THE VALUE OF MORPHOLOGICAL ANALYSIS IN DUODENAL ULCER THERAPY

by

MICHAEL ALFRED GREGORY

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ABSTRACT

This study was designed to examine two premises: that the morphological "severity" of duodenal ulcers (DU) may influence the incidence of drug mediated healing and the morphological "quality" of healing after curative therapy may influence the duration of remission.

Biopsies taken at endoscopy from five healthy volunteers and from 84 patients suffering from DU were examined by light and electron microscopy. The endoscopic and morphological appearance of the mucosa within 8mm of the DU or scar, before and up to 1 year after therapy with either sucralfate, cimetidine, pirenzipine or misoprostol are described. Irrespective of the mode of therapy or whether the biopsies were from normal, juxta-DU or scar mucosa, specimens could be divided into 2 primary morphological classes: gastric metaplastic and non-metaplastic. Based on the degree of metaplastic differentiation and non-metaplastic degeneration, these classes were further divided into 4 sub-classes. When correlated with the incidence of healing and duration of remission, metaplasia was generally found to be a positive and degenerative non-metaplasia a negative prognostic criterion. Scores were awarded to primary morphological criteria and weighted to give high totals to favourable (metaplastic) and low totals to non-favourable (degenerative non-metaplastic) prognostic features. The sum of scores expressed as a percentage was termed the morphological index. This proved useful as a means of correlating mucosal morphology with DU healing and duration of remission. It also facilitated comparison of morphology within and between groups of patients before and after each drug regimen. The results showed that the

morphological appearance of the ulcerative mucosa influenced healing and remission outcome.

Discriminant analysis was applied to the numeric data that described the juxta-DU (group 1) and scar (group 2) morphology of patients treated with cimetidine in 2 studies. Separation between healed and not healed DU was achieved in 92% of group 1 and 100% (remission - more or less than 6 months) of group 2. When applied to the juxta-DU data from patients treated with cimetidine in a third study, the formulae predicted correctly in 88% of cases. In addition to predicting outcome, the formulae were used as standards to accommodate for natural variations in the prognosis of individual DU of patients enrolled for comparative drug studies. These data show that morphological analysis may be usefully employed in duodenal ulcer therapy.

PREFACE

This study is my original work and has not been submitted in any form to another University for degree purposes. Reference to the work of others is duly acknowledged in the text.

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DEDICATION

For my dear wife Jenny

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PART 1: INTRODUCTION

Duodenal ulceration is a chronic, recurring, debilitating and sometimes fatal disease that causes suffering to millions of people around the world. The condition has no respect for age, gender, race or social class, although duodenal ulcers (DU) may be more or less prevalent in some of these categories. Proposed aetiologies include gastric acid and pepsin hypersecretion, reduced mucosal protection, bacterial or viral infection, genetic predisposition, or a combination of these factors. In some instances lesions heal spontaneously, in others they will not heal or remain healed even after the most intensive curative regimen. Different drugs with differing mechanisms of action have been developed to combat the disease. Most effect healing in between 60% and 90% of subjects over a 4 to 6 week period of therapy. However, the majority of patients relapse within 1 year of the termination of treatment.

Why should some DU heal and others not? Why should some patients remain in remission for long periods of time while others relapse within weeks of the end of treatment? Are there prognostic classes of DU, perhaps with differing aetiologies? Why should some drugs preferentially effect healing and/or extend the duration of remission while others do not - can they in some way alter the healing process or perhaps improve the morphological quality of scar mucosa? This study investigates the possibility that the answer to some or all of these questions may be found in the mucosa surrounding duodenal ulcers and scars.

Various studies have shown that the morphological appearance of the mucosa surrounding DU and scars is abnormal (Patrick,

Denham and Forrest 1974, Moshal et al. 1979). This study investigates the possibility that the type and/or degree of juxta-DU and scar pathomorphology influences healing and remission prognoses. In order to compare variations in the appearance of the ulcerative mucosa, it was necessary to establish a means of quantifying mucosal morphology. With the exception of myself, whose studies form the basis of this thesis (Gregory et al. 1982**a,b,c**, Gregory and Spitaels 1982, Gregory and Brouckaert 1985, Gregory and Simjee 1985, Gregory 1986**a,b**, Gregory and Simjee 1986), only Tovey et al. (1989**a,b**) have devised quantitative methods of comparative morphological analysis to investigate DU healing and remission prognoses in man.

