the canine stomach. *Veterinary Annual* **20**, 66-81.

Elwin, C.E. & Uvnäs, B. (1967) Distribution and local release of gastrin. In: *Gastrin*. Ed. M.I. Grossman, Berkeley, University of California Press. p69.

Ewing, G.O. (1972) Indomethacin associated gastrointestinal hemorrhage in a dog. *Journal of the American Veterinary Medical Association* **161**, 1665-1668.

Eyre-Brock, I.A., Holroyd, A.M. & Johnson, A.G. (1984) Is bile reflux at endoscopy a significant finding? *Scandanavian Journal of Gastroenterology* **19** (Suppl **92**), 203-205.

Faber, R.G., Russell, R.C.G., Royston, C.M.S., Whitefield, P. & Hobsley, M. (1974) Duodenal reflux during insulin-stimulated secretion. *Gut* **15**, 880-884.

Fallah, E., Schuman, B.M., Watson, J.H.L. & Goodwin, J. (1976) Scanning electron microscopy of gastroscopic biopsies. *Gastrointestinal Endoscopy* **22**, 137-144.

Fasano, A., Budillon, G., Guandalini, S., Cuoma, R., Parrilli, G., Cangiotti, A.M., Morroni, M. & Rubino, A. (1990) Bile acids reversible effects on small intestinal permeability: An *in vitro* study in the rabbit. *Digestion Disease and Sciences* **35**, 801-808.

Fawcett, D.W. (1981) Junctional Specializations. In: *The Cell*. 2nd edition. W.B. Saunders, Philadelphia. p124.

Feldman, M. & Richardson, C.T. (1986) Role of thought, site, smell, and taste of food in the cephalic phase of gastric acid secretion in humans. *Gastroenterology* **90**, 428-433.

Ferguson, A.M. (1928) A cytochemical study of the regeneration of gastric glands following the experimental removal of large areas of mucosa. *American Journal of Anatomy* **42**, 403-435.

Ferguson, W.W., Edmonds, A.W., Starling, J.R. & Wangensteen, S.L. (1973) Protective effect of prostaglandin E_1 (PGE_1) on lysosomal enzyme release in serotonin-induced gastric ulceration. *Annals of Surgery* **177**, 648-654.

Ferreira, S.H., Moncada, S. & Vane, J.R. (1971) Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature New Biology* **231**, 237-239.

Fiasse, R., Code, C.F. & Glass, G.B.J. (1968) Fractionation and partial purification of gastrin. *Gastroenterology* **54**, 1018-1031.

Finckh, E.S. & Milton, G.W. (1960) Regeneration of gastric mucosa from differentiated cells. *Journal of Pathology and Bacteriology* **80**, 134-145.

Fisher, R. & Cohen, S. (1973) Physiologic characteristics of the human pyloric sphincter. *Gastroenterology* **64**, 67-75.

Flemström, G. (1976) Properties of isolated gastric antral and SCN- inhibited fundic mucosa. In: *Gastric Hydrogen Ion Secretion*. Eds. D.K. Kasbekar, G. Sachs & W.S. Rehm. Marcel Dekker Inc., New York. p102.

Flemström, G. & Garner, A. (1982) Gastrodoudenal HCO_3^- transport: characteristics and proposed role in acidity regulation and mucosal protection. *American Journal of Physiology* **242**, G183-G193.

Flemström, G., Heylings, J.R. & Garner, A. (1982) Gastric and duodenal HCO_3^- transport: *in vitro* effects of hormones and local transmitters. *American Journal of Physiology* **242**, G100-G110.

Fletcher, D.G. & Harkins, H.H. (1954) Acute peptic ulcer as a complication of major surgery. *Surgery* **36**, 212-219.

Flower, R.J. (1974) Drugs inhibit prostaglandin biosynthesis. *Pharmacological Reviews* 26, 33-67.

Fontham, E., Zavala, D., Correa, P., Rodriquez, E., Hunter, F., Haenszel, W. & Tannenbaum, S.R. (1985) Diet and chronic atrophic gastritis: A case-control study. *Journal of the National Cancer Institute* **76**, 621-627.

Fork, F.Th., Haglund, U., Högström. H. & Wehlin, L. (1985) Primary gastric lymphoma versus gastric cancer. An endoscopic and radiographic study of differential diagnostic possibilities. *Endoscopy* **17**, 5-7.

Forte, J.G., Ganser, A.L. & Ray, T.K. (1976) The K^+-stimulated ATPase from oxyntic glands of gastric mucosa. In: *Gastric Hydrogen Ion Secretion*. Eds. D.K. Kasbekar, G. Sachs & W.S. Rehm. Marcel Dekker Inc., New York. p302.

Forte, T.M., Silen, W. & Forte, J.G. (1976) Ultrastructural lesions in the gastric mucosa exposed to deoxycholate: implications toward the barrier concept. In: *Gastric Hydrogen Ion Secretion*. Eds. D.K. Kasbekar, G. Sachs & W.S. Rehm. Marcel Dekker Inc., New York. p1.

Fox, S.M. & Burns, J. (1986) The effect of pyloric surgery on gastric emptying in the dog: Comparison of three techniques. *Journal of American Animal Hospital Association* **22**, 783-788.

Fox, J.G., Correa, D., Taylor, N.S., Lee, A., Murphy, J.C. & Rose, R. (1990) *Heliobacter mustelae* - associated gastritis in ferrets. An animal model of *Heliobacter pylori* gastritis in humans. *Gastroenterology* **99**, 352-361.

Frank, S.A., Walker C.O. & Fordtran, J.S. (1973) The effect of continuous pentagastrin (PG) infusion on the lower esophageal sphincter pressure (LES). *Gastroenterology* **64**, 728.

Franzin, G., Manfrini, C., Musola, R., Rodella, S. & Fratton, A. (1984) Chronic erosions of the stomach - a clinical, endoscopic and histological evaluation. *Endoscopy* **16**, 1-5.

Frenning, B. (1973) The effects of large osmolality variations on the gastric mucosal surface ultrastructure. *Scandanavian Journal of Gastroenterology* **8**, 185-192.

Frenning, B. & Öbrink, K.J. (1971) The effects of acetic and acetyl salicylic acids on the appearance of the gastric mucosal surface epithelium in the scanning electron microscope. *Scandanavian Journal of Gastroenterology* **6**, 605-612.

Fringes, B., Lorenz, D. & Oehlert, W. (1985) The cytoprotective effect of prostaglandin E_2 on taurocholate-induced erosions in gastric mucosa of the rat. *Pathological Research Practice* **179**, 478-486.

Frolich, J.C., Jeunet, F., Kunovits, G. & Scholz, H.J. (1984) Wirkung von Indometacin und Carprofen auf die gastrale Prostaglandin-Biosynthese. *Arzneimittelforschung* **34**, 1783-1785.

Fromm, D. (1981) Drug-induced gastric mucosal injury. *World Journal of Surgery* **5**, 199-208.

Fromm, D., Silen, M. & Robertson, R. (1976) Histamine effects on H^+ permeability by isolated gastric mucosa. *Gastroenterology* **70**, 1076-1081.

Fujita, T., Tanaka, K. & Tokunaga, J. (1981) Stomach. In: *SEM Atlas of Cells and Tissues*. Igaku-Shoin, Tokyo. p106.

Gad, A. (1986) Erosion: A correlative endoscopic histopathologic multicenter study. *Endoscopy* **18**, 76-79.

Gaffner, H., Florén, C.-H. & Nilsson, Ä. (1984) Conjugated bile acids in gastric aspirates after gastric resection. *Scandanavian Journal of Gastroenterology* **19**, 116-118.

Galbraith, R.A. & Michnovicz, J.J. (1989) The oxidative metabolism of estradiol: inhibition by cimetidine. *Transactions of the Association of American Physicians* **102**, 44-45.

Ganser, A.L. & Forte, J.G. (1973) K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochimica et Biophysica Acta* **307**, 169-180.

Garner, A. (1978) Mechanisms of action of aspirin on the gastric mucosa of the guinea pig. *Acta*

Physiologica Scandinavica (Special Supplement), Gastric Ion Transport. Eds. K.J. Öbrink & G. Flemström. p101.

Garnett W.R. (1982) Sucralfate--alternative therapy for peptic-ulcer disease. *Clinical Pharmacokinetics* **1**, 3073-3014.

Gasbarrini, G., Andreone, P., Barraldini, M. & Cursaro, C. (1990) Antacids in gastric ulcer treatment: Evidence of cytoprotection. *Scandanavian Journal of Gastroenterology* **25** (Suppl **174**), 44-47.

Gasster, M., Westwater, J.O. & Molle, W.E. (1954) Use of a defoaming agent in gastroscopy. *Gastroenterology* **27**, 652-655.

Gerber, J.G. & Nies, A.S. (1982) Canine gastric mucosal vasodilation with prostaglandins and histamine analogs. *Digestive Diseases and Sciences* **27**, 870-874.

Gerkins, J.F., Shand, D.G., Flexner, C., Nies, A.S., Oates, J.A. & Data, J.L. (1977) Effect of indomethacin and aspirin on gastric blood flow and acid secretion. *Journal of Pharmacology and Experimental Therapeutics* **203**, 646-652.

Gibbs, C. & Pearson, H. (1973) The radiological diagnosis of gastrointestinal obstruction in the dog. *Journal of Small Animal Practice* **14**, 61-82.

Gibson, R., Hirschowitz, D.I. & Hutchison, G. (1974) Actions of metiamide on H_2-histamine receptor antagonist, on gastrin H+ and pepsin secretion in dogs. *Gastroenterology* **67**, 93-99.

Gjeruldsen, S.T., Myren, J. & Fretheim, B. (1968) Alterations of gastric mucosa following a graded partial gastrectomy for duodenal ulcer. *Scandanavian Journal of Gastroenterology* **3**, 465-470.

Glikmanas, M., Souillac, P., Monteiro, I. & Gatineau-Saillant, G. (1984) Is the presence of a bilious mucus lake during endoscopy significant? A 24 hour gastric pH study. *Gastroenterologie Clinique et Biologique* **8**, 796-799.

Glise, H. (1990) Epidemiology in peptic ulcer disease: Current status and future aspects. *Scandanavian Journal of Gastroenterology* **25** (Suppl **175**), 13-18.

Goddard, P.J., Hills, B.A. & Lichtenberger, L.M. (1987) Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity? *American Journal of Physiology* **252**, G421-G430.

Goldblatt, M.W. (1933) A depressor substance in seminal fluid. *Journal of the Society of Chemical Industry* (London) **52**, 1056-1057.

Golenhofen, K., Lüdtke, F.E., Milenou, K. & Siewert, R. (1980) Excitatory and inhibitory effects on canine pyloric musculature. In: *Gastrointestinal Motility*. Ed. J. Christensen. Raven Press, New York. p203.

Goodman, A.A. & Osborne, M.P. (1972) Experimental model and clinical definition of stress ulceration. *Surgery Gynecology and Obstetrics* **134**, 563-571.

Goto, Y., Wakabayashi, T. & Murakami, M. (1985) Long-term treatment with cimetidine decreases rat gastric mucosal defence mechanisms. *Gastroenterology* **88**, 1401.

Gottschalk, A. & Menguy, R. (1970) Role of gastric acid in aspirin-induced erosive gastritis. *Proceedings of the Society for Experimental Biology and Medicine* **135**, 384-388.

Gough, M.J. (1985) Bile reflux and the gastric mucosal barrier after truncal vagotomy and drainage. *British Journal of Surgery* **72**, 853-858.

Graffner, H., Florén, C,-H. & Nilsson, Ä. (1984) Conjugated bile acids in gastric aspirates after gastric resection. *Scandanavian Journal of Gastroenterology* **19**, 116-118.

Grant, R. (1944) Conditions under which the epithelial cells of the gastric mucosa are shed and disintegrate. *Canadian Medical Association Journal* **51**, 577-578.

Grant, R. (1945) Rate of replacement of the surface epithelial cells of the gastric mucosa. *Anatomy Record* **91**, 175-185.

Grant, R., Grossman, M.I., Wang, K.J. & Ivy, A.C. (1951) The cytolytic action of some gastrointestinal secretions and enzymes on the epithelial cells of the gastric and duodenal mucosa. *Journal of Cellular and Comparative Physiology* **37**, 137-161.

Gregory, R.A. & Tracy, H.J. (1961) The preparation and properties of gastrin. *Journal of Physiology* **156**, 523-543.

Gregory, H., Hardy, R.M., Jones, D.S., Kenner, G.W. & Sheppard, R.C. (1964) Structure of gastrin. *Nature* **204**, 931-933.

Grisham, M.B. & Granger, D.N. (1988) Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Digestive Diseases and Sciences* **33**, 6S-15S.

Grönbech, J.E., Grong, K., Varhaug, J.E., Lekven, J. & Svanes, K. (1987) Gastric epithelial restitution at low luminal pH during influence of pentagastrin or cimetidine in the cat. *Gastroenterology* **93**, 753-764.

Grönbech, J.E., Matre, K., Stangeland, L., Svanes, K. & Varhaug, J.E. (1988) Gastric mucosal repair in the cat: role of the hyperemic response to mucosal damage. *Gastroenterology* **95**, 311-320.

Grossman, M.I. (1958) The names of the parts of the stomach. *Gastroenterology* **34**, 1159-1162.

Grossman, M.I. & Marks, N.I. (1960) Secretion of pepsinogen by the pyloric glands of the dog, with some observations on the histology of the gastric mucosa. *Gastroenterology* **38**, 343-351.

Gurll, N.J. & Damianos, A.J. (1981) The role of histamine and histamine receptors in the pathogenesis and treatment of erosive gastritis. *World Journal of Surgery* **5**, 181-187.

Guslandi, M. (1985) Sucralfate and gastric bicarbonate. *Pharmacology* **31**, 298-300.

Guth, P.H. (1984) Local metabolism and circulation in mucosal defense. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p253.