The following pages detail the preliminary work and the rationale behind the formulation of a morphological index that numerically describes the appearance of the ulcerative mucosa before and after 7 curative regimens. All prognostic correlates between morphology and DU healing and duration of remission were investigated. Discriminant analysis was employed to identify morphological criteria associated with healing and non-healing and duration of remission in patients treated with cimetidine. The discriminant formulae were then used to predict DU healing in patients treated with cimetidine in a further study. Having established that individual DU were not equal with regards potential for healing, the discriminant formulae were used to compensate for any disproportion of potentially good or bad healers in groups of patients enrolled for comparative drug studies.

CHAPTER 1

DUODENAL ULCER

In this chapter, in order to aquaint the reader with the magnitude of the world-wide problem of duodenal ulceration, the occurrence and epidemiology of DU is described. The chapter continues with an outline of the most important risk factors for developing DU and the current concepts on pathogenesis and mucosal protection. It concludes with a description of the reported efficacy and mechanism of action of the drugs employed in this study.

1.1. The Normal Duodenum

1.1.1. Structure

The duodenum is formed from the fusion of the most distal portion of the embryonic foregut and the most proximal portion of the midgut (Grand 1976). The adult duodenum is a "C" shaped muscular tube approximately 30 centimetres in length and situated between the stomach and the jejunum. In simple cross-section, the duodenum has outer longitudinal and inner circular smooth muscle layers. The walls are much folded and covered with numerous (10-40 per mm^2) finger, spade or leaf shaped villi that project approximately 250um to 650um into the lumen (Doniach and Shiner 1957, Ferguson et al. 1977). The crypts of Lieberkuhn are situated at the base of each villus and extend downward for approximately 230um into the basal layers of the mucosa, often reaching the muscularis mucosae (Hasan et al. 1981a). Three-dimensional studies have shown that three or four crypts open into a vestibule and a number of vestibules coalesce to

form a basin around each villus (Loehry and Creamer 1969). The submucosa is rich in branched and coiled Brunner's glands that synthesise a clear, viscous, alkaline mucosubstance (Florey 1962, Smits and Kramer 1984). The mucus is secreted into the duodenum via ducts which open into the bases of crypts of Lieberkuhn.

A continuous epithelium covers the villi and lines the crypts. The epithelium covering normal villi is composed of columnar absorptive and mucus secreting goblet cells in a ratio of approximately 5:1 (Morson and Dawson 1972). Within the crypts are the proliferative immature goblet and absorptive cells. Both types of cell contain secretory granules and produce an assortment of digestive enzymes. The immature cells migrate from the base of the crypts to exfoliate as mature enterocytes from the villus tip in between five and six days (MacDonald et al 1964). Concentrated near the base of crypts are occasional Paneth and enterochromaffin cells.

The duodenum is divided into four parts. The first extends for approximately 5cm from the gastro-pyloric sphincter. The second, or descending portion, is approximately 8cm in length. Appproximately 4cm along this portion is the ampulla of Vater, the point at which the major pancreatic and common bile ducts gain entry to the intestinal lumen. The third or horizontal part of the duodenum is approximately 10cm long. The fourth portion of approximately 5cm ascends along the left side of the aorta and then descends abruptly at the ligament of Treitz as the jejunum.

1.1.2. Function of Duodenum

The duodenum is employed in the digestion and absorption of food and neutralising of chyme. The duodenum receives partly digested food in the form of acidic chyme from the stomach via the pyloric canal. The presence of low pH chyme in the duodenum stimulates the gall bladder to secrete bile and the pancreas to secrete digestive enzymes into the duodenal lumen. Bile salts emulsify fats and thereby facilitate their digestion by increasing the surface area of lipid moieties. Pancreatic juices contain enzymes which hydrolyse starch to glucose and maltose, break down lipids into their separate glycerol and fatty acid units and break the peptide bonds of protein molecules thereby turning them into absorbable polypeptides. The pancreatic juice also contains mineral salts, especially alkaline sodium bicarbonate that in association with bile and the alkaline secretions from Brunner's glands, helps to neutralise the chyme. Digestion is completed just prior to absorption by enzymes located within the luminal plasma membrane of villous enterocytes. Amino acids and monosaccharides are transported from the lumen via absorptive cells to the intestinal capillaries and via the portal vein to the liver. Triglycerides pass into the intestinal lacteals and via the thoracic duct to the general circulation.

1.2 The Development Of Duodenal Ulcers In Peptic Ulcer Disease

The term "peptic ulcer" embraces a small group of common ulcerative upper gastrointestinal tract disorders. The principal types of peptic ulcer disease are gastric and duodenal ulcers. Both can be transient, recurrent or chronic. It remains unclear whether peptic ulcer is a homogeneous disease with a characteristic pathophysiology or

whether these entities represent a final expression of many and heterogenous causes (Blum et al. 1975). It is also unclear whether duodenal ulcer and gastric ulcer have the same pathogenesis (Rotter and Rimoin 1977).

A duodenal ulcer is defined as "a break in the mucosa of the duodenum extending through the muscularis mucosae with an ulcer crater surrounded by an acute and chronic cell infiltrate" (Soll 1989 p.814). Most are less than 1cm in diameter, but larger ulcers sometimes develop. Ninety percent of DU occur close to the pylorus in the first part of the duodenum (Oi and Sakurai 1959, Kirk 1968).

1.2.1. Occurrence

Prior to the 20th century, duodenal ulcers were infrequently recognised or described (Jennings 1940). Reports of their occurrence from most Western countries appear to have increased steadily until about 1960 (Soll 1989). The incidence, defined as the occurrence of new cases per population base in a given period of time, varies between countries, cultures within countries and the age and sex of the target group (Sonnenberg 1985). In Copenhagen County, Denmark, between 1963 and 1968, Bonnevie (1975) estimated the yearly incidence of DU to be 0.15% in males and 0,03% in females. The age specific incidence increased almost linearly reaching 0.3% in males between 75 and 79 years. In the County of York, UK, DU occurred in 0,21% of males and 0,06% females (Pulvertaft 1959). A national study in the USA undertaken between 1980 and 1983 showed an annual incidence of 0,29% DU for the entire population suggesting that between 200000 and 400000 new cases occurred each year (Soll 1989).

Prevalence of DU, defined as the proportion of the population with DU at a single point in time, over a period of time or over a lifetime, also varied with age, sex and culture. In Finland, an endoscopic survey of 358 apparently normal subjects found a point prevalence of active DU of 1,4% (Ihamaki et al. 1979). A National Health Interview survey suggested that in 1986, the 12 month prevalence for peptic ulcers in the USA was 1,8% for males and 1,7% for females (Soll 1989). As regards lifetime prevalence, Grossman (1980) reported that in 1976, DU occurred in about 10% of males and 4% of females in the USA. Based on presence of active DU, scarring or ulcer surgery, Ihamaki et al (1979) estimated a lifetime prevalence of 6% of the population in Finland. From the above, although 10% of the population may be affected by DU during their lifetime, the prevalence of active disease at any given point in time is probably in the range of 1% to 2%.

Data from England, Europe and the USA suggest there has been a marked decrease in the number of patients experiencing complications with DU since the 1960's (Brown et al. 1976, Coggon et al. 1981, Hollander and Tawanawski 1986). Data from the UK and USA showed that death as a consequence of DU dropped from approximately 154 per million in 1930, to 7 per million in 1976 (Sonnenberg et al. 1985**a**, Sonnenberg 1985**b**). Also, whereas the number of people hospitalised because of haemorrhage or perforation of DU is much the same now as previously, the number of patients hospitalised by uncomplicated DU has dropped by at least 43% in the USA, UK and much of Western Europe (Elashoff and Grossman 1980, Sonnenberg 1987). Although DU complications were declining prior to the introduction of H^2-receptor antagonist drugs (see 1.3. below), these positive data are probably a consequence of improved curative and maintenance drug therapy for DU.