Guth, P.H. (1987) Mucosal coating agents and other nonantisecretory agents, are they cytoprotective? *Digestive Diseases and Sciences* **32**, 647-654.

Guth, P.H. & Code, C.F. (1978) Histamine release and gastric mucosal damage. *Gastroenterology* **74**, 622-623.

Guth, P.H., Aures, D. & Paulsen, G. (1979) Topical aspirin plus HCl gastric lesions in the rat: Cytoprotection effect of prostaglandin, cimetidine, and probanthine. *Gastroenterology* **76**, 88-93.

Guth, P.H., Paulsen, G., Lynn, D. & Aures, D. (1976) Mechanism of prevention of aspirin-induced gastric lesions by bile duct ligation in the rat. *Gastroenterology* **71**, 750-753.

Guy, P.A. (1984) *Ollulanus tricuspis* in domestic cats - prevalence and methods of post-mortem diagnosis. *New Zealand Veterinary Journal* **32**, 81-84

Guyton, A.C. (1981) Secretory functions of the alimentary tract. In: *Textbook of Medical Physiology*. 6th edition. W.B. Saunders, Philadelphia. p801.

Haenszel, W., Kurihara, M. & Segi, M. (1972) Stomach cancer among Japanese in Hawaii. *Journal of the National Cancer Institute* **49**, 969-988.

Haglund, U. (1990) Stress ulcers. *Scandanavian Journal of Gastroenterology* **25** (Suppl **175**), 27-33.

Håkanson, R., Oscarson, J. & Sundler, F. (1986) Gastrin and the trophic control of gastric mucosa. *Scandanavian Journal of Gastroenterology* **21** (Suppl **118**), 16-30.

Hammond, A.L. (1971) Aspirin: new perspective on everyman's medicine. *Science* **174**, 48.

Hansen, D.G., Aures, D. & Grossman, M.I. (1978) Histamine augments gastric ulceration produced by intravenous aspirin in cats. *Gastroenterology* **74**, 540-543.

Happé, R.P. (1986) Gastrointestinal endoscopy in the dog. *Tijdschrift voor Diergeneeskunde* **11**, 195-198.

Happé, R.P., van den Brom, W.E. & van der Gaag, I. (1982) Duodenogastric reflux in the dog, a clinicopathological study. *Research in Veterinary Science* **33**, 280-286.

Happé, R.P. & van der Gaag, I. (1983) Endoscopic examination of esophagus, stomach and duodenum in the dog. *Journal of the American Animal Hospital Association* **19**, 197-206.

Happé, R.P., van der Gaag, I. & Wolvekamp, W.Th.C. (1981) Pyloric stenosis caused by hypertrophic gastritis in three dogs. *Journal of Small Animal Practice* **22**, 7-17.

Happé, R.P., van der Gaag, I., Lamers, C.B.H.W., van Toorebburg, J., Rehfeld, J.F. & Larsson, L.I.

(1980) Zollinger-Ellison syndrome in three dogs. *Veterinary Pathology* **17**, 177-186.

Harding, R.K. & Morris, G.P (1976) Pathological effects of aspirin and of haemorrhagic shock on the gastric mucosa of the rat. *Scanning Electron Microscopy/1976* **V**, 253-262.

Harding, K.R. & Morris, G.P. (1977) Cell loss from normal and stressed gastric mucosae of the rat. An ultrastructural analysis. *Gastroenterology* **72**, 857-863.

Hargis, A.M., Prieur, D.J. & Westcott, R.B. (1981) A gastric nematode (*Ollulanus tricuspis*) in cats in the Pacific Northwest. *Journal of the American Veterinary Medical Association* **178**, 475-478.

Harmon, J.W., Lewis, C.D. & Gadacz, T. (1981) Bile salt composition and concentration as determinants of canine gastric mucosal injury. *Surgery* **89**, 348-354.

Harrington, S.J., Schlegel, J.F. Code, C.F. (1981) The protective effect of sucralfate on the gastric mucosa of rats. *Journal of Clinical Gastroenterology* **3** (Suppl 2), 129-134.

Harris, J.B., Nigon, K. & Alonso, D. (1969) Adenosine-3', 5-monophosphate: Intracellular mediator for methyl xanthine stimulation of gastric secretion. *Gastroenterology* **57**, 377-384.

Harvey, B.C.H. (1906) A study of the structure of the gastric glands of the dog and of the changes which they undergo after gastroenterostomy and occlusion of the pylorus. *American Journal of Anatomy* **6**, 207-239.

Hasslinger, M.A. (1984) *Ollulanus tricuspis*, the stomach worm of the cat. *Feline Practice* **14**, 22-35

Hatfield, A.R.W., Slavin, G., Segal, A.W. & Levi, A.J. (1975) Importance of the site of endoscopic gastric biopsy in ulcerating lesions in the stomach. *Gut* **16**, 884-886.

Hayden, D.W. & Van Kruiningen, H.J. (1973) Eosinophilic gastroenteritis in German Shepherd dogs and its relationship to visceral larval migrans. *Journal of the American Veterinary Medical Association* **162**, 379-384.

Hazell, S.L. & Lee, A. (1986) *Campylobacter pyloridis*, urease, hydrogen ion back diffusion and gastric ulcers. *Lancet* **ii**, 15-17.

Hazell, S.L., Lee, A., Brady, L. & Hennessy, W. (1986) *Campylobacter pyloridis* and gastritis: Association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. *Journal of Infectious Diseases* **153**, 658-663.

Heading, R.C. (1983) Duodenogastric reflux. *Gut* **24**, 507-509.

Heatley, N.G. (1959) Mucosubstance as a barrier to diffusion. *Gastroenterology* **37**, 313-317.

Hill, M.J. Hawksworth, C. & Tattersall, G. (1973) Bacteria, nitrosamines and cancer of the stomach. *British Journal of Cancer* **28**, 562-567.

Hills, B.A. (1982) Water repellency induced by pulmonary surfactants. *Journal of Physiology* **325**, 175-186.

Hills, B.A., Butler, B.D. & Lichtenberger, L.M. (1983) Gastric mucosal barrier: hydrophobic lining to the lumen of the stomach. *American Journal of Physiology* **244**, G561-G568.

Hingson, D.J. & Ito, S. (1971) Effect of aspirin and related compounds on the fine structure of mouse gastric mucosa. *Gastroenterology* **61**, 157-171.

Hirschowitz, B.I. & Hutchison, G.A. (1977) Evidence for a histamine H_2 receptor that inhibits pepsin secretion in the dog. *American Journal of Physiology* **233**, E225-E228.

Hoare, A.M., Keighley, M.R.B., Starkey, B. & Alexander-Williams, J. (1978) Measurement of bile acids in fasting gastric aspirates: an objective test for bile reflux after gastric surgery. *Gut* **19**, 166-169.

Hoffman, A.F. & Small, D.M. (1967) Detergent properties of bile salts: correlation with physiological function. *Annual Review of Medicine* **18**, 333-376.

Hogben, C.A.M. (1955) Active transport of chloride by isolated frog gastric epithelium. Origin of the gastric mucosal potential. *American Journal of Physiology* **180**, 641-649.

Hollander, D. (1981) Efficacy of sucralfate for duodenal ulcers: A multicenter, double-blind trial. *Journal of Clinical Gastroenterology* **3** (Suppl 2), 153-157.

Hollander, F. (1954) Two-component mucous barrier: its activity in protecting gastrodoudenal mucosa against peptic ulceration. *Archives Internal Medicine* **93**, 107-129.

Hollander, F., Stein, J. & Lauber, F.U. (1946) The consistency, opacity and columnar cell content of gastric mucosa secreted under the influence of several mild irritants. *Gastroenterology* **6**, 576-595.

Hollander, D., Tarnawski, A., Gergely, H. & Zipser, R.D. (1984) Sucralfate protection of the gastric mucosa against ethanol-induced injury: a prostaglandin-mediated process? *Scandanavian Journal of Gastroenterology* **19** (Suppl **101**), 97-102.

Hollander, D., Tarnawski, A., Krause, W.J. & Gergely, H. (1985) Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat: Macroscopic, histologic, ultrastructural, and functional time sequence analysis. *Gastroenterology* **88**, 366-374.

Honsinger, R.W., Silverstein, D. & van Arsdel, P.P. (1972) The eosinophil and allergy: Why? *Journal of Allergy and Clinical Immunology* **49**, 142-153.

Houghton, P.W.J., Mortensen, N.J. MacC., Thomas, W.E.G., Cooper, M.J., Morgan, A.P. & Burton, P. (1986) Intragastric bile acids and histological changes in gastric mucosa. *British Journal of Surgery* **73**, 354-356.

Howson, C.P., Hiyama, T. & Wynder, E.L. (1986) The decline of gastric cancer: Epidemiology of an unplanned triumph. *Epidemiologic Reviews* **8**, 1-27.

Hunt, T.E. & Hunt, E.A. (1962) Radioautographic study of proliferation in the stomach of the rat using thymidine-H^3 and compound 48/80. *Anatomy Record* **142**, 505-517.

Hurley, J.W. & Crandall, L.A. (1964) The effects of salicylates upon the stomach of dogs. *Gastroenterology* **46**, 36-43.

Ichikawa, H. (1971) Detectability of early gastric carcinoma with indirect fluororadiography. In: *Early Gastric Cancer*. University of Tokyo Press, Tokyo. p27.

Ichikawa, H. (1973) Differential diagnosis between benign and malignant ulcers of the stomach. *Clinics in Gastroenterology* **2**, 329-343.

Ito, S. (1967) Anatomic structure of the gastric mucosa. In: *Handbook of Physiology*. Vol II. Ed. C.F. Code. American Physiological Society, Washington D.C. p705.

Ito, S. & Lacy, E.R. (1985) Morphology of rat gastric mucosal damage, defense, and restitution in the presence of luminal ethanol. *Gastroenterology* **88**, 250-260.

Ito, S. & Winchester, R.J. (1963) The fine structure of the gastric mucosa in the bat. *Journal of Cell Biology* **16**, 541-577.

Ivey, K.J., DenBesten, L. & Clifton, J.A. (1970) Effect of bile salts on ionic movement across the human gastric mucosa. *Gastroenterology* **59**, 683-690.

Ivy, A.C. (1955) Experimental observations on the etiology of gastric carcinoma. I. A theoretical analysis of the problem. *Gastroenterology* **28**, 325-344.

Ivy, K.J. (1971) Gastric mucosal barrier. *Gastroenterology* **61**, 247-257.

Jacobson, E.D., Linford, R.H. & Grossman, M.I. (1966) Gastric secretion in relation to mucosal blood flow studied by a clearance technic. *Journal of Clinical Investigation* **45**, 1-13.

Jacobson, E.D., Swan, K.G. & Grossman, M.I. (1967) Blood flow and secretion in the stomach. *Gastroenterology* **52**, 414-420.

Jakovljevic, S. (1988) Gastric radiology and gastroscopy in the dog. *Veterinary Annual* **28**, 172-182.

James, S.L. & Marriott, C. (1982) An investigation of the effect of an antacid mucosal protective on gastric mucus. *Pharmaceutica Acta Helvetiae* **57**, 265-267.

Janowitz, H.D., Hollander, F. & Jackson, C. (1951) Stimulation of cell-free gastric mucus by the topical application of acetylcholine. *Proceedings of the Society Experimental Biology and Medicine* **76**, 578-580.

Jensen, D. (1980) Gastrointestinal Motility. In: *The Principles of Physiology*. 2nd edition. Appleton-Century-Crofts, New York. p774.

Johansson, G. & Bergström, S. (1982) Prostaglandins and protection of the gastroduodenal mucosa.

Scandanavian Journal of Gastroenterology **17** (Suppl 77), 21-46.

Johnson, L.R. (1966) Histamine liberation by gastric mucosa of pylorus-ligated rats damaged by acetic or salicylic acids. *Proceedings of the Society for Experimental Biology and Medicine* **121**, 384-386.

Johnson, L.R. (1968) Source of the histamine released during damage to the gastric mucosa by acetic acid. *Gastroenterology* **54**, 8-15.

Johnson, L.R. (1971) Pepsin output from the damaged canine Heidenhain pouch. *American Journal of Digestive Diseases* **16**, 403-407.

Johnson, A.G. (1972) Pyloric function and gallstone dyspepsia. *British Journal of Surgery* **59**, 449-454.

Johnson, S.E. (1989) Pancreatic APUDomas. *Seminars in Veterinary Medicine and Surgery (Small Animal)* **4**, 202-211.

Johnson, A.G. & McDermott, S.J. (1974) Lysolecithin: A factor in the pathogenesis of gastric ulceration? *Gut* **15**, 710-713.

Johnson, L.R. & Overholt, B.F. (1967) Release of histamine into gastric venous blood following injury by acetic or salicylic acid. *Gastroenterology* **52**, 505-509.

Jones, B.R., Nicholls, M.R. & Badman, R. (1976) Peptic ulceration in a dog associated with an islet cell carcinoma of the pancreas and elevation of plasma gastrin level. *Journal of Small Animal Practice* **17**, 593-598.

Kammeraad, A. (1942) The development of the gastrointestinal tract of the rat. I. Histogenesis of the epithelium of the stomach, small intestine and pancreas. *Journal of Morphology* **70**, 323-351.

Kao, Y-C. J. & Lichtenberger, L.M. (1991) Phospholipid- and neutral lipid-containing organelles of rat gastroduodenal mucous cells. Possible origin of the hydrophobic mucosal lining. *Gastroenterology* **101**, 7-21.

Kao, Y-C, J., Goddard, P.J. & Lichtenberger, L.M. (1990) Morphological effects of aspirin and prostaglandin on the canine gastric mucosal surface: Analysis with a phospholipid-selective cytochemical stain. *Gastroenterology* **98**, 592-606.

Karvonen, A.-L., Sipponen, P., Lehtola, J. & Ruokonen, A. (1983) Gastric mucosal erosions. An endoscopic, histological, and functional study. *Scandanavian Journal of Gastroenterology* **18**, 1051-1056.

Kasbekar, D. (1972) Secretagogue interrelationships in amphibian gastric H^+ secretion. In: *Gastric Secretion*. Eds. G. Sachs, E. Heinz & K.L. Ullrich. Academic Press, London. p203.

Katz, L.B., Shriver, D.A. & Rosenthale, M.E. (1987) Selective gastric antilesion properties of rioprostil, a prostaglandin E_1 analog in rats and dogs. *Journal of Pharmacology and Experimental Therapeutics* **242**, 927-933.

Katz, L.B., Tobia, A.J. & Shriver, D.A. (1987) Effects of ORF 17583, other histamine H_2-receptor antagonists and omeprazole on gastric acid secretory states in rats and dogs. *Journal of Pharmacology and Experimental Therapeutics* **242**, 437-442.

Katz, L.B., Genna, T., Scott, C.K., Rosenthale, M.E. & Shriver, D.A. (1987a) Antigastrolesive, gastric antisecretory, diarrheagenic and mucus-stimulatory effects in rats following topically applied rioprostil, a synthetic prostaglandin E_1 analog. *Life Sciences* **41**, 1591-1598.

Katz, L.B., Genna, T., Greeely, G.H. & Shriver, D.A. (1987b) Antisecretory and antigastrin effects of rioprostil in gastric fistula dogs. *Digestive Diseases and Science* **32**, 1268-1274.

Kauffman, G.L. (1984) Mucosal damage to the stomach: how, when and why? *Scandanavian Journal of Gastroenterology* **19** (Suppl **105**), 19-28.

Kauffman, G.L. & Grossman, M.I. (1978) Prostaglandin and cimetidine inhibit the formation of ulcers by parenteral salicylates. *Gastroenterology* **75**, 1099-1102.

Kauffman, G.L., Reeve, J.J. & Grossman, M.I. (1980) Gastric bicarbonate secretion: effect of topical and intravenous 16,16-dimethyl prostaglandin E_2. *American Journal of Physiology* **239**, G44-G48.

Kauffman, G.L., Whittle, B.J.R., Aures, D., Vane, J.R. & Grossman, M.I. (1979) Effects of prostacyclin and a stable analoque $6_\beta PGI_1$, on gastric secretion, mucosal blood flow and blood pressure in conscious dogs. *Gastroenterology* **77**, 1301-1306.

Kawai, K., Miyaoka, T. & Kohli, Y. (1974) Evaluation of early gastric cancer from the clinical point of view. In: *Early Gastric Cancer: Current Status of Diagnosis.* Eds. E. Grundmann, H. Grunze & S. Witte. Springer-Verlag, Berlin. p63.

Kaye, M., Mehta, S.J. & Showalter, J.P. (1976) Manometric studies of the human pylorus. *Gastroenterology* **70**, 477-480.

Keighley, M.R.B., Asquith, P. & Alexander-Williams, J. (1975) Duodenogastric reflux: A cause of gastric mucosal hyperaemia and symptoms after operations for peptic ulceration. *Gut* **16**, 28-32.

Kekki, M., Ihämaki, T., Varis, K. & Siurala, M. (1991) Chronic gastritis profiles in sibs of probands of calculated to carry a highly increased risk of gastric carcinoma. *Scandanavian Journal of Gastroenterology* **26** (Suppl **186**), 29-32.

Kellosalo, J., Alavaikko, M. & Laitinen, S. (1991) Effect of biliary tract procedures on duodenogastric reflux and the gastric mucosa. *Scandanavian Journal of Gastroenterology* **26**, 1272-1278.

Kelly, K.A. (1980) Gastric emptying of liquids and solids: roles of proximal and distal stomach. *American Journal of Physiology* **239**, G71-G76.

Kelly, D.G., Code, C.F., Lechago, J., Bugaski, J. & Shelger, J.F. (1979) Physiological and morphological characteristics of progressive disruption of the canine gastric mucosal barrier. *Digestive Diseases and Science* **24**, 424-441.

Kent, P.W. & Allen, A. (1968) The biosynthesis of intestinal mucosubstances. The effects of salicylates on glycoprotein biosynthesis. *Biochemical Journal* **106**, 645-658.

Kenyon, G.S., Ansell, I.F. & Carter, D.C. (1977) Cimetidine and the gastric mucosal barrier. *Gut* **18**, 631-635.

Kilby, J. (1970) Duodenogastric reflux and pyloric surgery. *Gastroenterology* **58**, 593-595.

Kimmey, M.B., Martin, R.W., Haggitt, R.C., Wang, K.V., Franklin, D.W. & Silverstein, F.E. (1989) Histologic correlates of gastrointestinal ultrasound images. *Gastroenterology* **96**, 433-441.

Kirk, E.G. (1910) On the histogenesis of gastric glands. *American Journal of Anatomy* **10**, 473-520.

Kirk, R.M. (1970) Experimental gastric ulcers in the rat: The separate and combined effects of vagotomy and bile duct implantation into the stomach. *British Journal of Surgery* **57**, 521-524.

Kirsner, J.B. & Ford, N. (1955a) Phenylbutazone (Butazolidin) - studies on the stimulation of gastric secretion and formation of peptic ulcer in man. *Gastroenterology* **29**, 1-17.

Kirsner, J.B. & Ford, N. (1955b) Phenylbutazone (Butazolidin). Effect on basal gastric secretion and the production of gastro-duodenal ulcerations in dogs. *Gastroenterology* **29**, 18-23.

Kitamura, N., Yamada, J., Calingasan, N.Y. & Yamashita, T. (1984) Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Equine Veterinary Journal* **16**, 103-107.

Kivilaakso E., Fromm, D. & Silen, W. (1978) Relationship between ulceration and intramural pH of gastric mucosa during haemorrhagic shock. *Surgery* **84**, 70-78.

Klag, A.R., Giger, U. & Shofer, F.S. (1993) Idiopathic immune-mediated hemolytic anaemia in dogs: 42 cases (1986-1990). *Journal of the American Veterinary Medical Association* **202**, 783-788.

Konturek, S.J. (1974) Gastric Secretion. In: *Gastrointestinal Physiology.* Vol 4, eds. E.D. Jacobson & L.L. Shanbour. Butterworth & Co Ltd. London. p227.

Konturek, S.J. (1985) Prostaglandins in pathophysiology of peptic ulcer disease. *Digestive Diseases and Science* **30** (Suppl **11**), 105S-108S.

Konturek, S.J. & Robert, A. (1982) Cytoprotection of canine gastric mucosa by prostacyclin: possible mediation by increased mucosal blood flow. *Digestion* **25**, 155-163.

Konturek, S.J., Rayford, P.L. & Thompson, J.C. (1977) Effect of pH on gastric and intestinal meals on gastric acid and plasma gastrin and secretin responses in the dog. *American Journal of Physiology* **233**, E537-E543.

Konturek, S.J., Wysocki, A. & Oleksy, J. (1968) Effect of medical and surgical vagotomy on gastric response to graded doses of pentagastrin and histamine. *Gastroenterology* **54**, 329-400.

Konturek, S.J., Kaess, H. Kwiecien, N., Radecki, T., Dorner, M. & Techkentrupp, U. (1976) Characteristics of intestinal phase of gastric secretion. *American Journal of Physiology* **230**, E335-E340.

Konturek, S.J., Radecki, T., Brzozowski, T., Piastucki, I., Dembinska-Kiec, A. & Zmuda, A. (1981a) Gastric cytoprotection by prostaglandins, ranitidine and probanthine in rats. *Scandanavian Journal of Gastroenterology* **16**, 7-12.

Konturek, S.J., Piastucki, I., Brzozowski, T., Radecki, T., Dembinska-Kiec, A., Zmuda, A. & Gryglewski, R. (1981b) Role of prostaglandins in the formation of aspirin-induced gastric ulcers. *Gastroenterology* **60**, 4-9.

Konturek, S.J., Radecki, T., Brzozowski, T., Piastucki, I., Dembinska-Kiec, A., Zmuda, A. & Gryglewski, R. (1981c) Prostaglandin E_2 in gastric mucosa and its role in the prevention of ulcers induced by acetylsalicyclic acid in cats. *Digestion* **21**, 205-213.

Konturek, S., Brzozowski, T., Piastucki, I., Radecki, T., Dembinski, A. & Dembinska-Kiec, A. (1982a) Role of locally generated prostaglandins in adaptive gastric cytoprotection. *Digestive Diseases and Sciences* **27**, 967-971.

Konturek, S.J., Kwiecien, N., Obtulowicz, W., Dembinska-Kiec, A., Polanski, M., Kopp, B., Sito, E. & Oleksy, J. (1982b) Effect of carprofen and indomethacin on gastric function, mucosal integrity, and generation of prostaglandins in men. *Hepato-Gastroenterology* **29**, 267-270.

Konturek, S.J., Kwiecien, N., Obtulowicz, W., Zmuda, A., Polanski, M., Kopp, B., Sito, E. & Oleksy, J. (1983) The use of carprofen, a non-steroidal antiinflammatory agent, in peptic ulcer disease. *Hepato-Gastroenterology* **30**, 261-265.

Konturek, S.J., Tasler, J., Bilski, J., Kaminska, A. & Laskiewicz, J. (1984a) Role of prostaglandins in alkaline secretion from the gastroduodenal mucosa exposed to acid and taurocholate. *Scandanavian Journal of Gastroenterology* **19**, (Suppl **92**), 69-74.

Konturek, S.J., Bilski, J., Tasler, J. & Laskiewicz, J. (1984b) Gastroduodenal response to acid and taurocholate in conscious dogs. *American Journal of Physiology* **247**, G149-G154.

Konturek, S.J., Brzozowski, T., Garlicki, J., Majka, J., Stachura, J. & Nauert, C. (1991a) Intragastric pH in the gastroprotective and ulcer-healing activity of aluminium-containing antacids. *Digestion* **49**, 140-150.

Konturek, S.J., Brzozowski, T., Drozdowicz, D., Garlicki, J., Majka, J. & Pytko-Polonczyk, J. (1991b) Role of acid mileu in the gastroprotective and ulcer-healing activity of sucralfate. *American Journal of Medicine* **91**, (Suppl **2A**), 20-29.

Kosovsky, J.E., Matthiesen, D.T. & Patnaik, A.K. (1988) Small intestinal adenocarcinoma in cats: 32 cases (1978-1985) *Journal of the American Veterinary Medical Association* **192**, 233-235.

Kramer, J.W. (1980) Clinical Enzymology. In: *Clinical Biochemistry of Domestic Animals*. 3rd edition, ed. J.J. Kaneko. Academic Press, London. p175.

Krauser, K. (1985) Neoplasien des Magens beim Hund. *Berliner und Munchener Tierärztliche Wochenschrift* **98**, 48-53.

Kuo, Y-J. & Shanbour, L.L. (1976) Inhibition of ion transport by bile salts in canine gastric mucosa. *American Journal of Physiology* **231**, 1433-1437.

Kuwayama, H., Miyake, S. & Matsuo, Y. (1987) Effects of sucralfate, 15(R) 15-methyl prostaglandin E_2 and cimetidine on rat gastric mucosal damage induced by ethanol. *American Journal of Medicine* **83** (Suppl **3B**), 4-10.

La Brooy, S.J., Misiewicz, J.J., Edwards, J., Smith, P.M., Haggie, S.J., Libman, L., Sarner, M., Wyllie, J.H., Croker, J. & Cotton, P. (1979) Controlled trial of cimetidine in upper gastrointestinal haemorrhage. *Gut* **20**, 892-895.

Lacy, E.R. (1985) Prostaglandins and histological changes in the rat gastric mucosa. *Digestive Diseases and Sciences* **30** (Suppl **11**), 83-94.

Lacy, E.R. (1986) Effects of absolute ethanol, misoprostol, cimetidine and phosphate buffer on the morphology of rat gastric mucosa. *Digestive Diseases and Sciences* **31** (Suppl **2**), 101-107.

Lacy, E.R. (1987) Gastric mucosal defense after superficial injury. *Clinical and Investigative Medicine* **10**, 189-200.

Lacy, E.R. & Ito, S. (1982) Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. *Gastroenterology* **83**, 619-625.

Lacy, E.R. & Ito, S. (1984) Rapid epithelial restitution of the rat gastric mucosa after ethanol injury. *Laboratory Investigation* **51**, 573-583.

Laine, L. & Weinstein, W.M. (1988) Subepithelial hemorrhages and erosions of human stomach. *Digestive Diseases and Sciences* **33**, 490-503.

LaMont, J.T. & Szabo, S. (1984) Stimulatory effects of prostaglandin and cysteamine on gastric mucus glycoprotein secretion. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p241.

LaMont, J.T., Ventola, A.S., Maull, E.A., Szabo, S. (1983) Cysteamine and prostaglandin $F_{2\beta}$ stimulate rat gastric mucin release. *Gastroenterology* **84**, 306-313.

Lanciault, G., Shaw, J.E., Urquhart, J., Adiar, L.S. & Brooks, F.P. (1975) Response of the isolated perfused stomach of the dog to electrical vagal stimulation. *Gastroenterology* **68**, 294-300.

Lang, I.M., Srana, S.K. & Condon, R.E. (1986) Gastrointestinal correlates of vomiting in the dog: Quantification and characterization as an independent phenomenon. *Gastroenterology* **90**, 40-47.

Lanza, F.L. (1984) Endoscopic studies of gastric and duodenal injury after use of ibuprofen, aspirin, and other nonsteroidal antiinflammatory drugs. *American Journal of Medicine* **77** (Suppl **1A**), 19-24.

Lanza, F.L., Boyer, G.L., Nelson, R.S., Chen, T.T., Seckman, C.E. & Rack, M.F. (1979) Effects of ibuprofen, indomethacin, aspirin, naproxen and placebo on the gastric mucosa of normal volunteers. A gastroscopic and photographic study. *Digestive Diseases and Sciences* **24**, 823-828.