1.2.2. Epidemiology

Epidemiological data are less than perfect and incidence of DU is often based upon such indirect markers as hospitalisation and mortality rates. Factors which appear to influence the incidence of DU include age, sex and race together with less definable considerations such as occupation, social class and area of domicile (Sonnenberg 1985b). In general, the incidence of DU increases with age and peaks at about 60 years. The declining incidence of DU referred to above was associated with individuals under 50 years. Rates have remained stable for men over 60 but have been increasing for elderly women (Walt et al. 1986). The latter trend is probably a consequence of increased use of nonsteroidal anti-inflammatory drugs (NSAID) by elderly people (Collier and Pain 1985, Walt et al. 1986).

Bonnevie (1975) and Grossman (1980a) showed that duodenal ulceration was 1.5 to 3 times more prevalent in males than in females. A study performed by Kurata et al. (1985a) showed the male to female ratio for DU hospitalisation and mortality in the USA was 1,3:1.

Kurata et al. (1985b) reported an inverse relationship between incidence of DU and family income. They also reported that DU were more common among persons with a low level of educational achievement. These observations implicating class in the epidemiology of DU supported earlier work by Pulvertaft (1959) who reported that DU were slightly more common among unskilled workers than in professionals and executives (Pulvertaft 1959). The latter observation, however, may be partly explained by the higher frequency of cigarette smoking amongst these individuals (Friedman et al. 1974 - see 2.3.3).

Race may or may not be a factor in the incidence of DU. Using often incomplete data from hospitalisation and mortality records, statistics from the USA suggest that DU is more prevalent in whites than in non-whites (Kurata 1982). However, when age, sex and general social patterns were taken into consideration, there was little evidence to support the premise that race, per se, is an important factor in the incidence of DU.

Duodenal ulcer disease occurs world-wide (Tovey 1975). There does, however, appear to be a real geographical variation in prevalence of DU. For example, DU were more common in the inhabitants of the city of York than those populating the surrounding countryside (Pulvertaft 1959). Although somewhat dated, these observations suggest a link between prevalence of DU and urbanisation. More recently Hugh et al. (1984) supported this premise by showing that in Australia, the occurrence of DU in relatively densely populated New South Wales was greater than in Queensland or Western Australia. Whether such differences relate to genetic, dietary, social or environmental factors is as yet unknown. However, these and other factors are undoubtedly important in the predisposition of some sectors of a population to DU.

1.2.3. Risk Factors

Familial and socio-economic factors, the taking of some types of drugs, especially NSAIDs, cigarette smoking, diet and stress may all predispose towards duodenal ulceration.

1.2.3.1. Genetic

Twenty to 50% of patients with DU have a positive family

history compared with only 5% to 15% of non-ulcer subjects (Rotter 1983). This together with observations showing that concordance of DU is more common in monozygotic than dizygotic twins (McConnell 1980) supports the view that the risk of developing DU may be inherited. However, because the concordance for DU in monozygotic twins is less than 100%, factors other than genetic must be operating (Rotter 1983). It is interesting to note that individuals with blood group O have approximately a 30% increase in risk compared with those of blood groups A, B, or AB (Rotter 1983, McConnell 1980).

Some populations, perhaps as a consequence of a genetic predisposition, are more suceptible to DU than others. Tovey (1979) has shown that DU are more common among Southern than Northern Indians. Moshal et al. (1981) showed this predisposition to persist in the same populations 100 years after translocation to Natal in South Africa. The hereditary factor in DU disease may be multifactorial (Ellis 1985). For example, in some instances genetic factors are responsible for pepsinogen II hypersecretion, a phenomenon considered a factor in the aetiology of the disease (Pearson et al. 1986). In other cases, familial rapid gastic emptying (Rotter et al. 1979) and hypergastrinaemia (Taylor et al. 1981) are phenomena that may predispose some families to a higher incidence of DU.

1.2.3.2. Drugs

Aspirin and other NSAIDs are known to cause acute gastric mucosal damage (Lussier et al. 1978, Arnold et al. 1984). Less frequently, these drugs have also been shown to cause duodenal mucosal bleeding and DU (Lanza et al. 1980, Lockard et al. 1980, Lanza et al. 1981) especially if taken in high

doses (Levy 1974). There is a higher incidence of DU in subjects over 65 years who take these drugs regularly (Collier and Pain 1985, Walt et al. 1986). Corticosteroids are also thought to predispose the duodenum to DU. However, the association between this group of drugs and DU is more controversial; some studies showing a higher risk (Conn and Blitzer 1976) and others none (Messer et al. 1983).