Lanza, F.L., Boyer, G.L., Nelson, R.S., Seckman, C.E., Schwatrz, J.H., Rack, M.F. & Gernaat, C.M. (1986) Effects of flurbiprofen and aspirin on the gastric and duodenal mucosa: An endoscopic comparison. *American Journal of Medicine* **80** (Suppl **3A**), 31-35.

Lawson, H.H. (1964) Effect of duodenal contents on the gastric mucosa under experimental conditions. *Lancet* **i**, 469-472.

Lawson, H.H. (1981a) The production of chronic gastritis under experimental conditions. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 91-98.

Lawson, H.H. (1981b) The postoperative stomach as seen clinically and experimentally. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 157-160.

Leblond, C.P. & Walker, B.E. (1956) Renewal of cell populations. *Physiological Reviews* **36**, 255-276.

Lee, K.Y., Park, H.J. & Chey, W.J. (1985) Studies on the mechanism of retching and vomiting in dogs. Effect of peripheral dopamic blocker on myoelectric changes in antrum and upper small intestine. *Digestive Diseases and Sciences* **30**, 22-28.

Leeson, C.R., Leeson, T.S. & Paparo, A.A. (1985) The Stomach. In: *Textbook of Histology*. 11th edition. W.B. Saunders, Philadelphia. p331.

Lehy, T., Dubrasquet, M. & Bonfils, S. (1979) Effect of somatostatin on normal and gastric-stimulated cell proliferation in the gastric and intestinal mucosa of the rat. *Digestion* **19**, 99-109.

Leib, M.S., Wingfield, W.E. & Twedt, D.C. (1985) Gastric emptying of liquids in the dog: serial test meal and modified emptying-time techniques. *American Journal of Veterinary Research* **46**, 1876-1880.

Leib, M.S., Wingfield, W.E., Twedt, D.C. & Williams, A.R. (1986) Gastric emptying of liquids in the dog: Effect of volume. *American Journal of Veterinary Research* **47**, 1019-1021.

Lempinen, M., Pentillä, A. & Fock, G. (1968) Histochemical properties of regenerating mucous membrane in the rat. *Scandanavian Journal of Gastroenterology* **3**, 561-571.

Lev, R., Siegel, H.I. & Glass, G.B.J. (1972) Effects of salicylates on the canine stomach: A morphological and histochemical study. *Gastroenterology* **62**, 970-980.

Levine, R.A. (1970) The role of cyclic AMP in the hepatic and gastrointestinal function. *Gastroenterology* **51**, 280-300.

Levine, R.A. & Schwartzel, E.H. (1984) Effect of indomethacin on basal and histamine-stimulated

human gastric acid secretion. *Gut* **25**, 718-722.

Levine, R.A., Nandi, J. & King, R.L. (1990) Aspirin potentiates prestimulated acid secretion and mobilizes intracellular calcium in rabbit parietal cells. *Journal of Clinical Investigation* **86**, 400-408.

Levine, R.A., Sirinek, K.R. & Pruitt, B.A. (1979) Cimetidine protects against stress-induced gastric injury augmented by mucosal barrier breakers. *American Journal of Surgery* **137**, 321-328.

Lewi, H.J.E. & Carter, D.C. (1983) Bile salt-induced gastric mucosal damage and histamine receptor antagonists. *Gut* **24**, 33-37.

Lewin, M.J.M., Ghesquier, D., Soumarmon, A., Cheret, A.M., Grelac, F. & Gueson, J. (1978) Cytochrome b5 and K^+-PNPPase: Specific characterization in the isolated rat gastric parietal cell. *Acta Physiologica Scandinavia (Special Supplement)*, 267-282.

Lewin, K.J., Yang, K., Ulich, T., Elashoff, J.D. & Walsh, J. (1984) Primary gastrin cell hyperplasia. *American Journal of Surgery and Pathology* **8**, 821-832.

Lichtenberger, L.M., Richards, J.E. & Hills, B.A. (1985) Effect of 16,16-dimethyl prostaglandin E_2 on the surface hydrophobicity of aspirin-treated canine gastric mucosa. *Gastroenterology* **88**, 308-314.

Lichtenberger, L.M., Graziani, L.A., Dial, E.J., Butler, B.D. & Hills, B.A. (1983) Role of surface-active phospholipids in gastric cytoprotection. *Science* **219**, 1327-1329.

Lightdale, C.J. (1992) Endoscopic ultrasonography in the diagnosis, staging and follow-up of esophageal and gastric cancer. *Endoscopy* **24** (Suppl 1), 297-303.

Ligumsky, M., Karmski, F. & Rochmilewitz, D. (1984) Sucralfate stimulation of gastric PGE synthesis: possible mechanism to explain its effective cytoprotection. *Gastroenterology* **86**, 1164.

Lillibridge, C.B. (1964) The fine structure of normal human gastric mucosa. *Gastroenterology* **47**, 269-290.

Lind, T., Cederberg, C., Ekenved, G., Haglund, U. & Olbe, L. (1983) Effect of omeprazole - a gastric proton pump inhibitor on pentagastrin stimulated acid secretion in man. *Gut* **24**, 270-276.

Lingeman, C.H., Garner, F.M. & Taylor, D.O.N. (1971) Spontaneous gastric adenocarcinoma in dogs: A review. *Journal of the National Cancer Institute* **47**, 137-153.

Lipkin, M. (1985) Growth and development of gastrointestinal cells. *Annual Reviews in Physiology* **47**, 175-197.

Lipkin, M., Sherlock, P. & Bell, B. (1963) Cell proliferation kinetics in the gastrointestinal tract of man. II. Cell renewal in stomach, ileum, colon and rectum. *Gastroenterology* **45**, 721-729.

Lipowitz, A.J., Boulay, J.P. & Klausner, J.S. (1986) Serum salicylate concentrations and endoscopic evaluation of the gastric mucosa in dogs after oral administration of aspirin-containing products. *American Journal of Veterinary Research* **47**, 1586-1589.

Loiudice, T.A., Saleem, T. & Lang, J.A. (1981) Cimetidine in the treatment of gastric ulcer induced by steroidal and nonsteroidal anti-inflammatory agents. *American Journal of Gastroenterology* **75**, 104-110.

Lord, P.F., Schaer, M. & Tilley, L. (1975) Pulmonary infiltrates with eosinophilia in the dog. *Journal of American Veterinary Radiology Society* **16**, 115-120.

Lucas, C.E. (1981) Stress ulceration: The clinical problem. *World Journal of Surgery* **5**, 139-151.

Lucas, E., Sugawa, C., Friend, A. & Walt, A.J. (1972) Therapeutic implications of disturbed gastric physiology in patients with stress ulceration. *American Journal of Surgery* **123**, 25-34.

Luft, J.H. & Hechter, O. (1957) An electron microscopic correlation of structure with function in the isolated perfused cow adrenal. *Journal of Biophysical and Biochemical Cytology* **3**, 615-620.

Lynch, A., Shaw, H. & Milton, G.W. (1964) Effect of aspirin on gastric secretion. *Gut* **5**, 230-236.

MacDonald, W.C., Trier, J.S. & Everett, N.B. (1964) Cell proliferation and migration in the stomach, duodenum and rectum of man. *Gastroenterology* **46**, 405-417.

MacIntosh, F.C. (1938) Histamine as a normal stimulant of gastric secretion. *Quarterly Journal of Experimental Physiology* **28**, 87-98.

MacKercher, P.A., Ivey, K.J., Baskin, W.N. & Krause, W.J. (1977) Protective effect of cimetidine on aspirin-induced gastric mucosal damage. *Annals of Internal Medicine* **87**, 676-679.

McCloy, R.F. (1985) Endoscopy. *Current Opinion in Gastroenterology* **1**, 837-843.

McGraw, B.F. & Caldwell, E.G. (1981) Sucralfate. *Drug Intelligence and Clinical Pharmacy* **15**, 578-580.

McGraw, B.F., Hesterlee, E.J., Lanza F.L. & Tesler, M.A. (1981) *In vitro* and *In vivo* evaluations of a tableted antacid and sucralfate, a new antiulcer agent. *American Journal of Gastroenterology* **76**, 412-415.

McGreevy, J.M. & Moody, F.G. (1977) Protection of gastric mucosa against aspirin-induced erosions by enhanced blood flow. *Surgery Forum* **28**, 357-359.

McGuigan, J.E. (1968) Gastric mucosal intracellular localization of gastrin by immunofluorescence. *Gastroenterology* **55**, 315-327.

McGuigan, J.E. (1981) A consideration of the adverse effects of cimetidine. *Gastroenterology* **80**, 181-192.

McHardy, G.G. (1981) A multicenter double-blind trial of sucralfate and placebo in duodenal ulcer. *Journal of Clinical Gastroenterology* **3** (Suppl **2**), 147-152.

McKellar, Q.A., Pearson, T., Bogan, J.A., Galbraith, E.A., Lees, P., Ludwig, B. & Tiberghien, M.P. (1990) Pharmacokinetics, tolerance and serum thromboxane inhibition of carprofen in the dog. *Journal of Small Animal Practice* **31**, 443-448.

McMillen, M.A., Ambis, D. & Siegel, J.H. (1978) Cimetidine and mental confusion. *New England Journal of Medicine* **298**, 284-285.

McNally, P.R., Maydonovitch, C.L. & Wong, R.K.H. (1988) The effectiveness of simethicone in improving visibility during colonoscopy: A double-blind randomized study. *Gastrointestinal Endoscopy* 34, 255-258.

Maaroos, H.I., Rägo, T., Sipponen, P. & Siurala, M. (1991) *Heliobacter pylori* and gastritis in children with abdominal complaints. *Scandanavian Journal of Gastroenterology* **26** (Suppl **186**), 96-99.

Maclaurin, B.P., Watts, C. & Palmer, D.G. (1985) Protection by sucralfate against indomethacin induced gastric ulceration in the guinea pig. *Pathology* **17**, 408-411.

Main, I.H.M. & Whittle, B.J.R. (1973) The effects of E and A prostaglandins on gastric mucosal blood flow and acid secretion in the rat. *British Journal of Pharmacology* **49**, 428-436.

Mann, N.S. (1976) Bile-induced acute erosive gastritis: Its prevention by antacid, cholestyramine and prostaglandin E_2. *American Journal of Digestive Disease* **21**, 89-92.

Mann, S.G. (1987) The place of famotidine in anti-ulcer therapy. *Alimentary Pharmacology and Therapeutics* **1** (Suppl **1**), 504-509.

Mann, N.S. & Sachdev, A.J. (1976) Effect of aspirin, ketoprofen, ibuprofen and naproxen on gastric mucosa. *Gastroenterology* **70**, 913.

Mårdh, S. (1986) Omeprazole inhibits the formation of acid in the parietal cell by a direct inhibition of the H+K+ATPase, the acid pump of the stomach. *Scandanavian Journal of Gastroenterology* **21** (Suppl **118**), 49-51.

Marks, I.N., Lucke, W., Wright, J.P. & Girdwood, A.H. (1981) Ulcer healing and relapse rates after initial treatment with cimetidine or sucralfate. *Journal of Clinical Gastroenterology* **3** (Suppl **2**), 163-165.

Marshall, B.J. (1986) *Campylobacter pyloridis* and gastritis. *Journal of Infectious Diseases* **153**, 650-657.

Martin, B.K. (1963) Accumulation of drug anions in gastric mucosal cells. *Nature* **198**, 896-897.

Martin, G.P., Marriot, C. & Kellaway, I.W. (1978) Direct effect of bile salts and phospholipids on the physical properties of mucus. *Gut* **19**, 103-107.

Matsuyama, M. & Suzuki, H. (1970) Differentiation of immature mucous cells into parietal argyrophil and chief cells in stomach grafts. *Science* **169**, 385-387.

Matthiesen, D.T. & Walters, M.C. (1986) Surgical treatment of chronic hypertrophic pyloric gastropathy in 45 dogs. *Journal of the American Animal Hospital Association* **22**, 241-247.

Mattioli, S., Pilotti, V., Felice, V., Lazzari, A., Zannoli, R., Bacchi, M.L., Loria, P., Tripolo, A. & Gozzetti, G. (1990) Ambulatory 24-hr pH monitoring. *Digestive Diseases and Sciences* **35**, 929-938.

Mattsson, H. (1986) Protective effects of omeprazole in the gastric mucosa. *Scandanavian Journal of Gastroenterology* **21** (Suppl **118**), 86-88.

Menguy, R. (1969) Gastric mucus and the gastric mucous barrier. *American Journal of Surgery* **117**, 806-812.

Menguy, R. (1970) Pathophysiology of peptic ulcer. *American Journal of Surgery* **120**, 282-288.

Menguy, R. & Masters, Y.F. (1963) Effect of cortisone on mucoprotein secretion by gastric antrum of dogs: Pathogenesis of steroidal ulcers. *Surgery* **54**, 19-28.

Menguy, R. & Max, M.H. (1970) Influence of bile on the canine gastric-antral mucosa. *American Journal of Surgery* **119**, 177-182.

Menguy, R. & Thompson, A.E. (1967) Regulation of secretion of mucus from the gastric antrum. *Annals of the New York Academy of Science* **140**, 797-803.

Mersereau, W.A. & Hinchey, E.J. (1982) Role of gastric mucosal folds in formation of focal ulcers in the rat. *Surgery* **91**, 150-155.

Messier, B. (1960) Radioautographic evidence for the renewal of the mucous cells in the gastric mucosa of the rat. *Anatomy Record* **135**, 242.

Messier, B. & Leblond, C.P. (1960) Cell proliferation and migration as revealed by radioautography after injection of thymidine H^3 into male rats and mice. *American Journal of Anatomy* **106**, 247-265.

Miller, T.A. (1983) Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. *American Journal of Physiology* **245**, G601-G623.

Miller, T.A. & Jacobson, E.D. (1979) Gastrointestinal cytoprotection by prostaglandins. *Gut* **20**, 75-87.

Minuz, P., Cavallini, G., Brocco, G., Degan, M., Jeunet, F., Kunovits, G., Riela, A. & Velo, G.P. (1986) Effect of carprofen and indomethacin on gastric function and the content of prostaglandins E_2 and $F_{2\alpha}$ in human gastric juice. *Hepato-Gastroenterology* **33**, 20-22.

Miyabayashi, T. & Morgan, J.P. (1984) Gastric emptying in the normal dog: a contrast radiographic technique. *Veterinary Radiology* **25**, 187-191.

Moody, F.G. (1967) Gastric blood flow and acid secretion during direct intra-arterial histamine administration. *Gastroenterology* **52**, 216-224.

Moody, F.G. & Davis, W.L. (1970) Hydrogen and sodium permeation of canine gastric mucosa during histamine and sodium thiocyanate administration. *Gastroenterology* **59**, 350-359.

Moody, F.G., Zalewsky, C.A. & Larsen, K.R. (1981) Cytoprotection of the gastric epithelium. *World Journal of Surgery* **5**, 153-163.

Moody, F.G., McGreevy, J., Zalewesky, C., Cheung, L.Y. & Simons, M. (1978) The cytoprotective effect of mucosal blood flow in experimental erosive gastritis. *Acta Physiologica Scandinavia (Special Supplement)*, 35-43.

Moon, M. (1991) Pulmonary infiltrates with eosinophilia. *Journal of Small Animal Practice* **33**, 19-23.

Morris, G.P. (1985) The myth of the mucus barrier. *Gastroenterology and Clinical Biology* **9**, 106-107.

Morris, G.P. (1986) Prostaglandins and cellular restitution in the gastric mucosa. *American Journal of Medicine* **81** (Suppl **2A**), 23-29.

Morris, G.P. & Harding, R.K. (1974) Topography and fine structure of acute fundic mucosal erosions in the rat. *Laboratory Investigation* **30**, 639-646.

Morris, G.P. & Harding, R.K. (1984) Mechanisms of mucosal recovery from acute gastric damage: Role of extracellular mucus and cell migration. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p209.

Morris, G.P. & Wallace, J.L. (1981) The role of ethanol and acid in the production of gastric mucosal erosions in rats. *Virchows Archiv B [Cell Pathology]* **38**, 23-38.

Morris, G.P., Keenan, C.M., MacNaughton, W.K., Wallace, J.L. & Williamson, T.E. (1989) Protection of rat gastric mucosa by sucralfate. Effects of luminal stasis and inhibition of prostaglandin synthesis. *American Journal of Medicine* **86** (Suppl 6A), 10-16.

Morrissey, J.F. (1972) Gastrointestinal endoscopy. *Gastroenterology* **62**, 1241-1268.

Muir, A. & Cossar, I.A. (1955) Aspirin and ulcer. *British Medical Journal* **2**, 7-12.

Müller-Lissner, S.A. & Blum, A.L. (1984) To-and-fro movements across the canine pylorus. Scandanavian Journal of *Gastroenterology* **19** (Suppl **92**), 1-6.

Müller-Lissner, S.A., Schattenmann, G., Siewert, J.R. & Blum, A.L. (1983) Effect of a transpyloric tube on gastric emptying and duodenogastric reflux in the dog. *Digestion* **28**, 176-180.

Murray, H.S., Stroltman, M.P. & Cooke, A.R. (1974) Effect of several drugs on gastric potential difference. *British Medical Journal* **1**, 19-21.

Murray, M., Robinson, P.B., McKeating, F.J., Lauder, I.M. (1972a) Peptic ulceration in the dog: A clinico-pathological study. *Veterinary Record* **91**, 441-447.

Murray, M., Robinson, P.B., McKeating, F.J., Baker, G.J., Lauder, I.M. (1972b) Primary gastric neoplasia in the dog: A clinico-pathological study. *Veterinary Record* **91**, 474-479.

Nagashima, R. (1981a) Development and characteristics of sucralfate. *Journal of Clinical Gastroenterology* **3** (Suppl **2**), 103-110.

Nagashima, R. (1981b) Mechanisms of action of sucralfate. *Journal of Clinical Gastroenterology* **3** (Suppl **2**), 117-127.

Nagashima, R., Hoshino, E., Hinohara, Y., Sakai, K., Hata, S, & Nakano, H. (1983) Effect of sucralfate on ethanol-induced gastric mucosal damage in the rat. *Scandanavian Journal of Gastroenterology* **18** (Suppl **83**), 17-20.

Nakazawa, S., Nagashima, R. & Samloff, I.M. (1981) Selective binding of sucralfate to gastric ulcer in man. *Digestive Diseases and Science* **26**, 297-300.

Narama, I., Kuroda, J., Nagatani, M., Mirua, K., Katoh, T. & Tsuchitani, M. (1990) Superficial eosinophilic gastritis in labaoratory Beagle dogs attributable probably to diet. *Japanese Journal of Veterinary Science* **52**, 581-589.

Nezamis, J.E., Robert, A. & Stowe, D.F. (1971) Inhibition of prostaglandin E_1 of gastric secretion in the dog. *Journal of Physiology* **218**, 369-383.

Nicoloff, D.M. (1968) Indomethacin: Effect on gastric secretion, parietal cell population and ulcer provocation in the dog. *Archives of Surgery* **97**, 809-815.

Nobuhara, Y. & Takeuchi, K. (1984) Possible role of endogenous prostaglandins in alkaline response in rat gastric mucosa damaged by hypertonic Na Cl. *Digestive Diseases and Sciences* **29**, 1142-1147.

Norburn, J. & Tvedten, H.B. (1992) Diagnosis of microthrombocytosis and immune-mediated thrombocytopeni in dogs with thrombocytopenia: 68 cases (1987-1989). *Journal of the American Veterinary Medical Association* **200**, 368-372.

O'Brien, T. (1978) The Stomach. In: *Radiographic Diagnosis of Abdominal Disorders in the Dog and Cat*. W.B. Saunders, Philadelphia. p204.

O'Brien, P.E. & Carter, D.C. (1975) Effect of gastric secretory inhibitors on the gastric mucosal barrier. *Gut* **16**, 437-442.

O'Brien, W.M. & Bagby, G.F. (1987) Carprofen: a new nonsteroidal antiiflammatory drug. Pharmacology, clinical efficacy and adverse effects. *Pharmacotherapy* **7**, 16-24.

O'Brien, P.E., Frydman, G., Holmer, R., Malcontenti, C. & Phelan, D. (1990) Evaluation of putative cytoprotective properties of antiulcer drugs using quantative histological techniques. *Digestive Diseases and Sciences* **35**, 1130-1139.

Ogata, T. & Murata, F. (1969) Scanning electron microscopic study on the rat gastric mucosa. *Tohoku Journal of Experimental Medicine* **99**, 65-71.

Ohno, T., Ohtsuki, H. & Okabe, S. (1985) Effects of 16,16-dimethyl prostaglandin E_2 on ethanol-

induced and aspirin-induced gastric damage in the rat: Scanning electron microscopic study. *Gastroenterology* **88**, 353-361.

Okabe, S. & Nobuhara, Y. (1984) Effects of famotidine, a histamine H_2-receptor antagonist, on gastric secretion and gastric and duodenal lesions in rats. *Pharmacometrics* **27**, 563-569.

Okabe, S., Takeuchi, K., Murata, T. & Vrushidini, T. (1978) Effects of cimetidine on healing of chronic gastric and duodenal ulcers in dogs. *American Journal of Digestive Diseases* **23**, 166-168.

Okabe, S., Takeuchi, K., Kunimi, H., Kanno, M. & Kawashima, M. (1983) Effects of an antiulcer drug, sucralfate (a basic aluminium salt of sulfated disaccharide), on experimental gastric lesions and gastric secretion in rats. *Digestive Diseases and Science* **28**, 1034-1042.

Olsen, O. & Christiansen, J. (1990) Inhibition of human gastric acid secretion by peptide YY secretion. *Digestion* **47**, 156-159.

Osada, J., Goto, H., Tsukamoto, Y., Nakazawa, S., Sugiyama, S. & Ozawa, T. (1990) Role of leukotrienes in hydrochloric acid-induced gastric lesions in rats. *Digestive Diseases and Sciences* **35**, 186-192.

Ostro, M.J. (1987) Pharmacodynamics and pharmacokinetics of parenteral histamine (H_2)-receptor antagonists. *American Journal of Medicine* **83** (Suppl **6A**), 15-22.

Pantoja, J.L., Defilippi, C., Valenzuela, J.E. & Csondes, A. (1979) Nonsteroidal anti-inflammatory drugs: effect on pyloric sphincter and duodenogastric reflux. *American Journal of Digestive Diseases* **24**, 217-220.

Papageorges, M., Breton, L. & Bonneau, N.H. (1987b) Gastric drainage procedures: Effects in normal dogs II. Clinical observations and gastric emptying. *Veterinary Surgery* **16**, 332-340.

Parisio, C. & Clementi, F. (1976) Surface alterations induced by stress in gastric mucosa: Protective effect of zolimidine. A transmission and scanning electron microscope investigation. *Laboratory Investigation* **35**, 484-495.

Patnaik, A.M., Hurvitz, A.I. & Johnson, G.F. (1977) Canine gastrointestinal neoplasms. *Veterinary Pathology* **14**, 547-555.

Pendleton, R.G., Torchiana, M.L., Chung, C., Cook, P., Wiese, S. & Cline, B.V. (1983) Studies on MK-208 (YM-1170), a new slowly dissociable H_2-receptor antagonist. *Archives Internationales de Pharmacodynamie et de Therapie* **266**, 4-16.

Pe Thein, M. & Schofield, B. (1959) Release of gastrin from the pyloric antrum following vagal stimulation by sham feeding in dogs. *Journal of Physiology* **148**, 291-305.

Peura, D.A. & Freston, J.W. (1987) Evolving perspectives on parenteral H_2-receptor antagonist therapy. *American Journal of Medicine* **83** (Suppl **6A**), 1-2.

Pevsner, L. & Grossman, M.I. (1955) The mechanism of vagal stimulation of gastric acid secretion. *Gastroenterology* **28**, 493-499.

Pfeiffer, C.J. (1970a) Surface topology of the stomach in man and the laboratory ferret. *Journal of Ultrastructural Research* **33**, 252-262.

Pfeiffer, C.J. (1970b) Gastric surface morphology in man, monkey and ferret. Evidence for *in situ* surface cell degeneration. *Experimental and Molecular Pathology* **13**, 319-328.

Pfeiffer, C.J. & Weibel, J. (1973) The gastric mucosal response to acetylsalicylic acid in the ferret. An ultrastructural study. *American Journal of Digestive Diseases* **18**, 834-846.

Pihan, G., Majzoubi, D., Haudenschld, C., Trier, J.S. & Szabo, S. (1986) Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,16-dimethyl prostaglandin E_2 or sodium thiosulphate. *Gastroenterology* **91**, 1415-1426.

Pugh., M.C., Small, R.E., Garnett, W.R., Townsend, R.J. & Willis, H.E. (1984) Effect of sucralfate on ibuprofen absorption in normal volunteers. *Clinical Pharmacokinetics* **3**, 630-633.

Quadros, E., Ramsamooj, E. & Wilson, D.E. (1987) Role of mucus and prostaglandins in the gastric mucosal protective action of sucralfate against ethanol-induced injury in the rat. *American Journal of Medicine* **83** (Suppl **3B**), 19-23.

Rainsford, K. (1975) Electron-microscopic observations on the effects of orally administered aspirin and aspirin-bicarbonate mixtures on the development of gastric mucosal damage in the rat. *Gut* **16**, 514-527.

Rainsford, K.D. (1978) The role of aspirin in gastric ulceration. Some factors involved in the development of gastric mucosal damage induced by aspirin in rats exposed to various stress conditions. *Digestive Diseases and Sciences* **23**, 521-530.

Randall, L.O. & Baruth, H. (1976) Analgesic and anti-inflammatory activity of 6-chloro-alpha-methyl-carbazole-2-acetic acid (C-5720). *Archives Internationales de Pharmacodynamie et de Therapie* **220**, 94-114.

Rarnakrishnan, K.R., Garik, P., Turner, B.S., Bradley, J.D., Bansil, R., Stanley, H.E. & LaMont, J.H. (1992) Viscous fingering of HCl through the gastric mucin. *Nature* **360**, 458-461.

Rask-Madsen, J. (1987) The role of eicosanoids in the gastrointestinal tract. *Scandanavian Journal of Gastroenterology* **22** (Suppl **127**), 7-19.

Ray, T.K. & Tague, L.L. (1978) Role of K^+-stimulated ATPase in H^+ and K^+ transport by bull frog gastric mucosa *in vitro*. *Acta Physiologica Scandinavia* (*Special Supplement*), 283-292.

Read, A.M. & Johnstone, F.R.C. (1961) The distribution of parietal cells in the gastric mucosa of the cat. *Anatomy Record* **139**, 525-528.

Rees, W.D.W., Rhodes, J., Wheeler, M.H., Meek, E.M., Williams, B., Newcombe, R.G. (1977a) Effect of histamine receptor antagonists on bile damage to the gastric mucosa of canine Heidenhain pouches. *Gut* **18**, 821-826.

Rees, W.D.W., Rhodes, J., Wheeler, M.H., Meek, E.M. & Newcombe, R.G. (1977b) The role of histamine receptors in the pathophysiology of gastric mucosal damage. *Gastroenterology* **72**, 67-71.

Rees, W.D.W., Gibbons, L.C. & Turnberg, L.A. (1984) Alkali secretion by isolated rabbit gastric mucosa: Effects of non-steroidal anti-inflammatory drugs and prostaglandins. *Scandanavian Journal of Gastroenterology* **19** (Suppl **92**), 63-68.

Reitemeier, R.J., Code, C.F. & Orvis, A.L. (1957) Barrier offered by gastric mucosa of healthy persons to absorption of sodium. *Journal of Applied Physiology* **10**, 261-266.

Rhodes, J., Barnado, D.E., Phillips, S.F., Rovelstad, R.A. & Hofman, A.F. (1969) Increased reflux of bile into the stomach in patients with gastric ulcer. *Gastroenterology* **57**, 241-252.