1.2.3.3. Tobacco Smoking

There is convincing evidence to support the premise that tobacco smoking in general and cigarette smoking in particular is a factor that predisposes for the development of DU (Friedman et al. 1974). There is also evidence to suggest that risk of developing a DU is proportional to the amount smoked (McCarthy 1984). Smoking has also been shown to impair DU healing (Korman et al. 1981, Baraket et al. 1984), promote recurrences (Sontag et al. 1984) and increase the risk of complications which may lead to death (Kurata et al. 1986). Although the mechanisms by which tobacco smoking exacerbates DU is uncertain, the link between cigarette smoking and DU is convincing.

1.2.3.4. Psychodynamic Factors

The importance of psychodynamic factors in the genesis of DU remains controversial despite decades of investigation (Weisman 1959, Fordtran 1979, Feldman et al. 1986). Weiner (1957) provided evidence that dependency-independency personality conflicts predispose to DU whereas others reject the notion that such conflicts are a predisposing factor (Weisman 1956, Dotevall 1984). One factor that does appear to play a part in the genesis of DU is the way individuals accommodate for both physical and mental stress (Peters and Richardson 1983, Dotevall 1984).

1.2.3.5. Diet

Although folklore has incriminated dietary indiscretion as a cause of DU, there are no convincing data to support a premise that diet may cause, perpetuate or reactivate DU. Some have tried to link diet to DU via the prevalence of the disease in a particular region. In areas of Southern India where rice is the staple diet, DU occur more frequently than in areas of Northern India where wheat is eaten (Malhotra 1978, Tovey 1979). However, in these and other studies, the many other regional socio-economic factors made it impossible to conclude that diet was a key factor in the incidence of DU (Rydning and Berstad 1986). Caffeine, tea and some non caffeine-containing soft drinks are potent stimulators of acid secretion (McArthur et al. 1982, Dubey et al. 1984, McCloy et al. 1984). In spite of causing dyspepsia in some subjects, there is no evidence to suggest that consumption of these beverages imparts an increased risk for duodenal ulcer.

Wine, beer and other alcoholic beverages are acid secretagogues (Lenz et al. 1983). However, the stimulation of acid secretion often reflects constituents other than alcohol in the drink (Peterson et al. 1986). Although alcohol in high concentration can cause damage to the gastric mucosa, there is no convincing evidence to suggest that alcohol is a predisposing factor in DU (Friedman et al. 1974). In fact Sonnenberg et al (1981) showed that a moderate consumption of alcohol favoured DU healing.

Although there are no unequivical data to support a link between the consumption of a particular food or beverage with the development of DU, a number of studies suggest that the consumption of particular foods helps prevent DU in

subjects at risk. Hollander and Tarnawski (1986) suggested that the reduction in the incidence of DU in recent years may be a consequence of an increased ingestion of vegetable oils (olive and sunflower oil for cooking and in margarines). Jayaraj et al. (1980) suggested that the ingestion of foods containing lipid or liposoluble substances may "protect" the mucosa from ulceration. Tovey (1974), found that wheat and rice bran had greater acid buffering capacity than refined carbohydrate foods and that DU were rare in areas of the world where the intake of unrefined dietary fibre was high (Tovey and Tunstall 1975, Tovey 1979). Rydning and Berstad (1985) showed that vegetable fibre bound bile acids, reduced pepsin concentration, improved the postprandial pH curve in DU patients and supported the results of earlier studies that suggested a diet rich in fibre may reduce the probability of developing DU.

1.2.4. Pathogenesis

Attempts to establish a single pathophysiological mechanism in DU have proved unsuccessful. The consensus of opinion is that two primary factors predispose the duodenal mucosa to ulceration: the presence of elevated levels of low pH gastric acid and pepsin in the duodenal lumen, together with a local or general breakdown of mucosal defence mechanisms (Lam et al. 1982, Wormsley 1983, Blair et al. 1987). Although generally independent of each other, both pathogenetic modalities are influenced by endogenous prostaglandins (Ruppin et al. 1979, Konturek 1985). Defective prostaglandin synthesis and/or secretion has therefore been proposed as an important factor in the genesis of DU (Ahlquist et al. 1983). Viral and bacterial aetiologies have been proposed as either a primary or secondary cause of both duodenal and gastric ulceration in

man (Vestergaard and Rune 1980, Goodwin et al. 1986a, Bode et al. 1987, Graham 1991).