Ritchie, W.P. (1975) Acute gastric mucosal damage induced by bile salts, acid, and ischaemia. *Gastroenterology* **68**, 699-707.

Ritchie, W.P. (1977) Bile acids, the "barrier", and reflux-related clinical disorders of the gastric mucosa. *Surgery* **82**, 192-200.

Ritchie, W.P. (1981a) Bile acid-induced acute gastric mucosal damage: A useful experimental model. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 99-101.

Ritchie, W.P. (1981b) Role of bile acid reflux in acute haemorrhagic gastritis. *World Journal of Surgery* **5**, 189-198.

Ritchie, W.P. & Cherry, K.J. (1979) Influence of hydrogen ion concentration on bile induced acute gastric mucosal ulcerogenesis. *Annals of Surgery* **189**, 637-642.

Ritchie, W.P. & Shearburn, E.W. (1976) Acute gastric mucosal ulcerogenesis is dependent on the concentration of bile salt. *Surgery* **80**, 98-105.

Ritchie, W.P., Barzilai, A. & Delaney, J.P. (1966) Mucosal cellular populations and distribution in the normal canine stomach. *Anatomical Record* **155**, 111-115.

Robbins, P.L., Boradie, R.A., Sosin, H. & Delaney, J.P. (1976) Reflux gastritis: The consequences of intestinal juice in the stomach. *American Journal of Surgery* **131**, 23-29.

Robert, A. (1971) Proposed terminology for the anatomy of the rat stomach. *Gastroenterology* **60**, 344-345.

Robert, A. (1974) Effects of prostaglandins on the stomach and the intestine. *Prostaglandins* **6**, 523-532.

Robert, A. (1976) Antisecretory, antiulcer, cytoprotective and diarrhoegenic properties of

prostaglandins. In: *Advances in Prostaglandin and Thromboxane Research*. Vol 2, eds. B. Amuelson & R. Paolette. New York, Raven Press. p507.

Robert, A. (1981) Prostaglandins and the gastrointestinal tract. In: *Physiology of the Gastrointestinal Tract*. Ed. L.R. Johnson, Raven Press, New York. p1407.

Robert, A., Nezamis, J.E. & Phillips, J.P. (1967) Inhibition of gastric secretion by prostaglandins. *American Journal of Digestive Diseases* **12**, 1073-1076.

Robert, A., Lancaster, C. Hanchar, A.J. & Nezamis, J.E. (1978) Mild irritants prevent gastric necrosis through prostaglandin formation. Histological study. *Gastroenterology* **74**, 1086.

Romano, M., Razandi, M. & Ivey, K.J. (1990) Effect of sucralfate and its components on taurocholate induced damage to rat mucosal cells in tissue culture. *Digestive Diseases and Sciences* **35**, 467-476.

Roth, G.J., Stanford, N. & Majerus, P.W. (1975) Acetylation of prostaglandin synthetase by aspirin. *Proceedings of the National Academy of Science* **72**, 3073-3076.

Roth, J.L.A., Valdes-Dapena, A., Pieses, B. & Buchanan, E. (1963) Topical action of salicylates in gastrointestinal erosion and hemorrhage. *Gastroenterology* **44**, 146-158.

Roth, L., Leib, M.S., Davenport, D.J. & Monroe, W.E. (1990) Comparisons between endoscopic and histologic evaluation of the gastrointestinal tract in dogs and cats: 75 cases (1986-1987). *Journal of the American Veterinary Medical Association* **196**, 635-638.

Roudebush, P. & Morse, G. (1981) Naproxen toxicosis in a dog. *Journal of the American Veterinary Medical Association* **179**, 805-806.

Rubio, F., Seawall, S., Pocelinko, R., DeBarbier, B., Benz, W., Berger, L., Morgan, L., Pao, J., Williams, T.H. & Koechlin, B. (1980) Metabolism of carprofen, a nonsteroid anti-inflammatory agent, in rats, dogs, and humans. *Journal of Pharamceutical Sciences* **69**, 1245-1253.

Rudick, R., Werther, J.L., Chapman, M.L., Danowitz, H.D. & Kark, A.E. (1972) Hydrogen permeability of canine gastric antral and fundic mucosa following exposure to pancreatic juice. *Journal of Surgical Research* **2**, 72-76.

Ruding, R. (1967) Gastric ulcer and antral border. *Surgery* **61**, 495-497.

Ryan, G.R., Copeland, E.M. & Johnson, L.R. (1977) Effects of gastrin and vagotomy on DNA synthesis in canine fundic mucosa. *Gastroenterology* **72**, 1124.

Sachs, G. (1986) The parietal cell as a therapeutic target. *Scandanavian Journal of Gastroenterology* **21** (Suppl **118**), 1-10.

Sachs, G., Carlsson, E., Londberg, P. & Wallmark, B. (1988) Gastric H^+,K^+-ATPase as therapeutic target. *Annual Review Pharmacology Toxicology* **28**, 269-284.

Saffouri, B., Weir, G., Bitar, K. & Makhlouf, G. (1979) Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. *Life Sciences* **25**, 1749-1753.

Salenius, P. (1962) On the ontogenesis of the gastric epithelial cells. *Acta Anatomica* **50** (Suppl **46**), 1-76.

Samloff, I.M. (1983) Inhibition of peptic aggression by sucralfate. The view from the ulcer crater. *Scandanavian Journal of Gastroenterology* **18** (Suppl **83**), 7-11.

Samloff, I.M. & O'Dell, C. (1985) Inhibition of peptic activity by sucralfate. *American Journal of Medicine* **79** (Suppl **2C**), 15-18.

Sasaki, H., Tsunoda, Y., Hinohara, Y., Nakano, H., Miura, K. & Hirayama, C. (1983) Specific binding of sucralfate in gastric ulcer and gastritis. *Clinical Therapeutics* **5**, 545-557.

Sauerbruch, T., Schreiber, M.A. & Schüssler, P. (1984) Endoscopy in the diagnosis of gastritis. Diagnostic value of endoscopic criteria in relation to histological diagnosis. *Endoscopy* **16**, 101-104.

Sautter, H. & Hanlon, G.F. (1975) Gastric neoplasms in the dog: A report of 20 cases. *Journal of the American Veterinary Medical Association* **166**, 691-696.

Scanziani, E., Giusti, A.M., Gualtieri, M. & Fonda, D. (1991) Gastric carcinoma in the Belgian shepherd dog. *Journal of Small Animal Practice* **32**, 465-469.

Scheiman, J.M. (1992) Pathogensis of gastroduodenal injury due to nonsteroidal antiinflammatory drugs: implications for prevention and therapy. *Seminars in Arthritis and Rheumatology* **21**, 201-210.

Scheurer, U.C., Schleger, J.F., Kelly, D.G. & Code, C.F. (1981) Chronic bile exposure increases resistance of canine gastric mucosa to bile. *Scandanavian Journal of Gastroenterology* **16** (Suppl **67**), 205-210.

Schmidt, K.L., Henagan, J.M., Smith, G.S., Hilburn, P.J. & Miller, T.A. (1985) Prostaglandin cytoprotection against ethanol-induced gastric injury in the rat. A histologic and cytologic study. *Gastroenterology* **88**, 649-659.

Schmitt, M. & Guentert, T.W. (1990) Biopharmaceutical evaluation of carprofen following single intravenous, oral, and rectal doses in dogs. *Biopharmaceutical and Drug Disposition* **7**, 585-594.

Scholes, P. & Lee, J. (1978) Properties of a K^+/H^+ ATPase from dog gastric mucosa. *Acta Physiologica Scandinavia (Special Supplement)*, 427-434.

Schunack, W. (1989) Pharmacology of H_2-receptor antagonists: an overview. *Journal of International Medical Research* **17** (Suppl 1), 9-16.

Sedar, A.W. (1964) Stomach and Intestinal Mucosa. In: *Electron Microscopic Anatomy*. 1st edition, ed. S.M. Kurtz. Academic Press, New York. p123.

Semple, P.F. & Russell, R.I. (1975) Role of bile acids in the pathogenesis of aspirin-induced gastric mucosal hemorrhage in rats. *Gastroenterology* **68**, 67-70.

Sepelyak, R.J., Feldkamp, J.R., Regneir, F.E., White, J.L. & Hem, S.L. (1984) Adsorption of pepsin by aluminium hydroxide. II. pepsin inactivation. *Journal of Pharmaceutical Sciences* **73**, 1517-1522.

Seppala, E., Nissila, M., Isomaki, H., Wuorela, H. & Vapaatalo, H. (1990) Effects of non-steroidal anti-inflammatory drugs and prednisolone on synovial fluid white cells, prostaglandin E_2, leukotriene B4 and cyclic AMP in patients with rheumatoid arthritis. *Scandanavian Journal of Rheumatology* **19**, 71-75.

Shay, H.S., Komarov, S.A., Fels, S.S., Meranze, D., Grunstein, M. & Siplet, H. (1945) A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology* **5**, 43-47.

Shea-Donohue, T., Steel, L., Montcalm, E. & Dubois, A. (1986) Gastric protection by sucralfate. Role of mucus and prostaglandins. *Gastroenterology* **91**, 660-666.

Sherding, R.C. (1989) Diseases of the small bowel. In: *Textbook of Veterinary Internal Medicine*. 3rd edition, ed. S.J. Ettinger. W.B. Saunders, Philadelphia. p1323.

Shoemaker, R.L. & Buckner, E.B. (1976) Stimulatory pathways for gastric secretion *in vitro*. In: *Gastric Hydrogen Ion Secretion*. Eds. D.K. Kasbekar, G. Sachs, & W.S. Rehm. Marcel Dekker Inc., New York. p146.

Shorrock, C.J. & Rees, W.D.W. (1989) Effect of sucralfate on human gastric bicarbonate secretion and local prostaglandin E_2 metabolism. *American Journal of Medicine* **86** (Suppl **6A**), 2-4.

Sikes, R.I., Birchard, S., Patnaik, A. & Bradley, R. (1986) Chronic hypertrophic pyloric gastropathy: A review of 16 cases. *Journal of the American Animal Hospital Association* **22**, 99-104.

Silen, W. (1977) New concepts of the gastric mucosal barrier. *American Journal of Surgery* **133**, 8-12.

Silen, W. & Ito, S. (1985) Mechanisms for rapid re-epithelialization of the gastric mucosal surface. *Annual Review of Physiology* **47**, 217-229.

Silverstein, F.E., Martin, R.W., Kimmey, M.B., Jironek, G.C., Franklin, D.W. & Proctor, A. (1989) Experimental evaluation of an endoscopic ultrasound probe: *In vitro* and *in vivo* canine studies. *Gastroenterology* **96**, 1058-1062.

Simon, L.S. & Mills, J.A. (1980a) Nonsteroidal antiinflammatory drugs: Part 1. *New England Journal of Medicine* **302**, 1179-1185.

Simon, L.S. & Mills, J.A. (1980b) Nonsteroidal antiinflammatory drugs: Part 2. *New England Journal of Medicine* **302**, 1237-1243.

Siurala, M. & Tawast, M. (1956) Duodenal regurgitation and the state of the gastric mucosa, with special reference to the occurence of surface-lowering factors in the gastric contents of cases with chronic

atrophic gastritis. *Acta Medica Scandinavica* **153**, 451-458.

Sjostrand, S.E., Ryberg, B. & Olbe, L. (1978) Stimulation and inhibition of acid secretion in the isolated guinea pig gastric mucosa. *Acta Physiologica Scandinavica (Special Supplement)*, Gastric Ion Transport. Eds. K.J. Obrink and G. Flemström. p181.

Slomiany, B.L., Laszewicz, W. & Slomiany, A. (1986) Effect of sucralfate on the viscosity of gastric mucus and the permeability to hydrogen ion. *Digestion* **33**, 146-151.

Slomiany, A., Yano, S., Slomiany, B.L., Glass, G.B.J. (1978) Lipid composition of the gastric mucous barrier in the rat. *Journal of Biological Chemistry* **253**, 3785-3791.

Slomiany, B.L., Laszewicz, W., Murty, V.L., Kosmala, M. & Slomiany, A. (1985) Effect of sucralfate on the viscosity and retardation of hydrogen ion diffusion by gastric mucus glycoprotein. *Comparative Biochemistry and Physiology* **82**, 311-314.

Slomiany, B.L., Piotrowski, J., Okazaki, K., Grzelinski, E. & Slomiany, A. (1989) Nature of enhancement of the protective qualities of gastric mucus by sucralfate. *Digestion* **44**, 222-231.

Smith, G.M. (1914) An experimental study of the relation of bile to ulceration of the mucous membrane of the stomach. *Journal of Medical Research* **30**, 147-183.

Smith, B.M., Skillman, J.J., Edwards, B.G. & Silen, W. (1971) Permeability of the human gastric mucosa. Alteration by acetylsalicylic acid and ethanol. *New England Journal of Medicine* **285**, 716-721.

Smith, P., O'Brien, P., Fromm, D. & Silen, W. (1977) Secretory state of gastric mucosa and resistance to injury by exogenous acid. *American Journal of Physiology* **233**, 81-85.

Smith, J.L., Gamal, M.A., Chremos, A.N. & Graham, D.Y. (1985) Famotidine, a new H_2-receptor antagonist. Effect on parietal, nonparietal, and pepsin secretion in man. *Digestive Disease and Sciences* **30**, 308-312.

Soll, A. (1977) Studies on the actions and interactions of secretagogues on isolated mammalian parietal cells as reflected in changes in oxygen consumption and aminopyrine uptake. *Gastroenterology* **73**, 899.

Soll, A.H. (1980a) Specific inhibition by prostaglandins E_2 and I_2 of histamine-stimulated [^{14}C]Aminopyrine accumulation and cyclic adenosine monophosphate generation by isolated canine parietal cells. *Journal of Clinical Investigation* **65**, 1222-1229.

Soll, A.H. (1980b) Secretagogue stimulation of ^{14}C-aminopyrine accumulation by isolated canine parietal cells. *American Journal of Physiology* 238, G366-G375.

Soll, A.H. (1981) Extracellular calcium and cholinergic stimulation of isolated canine parietal cells. *Journal of Clinical Investigation* **68**, 270-278.

Soll, A.H. & Berglindhi, T. (1987) Physiology of isolated gastric glands and parietal cells: receptors and effectors regulating function. In: *Physiology of the Gastrointestinal Tract.* 2nd edition, ed. L.R. Johnson. Raven Press, New York. p883.

Sommerville, K.W., Faulkner, G. & Langman, M.J.S. (1986) Non-steroidal anti-inflammatory drugs and bleeding peptic ulcer. *Lancet* **i**, 462-464.

Sonnenberg, A., Schattenmann, G., Lepsien, G., Hollinger, A., Siewert, J.R. & Blum, A.L. (1980) The determinants of duodeno-gastric reflux - A quantitative measurement in the dog. In: *Gastrointestinal Motility*. Ed. J. Christensen. Raven Press, New York. p169.

Sonnenberg, A., Müller-Lissner, S.A., Schattenmann, G., Siewert, J.R. & Blum, A.L. (1982) Duodenogastric reflux in the dog. *American Journal of Physiology* **242**, G603-G607.

Spiro, H.M. (1982) Pharmacology, clinical efficacy, and adverse effects of sucralfate, a nonsystemic agent for peptic ulcer. *Pharmacotherapy* **2**, 67-71.

St. John., D.J.B., Yeomans, N.D., McDermott, F.T. & DeBoer, W.G.R.M. (1973) Adaptation of the gastric mucosa to repeated administration of aspirin in the rat. *Digestive Diseases and Sciences* **18**, 881-886.

Stanton, M.E. & Bright, R.M. (1989) Gastroduodenal ulceration in dogs: Retrospective study of 43 cases and literature review. *Journal of Veterinary Internal Medicine* **3**, 238-244.

Stanton, M.E., Bright, R.M., Toal, R., DeNovo, R.C., McCraken, M. & McLauren, J.B. (1987) Effects

of the Y-U pyloroplasty on gastric emptying and duodenogastric reflux in the dog. *Veterinary Surgery* **16**, 392-397.

Steiner, K., Buhring, K.U., Faro, H.P., Garbe, A. & Nowak, H. (1982) Sucralfate: pharmokinetics, metabolism and selective binding to experimental gastric and duodenal ulcers in animals. *Arzneimittelforschung* **32**, 512-518.

Stern, A.I., Ward, F. & Hartley, G. (1987) Protective effect of sucralfate against aspirin-induced damage to the human gastric mucosa. *American Journal of Medicine* **83** (Suppl **3B**), 83-85.

Stone, E. (1763) An account of the success of the bark of the willow in the cure of the agues. *Philosophical Transactions of the Royal Society* **53**, 195-200.

Strombeck, D.R. & Guilford, W.G. (1991) *Small Animal Gastroenterology*. 2nd edition, Wolfe Publishing Ltd, London.

Strub, K.M., Aeppli, L., Müller, R.K.M. (1982) Pharmacological properties of carprofen. *European Journal of Rheumatology and Inflammation* **5**, 478-487.

Sullivan, M. & Miller, A. (1985) Endoscopy (fibreoptic) of the oesophagus and stomach in the dog with persistent regurgitation or vomiting. *Journal of Small Animal Practice* **26**, 369-379.

Sullivan, M., Lee, R., Fisher, E.W., Nash, A.S. & McCandlish, I.A.P. (1987) A study of 31 cases of gastric carcinoma in dogs. *Veterinary Record* **120**, 79-83.

Sutherland, E.W., Øye, I. & Butcher, R.W. (1965) The action of epinephrine and the role of adenyl cyclase system in hormone action. *Recent Progress in Hormone Research* **21**, 623-642.

Svanes, K., Ito, S., Takeuchi, K. & Silen, W. (1982) Restitution of the surface epithelium the *in vitro* frog gastric mucosa after damage with hyperosmolar sodium chloride: Morphologic and physiologic characteristics. *Gastroenterology* **82**, 1409-1426.

Svanes, K., Critchlow, J., Takeuchi, K., Magee, D., Ito, S. & Silen, W. (1984) Factors influencing reconstitution of the frog gastric mucosa: Role of prostaglandins. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p33.

Szabo, S. (1987) Mechanisms of mucosal injury in the stomach and duodenum: Time-sequence analysis of morphologic, functional, biochemical and histochemical studies. *Scandanavian Journal of Gastroenterology* **22** (Suppl **127**), 21-28.

Szabo, S. & Goldberg, I. (1990) Experimental pathogenesis: Drugs and chemical lesions in the gastric mucosa. *Scandanavian Journal of Gastroenterology* **25** (Suppl **174**), 1-8.

Szelenyi, I. & Engler, H. (1986) Cytoprotective role of gastric surfactant in the ethanol produced gastric mucosal injury in the rat. *Pharmacology* **33**, 199-205.

Takagi, T., Takeda, M. & Moore, H. (1982) Effect of a new potent H_2-blocker 3-[[[2-[(diaminomethylene) amino]-4-thiazolyl] methyl]-thio]-N_2-Sulfamoylpropionamide (YM-1170) on gastric secretion induced by histamine and food in conscious dogs. *Archives Internationales de Pharmacodynamie et de Therapie* **256**, 49-58.

Takagi, T., Takebayshi, S., Tokuyashu K. & Tsuji, K. (1974) Scanning electron microscopy on the human gastric mucosa: fetal, normal and various pathological conditions. *Acta Pathologica Japonica* **24**, 233-247.

Takahashi, M. & Hasegawa, R. (1986) Enhancing effects of dietary salt on both initiation and promotion of rat carcinogenesis. In: *Diet, Nutrition and Cancer*. Ed. Y. Hayashi, Japan Scientific Society Press, Tokto/ VHU Science Press, Utrecht. p169.

Takahashi, M., Furukawa, F., Toyoda, K., Sato, H., Hasegawa, R.& Hayashi, Y. (1986) Effects of four antioxidants on N-methyl-N'-nitro-N-nitrosoquanidine initiated gastric tumour development in rats. *Cancer Letters* **30**, 161-168.

Takeuchi, K. & Johnson, L.R. (1979) Pentagastrin protects against stress ulceration in rats. *Gastroenterology* **76**, 327-334.

Takeuchi, K. & Johnson, L.R. (1982) Effect of cell proliferation and cell loss on aspirin-induced gastric damages in the rat. *American Journal of Physiology* **243**, G463-G468.

Talley, N.J., Shorter, R.G., Phillips, A.F. & Zinsmeister, A.R. (1990) Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. *Gut* **31**, 54-58.

Taor, R.E., Fox, B., Ware, J. & Johnson, A.G. (1975) Gastritis - Gastroscopic and microscopic. *Endoscopy* **7**, 209-215.

Tarnawski, A., Hollander, D., Stachura, J., Kranse, W.J. & Gergely, H. (1985a) Prostaglandin protection of the gastric mucosa against alcohol injury - A dynamic time-related process. *Gastroenterology* **88**, 334-352.

Tarnawski, A., Hollander, D., Gergely, H. & Stachura, J. (1985b) Comparison of antacid, sucralfate, cimetidine, and ranitidine in protection of the gastric mucosa against ethanol injury. *American Journal of Medicine* **79**, 19-23.

Tarnawski, A., Hollander, D., Krauses, W.J., Zipser, R.D., Stachura, J. & Gergely, H. (1986) Does sucralfate affect the normal gastric mucosa? Histologic, ultrastructural, and functional assessment in the rat. *Gastroenterology* **90**, 893-905.

Tasman-Jones, C., Morrison, G., Thomsen, L. & Vanderwee, M. (1989) Sucralfate interactions with gastric mucus. *American Journal of Medicine* **86** (Suppl **6A**), 5-9.

Taylor, I.L. (1985) Distribution and release of peptide YY in dog measured by specific radioimmunoassay. *Gastroenterology* **88**, 731-737.

Taylor, L.A. & Crawford, L.M. (1968) Aspirin-induced gastrointestinal lesions in dogs. *Journal of the American Veterinary Medical Association* **152**, 617- 619.

Teorell, T. (1939) On the permeability of the stomach mucosa for acids and some other substances. *Journal of General Physiology* **23**, 263-274.

Terano, A., Shiga, J., Hiraishi, H., Ota, S. & Sugimoto, T. (1986) Protective action of tetraprenylacetone against ethanol-induced damage in rat gastric mucosa. *Digestion* **35**, 182-188.

Tesler, M.A. & Lim, E.S. (1981) Protection of gastric mucosa by sucralfate from aspirin-induced erosions. *Journal of Clinical Gastroenterology* **3** (Suppl **2**), 175-179.

Thomas, N.W. (1987) Piroxicam-associated gastric ulceration in a dog. *Compendium of Continuing Education* **9**, 1004-1005.

Thompson, D.G. & Malagelada, J.-R. (1982) Vomiting and the small intestine. *Digestive Diseases and Sciences* **27**, 1121-1125.

Thomson, A.B.R. (1984) Unstirred layers: Possible adaptive and cytoprotective function. In: *Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract*. Eds. A. Allen, G. Flemström, A. Garner, W. Silen & L.A. Turnberg. Raven Press, New York. p233.

Thun, R., Eggenberger, E., Zerobin, K., Rehm, W.F. & Ludwig, B. (1989) Carprofen in veterinary medicine. II. Inhibitory effect on the release of PGF_2 alpha in the early postpartum cow. *Schweizer Archiv fur Tierheilkunde* **131**, 205-212.

Toner, P.G. & Carr, K.E. (1971) Biological membranes and the cell surface. In: *Cell Structure*. 2nd edition, Churchill Livingstone, Edinburgh. p4.

Townsend, S.F. (1961) Regeneration of gastric mucosa in rats. *American Journal of Anatomy* **109**, 133-148.

Trabucchi, E., Foschi, D., Colombo, R., Baratti, C., Del Soldato, P., Centemero, E., Rizzitelli, E. & Montorsi, W. (1986) Cytoprotection by PGE_2, pirenzepine or vagotomy: A transmission and scanning electron microscopic study in rats. *Pharmacological Research Communications* **18**, 357-369.

Tsukamoto, Y., Nakazawa, S., Segawa, K., Goto, H., Kurita, Y., Fukui, A., Takano, K. & Hase, S. (1987) The role of H_2 receptors in gastric mucosal blood flow. *Scandanavian Journal of Gastroenterology* **22**, 250-252.

Turnberg, L.A. & Ross, I.N. (1984) Studies of the pH gradient across gastric mucus. *Scandanavian Journal of Gastroenterology* **19** (Suppl **92**), 48-50.

Tytgat, G.N.J. (1987) Clinical efficacy of sucralfate in reflux esophagitis: Comparison with cimetidine.

American Journal of Medicine **83** (Suppl **3B**), 38-42.

Ungethum, W. (1991) Study of the interaction between sucralfate and diclofenac/piroxicam in healthy volunteers. *Arzneimittelforschung* **41**, 797-800.

Urquhart, J. (1986) Two cheers for NSAIDs. *Gut* **27**, 1287-1291.

Uvnäs-Wallensten, K. (1977) Occurrence of gastrin in gastric juice, in antral secretion and in antral perfusates in cats. *Gastroenterology* **73**, 487-491.

Uvnäs-Wallensten, K., Uvnäs, B & Nilsson, G. (1976) Quantitative aspects of the vagal control of gastrin release in cats. *Acta Physiologica Scandinavica* **96**, 19-28.

Vane, J. (1972) Inhibition of prostaglandin synthesis as a mechanism for aspirin-like drugs. *Nature New Biology* **231**, 232-235.

Vane, J. & Botting, R. (1978) Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB Journal* **1**, 89-96.

Van Heerden, J.A., Priestley, J.T., Farrow, G.M. & Phillips, S.F. (1969) Post-operative alkaline reflux gastritis. Surgical implications. *American Journal of Surgery* **118**, 427-433.

Van Thiel, D.H., Gavaler, J.S., Smith, W.I. & Paul, G. (1979) Hypothalamic-pituitary-gonadal dysfunction in men using cimetidine. *New England Journal of Medicine* **300**, 1012-1015.

Varró, V., Csernay, L. & Jávor, T. (1959) Experimental phenylbutazone ulcer in dogs. *Gastroenterology* **37**, 463-467.

Vergin, H. & Kori-Lindner, C. (1990) Putative mechanisms of cytoprotective effect of certain antacids and sucralfate. *Digestive Disease and Science* **35**, 1320-1327.

Wallace, J.L. (1991) Possible mechanisms and mediators of gastritis associated with *Heliobacter pylori* infection. *Scandanavian Journal of Gastroenterology* **26**, 65-70.

Wallace, J.L. & Granger, D.N. (1992) Pathogenesis of NSAID gastropathy: are neutrophils the culprits? *TIPS* **13**, 129-131.

Wallace, J.L. & McKnight, G.W. (1990) The mucoid cap over superficial gastric damage in the rat: A high-pH microenvironment dissipated by non-steroidal antiinflammatory drugs and endothelin. *Gastroenterology* **99**, 295-304.

Wallace, J.L. & Whittle, B.J.R. (1986) Role of mucus in the repair of gastric epithelial damage in the rat. Inhibition of epithelial recovery by mucolytic agents. *Gastroenterology* **91**, 603-611.

Wallace, M.S., Zawie, D.A. & Garvey, M.S. (1991) Gastric ulcerations in the dog secondary to the use of non-steroidal anti-inflammatory drugs. *Journal of the American Animal Hospital Association* **26**, 467-472.

Wallmark, B. (1986) Mechanism of action of omeprazole. *Scandanavian Journal of Gastroenterology* **21** (Suppl **118**), 11-16.

Walsh, J.H. & Grossman, M.I. (1975) Gastrin (Part 1). *New England Journal of Medicine* **292**, 1324-1334.