1.2.4.1. Gastric secretion.

Gastric acid is secreted by parietal cells situated in the oxyntic glands beneath the gastric pits in the fundus of the stomach. Gastric acid secretion is controlled by three distinct pathways (Wolfe and Soll 1988). The vagus nerve delivers acetylcholine to muscarinic receptors on the parietal cells which are then stimulated to produce acid by a rise in intracellular calcium. The second pathway involves gastrin which, after secretion by the antral G cells, circulates in the blood to bind to gastrin receptors on the parietal cells. Again, cell stimulation to secrete acid occurs through a rise in intracellular calcium. The third pathway involves histamine secretion by mast-like cells that lie adjacent to the parietal cells. Histamine acts through a histamine H^2 receptor on the parietal cell to activate adenylate cyclase. Acid production is then stimulated by a rise in cyclic AMP.

In association with HCl, pepsinogen is converted to the enzyme pepsin. Pepsin has an optimum "working" pH of pH 1.5 - pH 3.0 and is virtually inactive at pH 5.0 (Calam 1985). Acid is a weak stimulator of pepsinogen secretion. Pepsinogens 1 and/or 2 are secreted by chief cells primarily situated in the glands of the stomach.

Gastric acid secretion in response to stimulation by a number of secretagogues is, on average, higher in DU patients than in normal subjects (Lam 1984a, Blair et al. 1987). Also, it has been reported that the postprandial acid

secretory response is prolonged in DU subjects, the response outlasting the buffering effects of food (Malagelada et al. 1977, Blair et al. 1987). It is thought that in some patients, the rapid postprandial emptying of low pH, poorly buffered gastric contents may predispose some individuals to the formation of DU (Malagelada et al. 1977, Lam et al. 1982). Basal and nocturnal acid secretions are also increased in some DU patients (Dragstedt 1967, Feldman and Richardson 1986) as is total 24hr secretion (Feldman and Richardson 1986). The maximal capacity of the stomach to secrete acid is a function of the total parietal cell mass (Grossman and Elashoff 1980) and this mass has been reported to be 1.5 to 2 times greater in DU patients than in control subjects (Cox 1952). In addition to having the capacity for hypersecretion, approximately 33% of DU patients also have hyperpepsinogenaemia I (Simloff et al. 1986) and up to 10% may have hypergastrinaemia (Cooper et al. 1985). The relevance of the latter finding to genesis of DU remains unclear.

From the above, it appears that some DU occur consequent to gastric acid and/or pepsin hypersecretion. This premise is further supported by data showing that DU generally heal when these factors are reduced or removed (Peterson et al. 1977). However, although acid is a permissive factor for most DU, and low pH acid plus pepsin a more corrosive mucosal degenerative combination (Joffe et al. 1980), hypersecretion of acid only occurs in between 20% and 50% of patients with DU (Cox 1952). In many instances, DU develops in a normal acid milieu or persists during therapeutic inhibition of acid secretion (Soll 1989 p. 824). These observations suggest that perhaps a more important ulcerogenic aberration is a breakdown in the resistance of the duodenal mucosa to even normal concentrations of acid and/or pepsin in the chyme or perhaps a breakdown of the

mechanisms that are continually being called on to repair superficial injury (Wormsley 1983).

1.2.4.2. Mucosal resistance.

Several factors are involved in the maintenance of duodenal mucosal integrity in an acid-peptic environment. These include mucus and mucosal bicarbonate secretion, mucosal blood flow and cell restitution and renewal. Endogenous prostaglandins are thought to orchestrate the multi-factorial mechanisms of mucosal defence (see 1.2.4.3.). Impairment of one or more of these factors may enable corrosive luminal fluids to breach the mucosal barrier thereby establishing a focus for mucosal erosion and ulceration.