Walter, M.C., Goldschmidt, M.H., Stone, E.A., Dougherty, J.F. & Matthiesen, D.T. (1985) Chronic hypertrophic pyloric gastropathy as a cause of pyloric obstruction in the dog. *Journal of the American Veterinary Medical Association* **186**, 157-161.

Warwick, R. & Williams, P.L. (1980) Splanchnology: Alimentary system. In: *Gray's Anatomy*. 36th edition, Churchill Livingstone, Edinburgh. p1267.

Wassef, M.K., Lin, Y.N. & Horowitz, M.I. (1979) Molecular species of phosphatidylcholine from rat gastric mucosa. *Biochimica et Biophysica Acta* **573**, 22-226.

Watt, J. & Wilson, C.W.M. (1959) Phenylbutazone and gastric ulceration in the guinea pig II. *Gastroenterology* **37**, 96-102.

Watt, P.C.H., Sloan, J.M. & Kennedy, T.L. (1983) Histology of the postoperative stomach before and after diversion of bile. *British Medical Journal* **287**, 1410-1412.

Watt, P.C.H., Sloan, J.M. & Kennedy, T.L. (1984) Relationship between intragastric bile acid

concentration and mucosal abnormality in the stomach after vagotomy and gastroenterostomy for duodenal ulcer. *Journal of Clinical Pathology* **37**, 506-510.

Weiss, W., Brunner, H., Buttner, G.R., Gabor, M., Miederer, S. & Mittelstaedt, A., Olbermann, M., Schwamberger, K. & Witzel, L. (1983) Treatment of reflux esophagitis with sucralfate. *Deutsche Medizinische Wochenschrift* **108**, 1706-1711.

Werther, J.L., Janowitz, H.D., Dyck, W.P., Chapman, M.L. & Rudick, J. (1970) The effect of bile on electrolyte movement across canine gastric antral and fundic mucosa. *Gastroenterology* **59**, 691-697.

Whitehead, R., Truelove, S.C. & Gear, M.W.L. (1972) The histological diagnosis of chronic gastritis in fibreoptic gastroscope biopsy specimens. *Journal of Clinical Pathology* **25**, 1-11.

Whittle, B.J.R. (1976) Relationship between the prevention of rat gastric erosions and the inhibition of acid secretion by prostaglandins. *European Journal of Pharmacology* **40**, 233-239.

Whittle, B.J.R. (1977) Mechanisms underlying gastric mucosal damage induced by indomethacin and bile-salts and the actions of prostaglandins. *British Journal of Pharmacology* **60**, 455-460.

Whittle, B.J.R. & Steel, G. (1985) Evaluation of the protection of rat gastric mucosa by a prostaglandin analoque using cellular enzyme marker and histologic techniques. *Gastroenterology* **88**, 315-327.

Williams, A.W. (1953) Observation of the healing experimental gastric ulcers in small laboratory animals. *British Journal of Surgery* **41**, 319-326.

Wilson, D.E. & Levine, R.A. (1972) The effect of prostaglandin E_1 on canine gastric acid secretion and gastric mucosal blood flow. *American Journal of Digestive Diseases* **17**, 527-532.

Wilson, R.B. & Presnell, J.C. (1990) Chronic gastritis due to *Ollulanus tricuspis* infection in a cat. *Journal of the American Animal Hospital Association* **26**, 137-139.

Winans, C.S. (1976) The fickle pylorus. *Gastroenterology* **70**, 622-623.

Winborn, W.B., Guerrero, D.L. & Hodge, E.E. (1976) Scanning electron microscopic studies on the effects of bile on the gastric mucosa of the rat. *Gastroenterology* **70**, 966.

Wittenberg, J. & Kantrowitz, P.A. (1970) Current diagnostic approaches to gastroduodenal disease. *American Journal of Surgery* **120**, 289-294.

Wlodek, G.K. & Leach, R.K. (1966) Effects of histamine, feeding and insulin hypoglycemia on net ionic fluxes in gastric pouches. *Archives of Surgery* **93**, 175-181.

Wolfe, M.M. & Soll, A.H. (1988) Physiology of gastric acid secretion *New England Journal of Medicine* **319**, 1707-1715.

Wood, L.R. & Dubois, A. (1981) SEM studies of gastric cell surface epithelium: An evaluation of various methods of preparation. *Virchows Archiv B [Cell Pathology]* **35**, 207-212.

Wood, L.R. & Dubois, A. (1983) Scanning electron microscopy of the stomach during modifications of acid secretion. *American Journal of Physiology* **244**, G475-G479.

Woodings, E.P., Dixon, G.T., Harrison, C., Carey, P. & Richards, D.A. (1980) Ranitidine - a new H_2-receptor antagonist. *Gut* **21**, 187-191.

Wormsley, K.G. (1972) Aspects of duodenogastric reflux in man. *Gut* **13**, 243-250.

Yeomans, N.D. (1976) Electron microscopic study of the repair of aspirin-induced gastric erosions. *American Journal of Digestive Diseases* **21**, 533-541.

Yeomans, N.D., St. John, D.J.B. & Boer, W.G.R.M. (1973) Regeneration of gastric mucosa after aspirin-induced injury in the rat. *American Journal of Digestive Diseases* **18**, 773-780.

Zalewsky, C.A. & Moody, F.G. (1979) Mechanisms of mucus release in exposed canine gastric mucosa. *Gastroenterology* **77**, 719-729.

Zeisel, S.H., DaCosta, K-A., Edrise, B.M. & Fox, J.G. (1986) Transport of dimethylamine, a precursor of nitrosodimethylamine, into the stomach of ferret and dog. *Carcinogenesis* **7**, 775-778.

Zerbe, C.A., Boosinger, T.R., Grabau, J.H., Pletcher, J.M. & O'Dorisio, T.M. (1989) Pancreatic polypeptide and insulin-secreting tumour in a dog with duodenal ulcers and hypertrophic gastritis. *Journal of Internal Veterinary Medicine* **3**, 178-182.

Zoli, G., Pasquinelli, G., Bonvicini, F., Gasbarrini, G. & Laschi, R. (1986) S.E.M. study I: Gastric and duodenal lesions induced by non-steroidal anti-inflammatory drugs (aspirin, piroxicam) in man. *International Journal of Tissue Reactions* **8**, 47-54.

Zontine, W.J. (1973) Effect of chemical restraint drugs on the passage of barium sulphate through the stomach and duodenum of dogs. *Journal of the American Veterinary Medical Association* **162**, 878-884.

9. Appendices

9.1 Appendix 1 Fixatives

Karnovsky's: Solution A was prepared from 10g of paraformaldehyde in 100ml of distilled water in 10 drops of 1N sodium hydroxide. This mixture was placed in an oven at 60°C until the solids were dissolved. The resulting solution was then allowed to cool. Solution B, the stock solution, was made from 250ml 0.2M sodium cacodylate, 50ml 25% gluteraldehyde, 100ml distilled water. Solution B was then added to solution A.

Buffered Normal Formalin 4% (BNF) was prepared by dissolving 4g of sodium dihydrogen orthophosphate (NaH_2PO_4 anhydrous) and 8g of potassium hydrogen phosphate (K_2HPO_4) in 900ml of tap water. To this was added 40% formaldehyde (formalin) in 100ml of water.

Gluteraldehyde 2% was made by first making the phosphate buffer with 8g disodium hydrogen orthophosphate and 2g potassium dihydrogen orthophosphate in 1000ml of water. To 23ml of this buffer was added 2ml of commercial 25% stock gluteraldehyde.

Isotonic cacodylate buffer (0.075M pH7.3) was made from 16.05g sodium cacodylate, 3.8g sodium chloride, 0.055g calcium chloride and 0.012g magnesium chloride dissolved in water to make 1000ml. The pH was adjusted to 7.3 with 1M hydrochloric acid.

9.2 Appendix 2 Tissue Processing

9.2.1 Light Microscopy

The tissues were dehydrated through ascending concentrations of alcohol, double embedded in 1% celloidin in methyl benzoate and cleared with xylene. The resulting samples were then embedded in paraffin wax.

The periodic acid Schiff & Alcian blue staining was carried out as follows. The wax was dissolved using histoclear and the sections hydrated first with absolute alcohol and then with methylated spirits before being immersed in water. With the wax removed, the sections were immersed in iodine, rinsed in water, bleached in 5% sodium thiosulphate and rinsed in water to remove any mercuric precipitate. The sections were then stained in 0.5% Alcian blue 8GX in 3% acetic acid for 30 minutes. Following a wash in water, the sections were oxidised in 1% periodic acid for 5 minutes, rinsed in water and placed in Schiff reagent (B.D.H.) for 20-30 minutes depending upon age of the reagent. The sections were washed in water for 5 minutes and counterstained in Mayer's haemalum for 1 minute and rinsed in water. Following blueing in Scott's tap water substitute for 10 seconds, the sections were rinsed again in water. The resulting sections were routinely dehydrated through methylated spirits, absolute alcohol and Histoclear and mounted in D.P.X.

9.2.2 Scanning Electron Microscopy

The tissues were removed from the Karnovsky's fixative and washed in 0.1M sodium cacodylate buffer (pH7.4) for a minimum of 4 hours. The buffer was 2.1403g sodium cacodylate made up to 100ml with distilled water. The pH was checked and adjusted if necessary with 1M hydrochloric acid. The samples were removed from this and stepped through a series of acetone baths; 70% acetone for 4 hours, 90% acetone for 2 hours, 100% acetone for 2 hours, and finally 100% acetone overnight.

The last acetone was replaced with fresh 100% acetone prior to placing the samples in acetone-filled boats for critical point drying.

The specimens were dessicated by liquid carbon dioxide (CO_2) substitution using a Polaron critical point dryer. The inlet and vent valves were opened periodically to remove acetone and replace it with CO_2, until acetone could no longer be seen, indicating complete replacement by CO_2. Once all the acetone had been removed, the dryer was warmed to 48°C at 8963kPa. This changed the carbon dioxide from a liquid to a gaseous phase. The CO_2 was vented slowly from the dryer. Once the CO_2 had been exhausted, the boats were removed from the dryer.

Following drying the samples were mounted on aluminium stubs. To aid adhesion

and bond the samples to the stubs, the stub was first coated with silver paint and the sample placed mucosal side up on the stub before the paint dried. The stubs and samples were placed in an Emscope SC500 sputter coater. The sample height was set at 20mm. The air was removed and replaced by argon gas at 34.5kPa. The stubs were then coated with gold/palladium for 3 minutes. The stubs were stored in an oven set at 38°C until examined.

9.2.3 Transmission Electron Microscopy

Samples were stored in a refrigerator at 4°C until processed. The gluteraldehyde-fixed samples were then post-fixed. The samples were removed from the gluteraldehyde and washed in isotonic cacodylate buffer for 30 minutes. They were secondarily fixed in 1% osmium tetroxide in isotonic cacodylate buffer for 2 hours, then washed in isotonic sodium cacodylate buffer. The samples were dehydrated through a series of alcohols; 50%, 70%, 90% & absolute alcohol for 15 minutes in each. This was completed by two further 20 minute rinsings in absolute alcohol. The samples were removed and washed in propylene oxide twice for 15 minutes and left overnight in 1:1 resin/propylene oxide. The following morning, the mixture was replaced with 3:1 resin/propylene oxide for the rest of the day. The next day the caps of the bottles were removed to allow the propylene oxide to evaporate. The blocks were embedded overnight in pure resin and polymerised in a 60°C oven.

The Araldite resin consisted of 30g Araldite CY212, 25.2g dodenyl succinic anhydride (DDSA) as a hardener, 1.2ml 2,4,6,-tri(dimethylaminomethyl) phenol (DMP 30) as an accelerator and 1ml dibutylphthalate as a plasticiser.

Semi-thin sections (1μ) were stained with methylene blue/ azur II (1% methylene blue and 1% azur II in 1% borax) and examined by light microscopy to determine the best areas for ultrastructural scrutiny. Ultra-thin sections were cut using a Reichert OmU3 ultratome or Reichert ultracut E, supported on 200 mesh copper grids and stained with uranyl acetate and Reynolds lead citrate.

The uranyl acetate used was 20% uranyl acetate in 50% alcohol centrifuged before use. The grids were placed in stain for 5min in the dark. The grids were then rapidly rinsed six times in 50% alcohol and in water several times before being blotted dry.

Lead citrate was made with 1.33g lead nitrate and 1.76 sodium citrate. Each salt was dissolved in 15ml distilled water and the two solutions mixed. This solution was left for 30 minutes, but shaken occasionally. Eight millilitres of 1N NaOH was added to dissolve the precipitate and then made up to 50ml with distilled water. The resulting

solution was filtered before use.

Staining was carried out in a sodium hydroxide chamber. The grids were placed face down on drops of the staining solution for 5 minutes, then rinsed rapidly in 0.02N sodium hydroxide once, followed by rapid rinsing in distilled water and blotted dry.

10. Glossary

<	less than
>	greater than
⩾	more than or equal to
%	per cent
am	ante meridiem
Cl^-	chlorine ion
UL	Units per Litre
cm	centimetre
µg	microgram
µm	micron
GMT	Greenwich Mean Time
gravel sign	produced by the retention of mineralised material in the alimentary tract indicative of partial obstruction
H^+	hydrogen ion
H_2	Histamine type 2 receptor
Hampton line	radiolucent line seen near the neck of a peptic ulcer
HCl	hydrochloric acid
kg	kilogram
kHz	kiloHerz
kPa	kiloPascals
L	Litre
m	metre
M	Molar
mg	milligram
min	minutes
ml	millilitre
min	minimum
max	maximum
mm	millimetre
mM	millimolar
N	Normal
o	degrees
PG	prostaglandin
pH	the negative value of the logarithm of the hydrogen ion concentration
pK_a	the negative logarithm of the ionization constant
pm	post meridiem
SEM	scanning electron micrograph
TEM	transmission eletron micrograph
w/v	weight volume