Mucus, a viscous, water insoluble glycoprotein gel, adheres to and blankets the entire surface of the healthy gastroduodenal mucosa. The high viscosity and gel-forming properties of mucus are imparted by high molecular weight glycoproteins which are characterised by their high ratio of carbohydrate to protein (Allan 1978). In the stomach, a neutral mucosubstance is secreted by surface mucus secreting cells (Pearson et al. 1980) whereas in the duodenum, the surface mucus coat is comprised largely of an acid non-sulphated mucosubstance derived from villous goblet cells (Gad 1969) together with alkaline/neutral non-sulphated secretions from the mucus cells of the glands of Brunner (Smits and Kramer 1984).

In the stomach, the mucus coat ranges from 0,2um to 0,6um and is of a similar thickness in the proximal duodenum (Bickel and Kauffman 1981, Kerss et al. 1982). Mucus keeps

the epithelial surface lubricated and protects the underlying mucosa from shear during the passage of chyme through the duodenum thereby avoiding mechanical and abrasive injury. Mucus, although reported to block the passage of macromolecules and pepsin (Edwards 1978, Venables 1986), is permeable to acid and therefore does not directly protect the underlying enterocytes from corrosive attack (Rees 1987). The thick mucus gel does, however, provide an unstirred water layer that retards the diffusion of H^+ from the lumen to the epithelial cell surface (Williams and Turnberg 1980, Pfeiffer 1980). Mucus, therefore, is an important component of the "mucus-bicarbonate barrier".

Small ammounts of bicarbonate pass from the blood to the duodenal lumen via the enterocyte by transcellular transport and/or chloride-bicarbonate exchange (Garner et al 1983,). Bicarbonate also reaches the lumen/epithelial cell interface by passive diffusion between mucosal cells (Garner 1988). The bicarbonate forms a thin film over the surface of enterocytes. Adherent mucus gel provides a stable unstirred layer over the mucosal surface which prevents the immediate admixture of bicarbonate and lumenal acid/pepsin. Imposition of a "mixing barrier" between the alkali situated on the apical membranes of surface cells and the vast excess of acid in the lumen explains how such a relatively small ammount of bicarbonate can contribute to mucosal protection (Flemstrom and Kivilaakso 1983). Reaction between bicarbonate and H^+ in the unstirred mucus layer then gives rise to a pH gradient through the thickness of the gel from pH 4.5 at the luminal interface to pH 7.0 near the surface of the cell (Quigley and Turnberg 1987).

In patients with DU, there may be reduced mucosynthesis and/or mucosecretion or the mucus may be biochemically

defective (Younan et al. 1982). Mucus gel in DU patients has been reported to have an increased proportion of lower molecular weight glycoproteins than in normal patients (Younan et al. 1982). This causes a less viscous, possibly "weaker" gel to be formed, a phenomenon which may enable more rapid permeation of H^+ through a less effective unstirred diffusion barrier. There is evidence to suggest that bicarbonate secretion may be reduced in some patients with DU (Isenberg et al. 1987). This together with alterations in mucus thickness or composition could reduce the efficiency of the mucus/bicarbonate layer to shield the mucosa against low pH chyme.

The mucus/bicarbonate layer is the first line of defence against low pH fluids in the duodenal lumen. The second line of defence is the "body" of the epithelial enterocytes themselves. In the duodenum, stem cells in the base of crypts of Lieberkuhn divide and differentiate into absorptive and mucus secreting cells. During maturation they migrate to the tip of villi where they exfoliate into the lumen. The total turnover time from mitosis to exfoliation is approximately 5 days (MacDonald et al. 1964). The balance between cell loss and cell replacement is an important factor in mucosal defence. An increase in cell loss or decrease in cell renewal may precipitate the formation or hinder the healing of DU.

Turnover of cells in the gut is regulated by a number of factors. These include hormones, neuropeptides and locally produced peptides. Epidermal growth factor (EGF), or urogastrone has long been known to stimulate protein and DNA synthesis and cell division in gastroduodenal cells (Leblond and Carriere 1955). Urogastrone is the best characterised gastrointestinal growth factor and is produced in the

salivary and Brunner's glands (Hirata and Orth 1979) from where it is secreted directly into the duodenal lumen (Gregory et al. 1979). Although there are no studies that show DU to have occurred directly as a consequence of the reduction or absence of EGF, administration of EGF has been shown to accelerate the healing of mucosal lesions in the gastroduodenum (Lee 1990).

The microvasculature plays an essential role in transporting oxygen and nutrients to various layers of the mucosa and its integrity is a prerequisite for the maintenance of the basal lamina and normal turnover of healthy enterocytes. Focal or generalised ischaemia has for many years been suggested as a cause of duodenal ulceration (Virchow 1853, Reeves 1920, Kirk 1968, Konturek 1985). Certainly, the shunting of blood away from the gastrointestinal tract following severe burns or head trauma, have been associated with the production of acute DU (Sevitt 1967). There is less evidence, however, to support the view that a focal or generalised reduction in blood flow is a major cause of DU in peptic ulcer disease.

1.2.4.3. Prostaglandins in health and peptic ulcer disease

Prostaglandins (PG) are naturally occurring long-chain fatty acids with ring structures classified by the letters A to I and with side-chain double bonds classified by numerical subscripts. The gastroduodenal mucosa is capable of producing PG from the common precursor arachidonic acid. Indeed, after appropriate stimulation, PG are synthesised and secreted on demand by all nucleated cells (Johansson et al. 1985). In the gastroduodenal mucosa, the most well studied PG are the E type prostaglandins (PGE).

Prostaglandins are involved in the normal secretion of acid by the parietal cells of the stomach (Wolfe and Soll 1988). In addition, PGE are involved in many of the previously described mechanisms of mucosal cytoprotection. They have been shown to be associated with mucus synthesis and secretion (Ruppin et al. 1979) and bicarbonate secretion and cell turnover via stimulation of EGF (Main and Whittle 1975, Brand et al. 1985). In addition, the secretion of E-series prostaglandins increases mucosal blood flow (Konturek 1985). The literature suggests that PG are an important factor for maintaining the integrity of the gastroduodenal mucosa in man.

From the above, insufficient or aberrant PG synthesis and/or secretion may be a pathogenic factor for DU. This premise is supported by many studies that show that prostaglandin inhibitors such as NSAIDs have a negative, ulcerative effect on the duodenum (Lanza et al. 1980, Lanza et al. 1981). In spite of reports that suggest prostaglandin deficiency as a possible cause of DU (Ahlquist et al. 1983) and PG replacement as a potential cure (Rachmilewitz et al. 1986), the presence of ulcerative lesions in the duodenal mucosa is not necessarily associated with a marked decrease in duodenal PG production (Hillier et al 1985, Hawkey et al. 1985). From this it is clear that duodenal ulcerogenesis and the maintenance of mucosal cytoprotection are not entirely prostaglandin-dependent. Furthermore, the data suggest that prostaglandin-independent mechanisms may well be sufficient to maintain mucosal integrity in the face of acid/pepsin attack.

1.2.4.4. Infections.

As the natural history of duodenal ulcer disease,

characterised by frequent remissions and relapses, can not be completely explained by either the "acid" or defective "mucosal resistance" hypotheses, viral and/or bacterial aetiologies have been considered. Supporting evidence has included findings that Herpes type 1 viruses are more frequently present and in higher titre in DU patients than in control subjects (Vestergaard and Rune 1980). To date, however, Herpes virus has not been found in the ulcerative mucosa. Cytomegalovirus has been isolated from duodenal and gastric ulcers in patients receiving immunosuppressants after renal transplantation (Franzin et al 1981, Cohen et al 1985). Although there is little to support a viral aetiology, studies suggest that a spiral bacillus - Helicobacter pylori - may play an important role in the aetiology of DU. (Goodwin et al. 1986a, Bode et al. 1987, Graham 1991).

Helicobacter pylori has been found exclusively in association with the surface mucus secreting cells of the stomach (GSMC) and the metaplastic gastric mucus secreting cells (MSC) found in inflamed areas of the duodenum in patients with duodenitis and DU (Anderson et al. 1987, Wyatt et al. 1987, Caselli et al. 1988). H. pylori is a robust, strongly urease positive, micro-aerophilic, non-fimbriate spiral organism ranging from 2um to 6,5um in length and 0,5um to 0,6um in width which undergoes 1 to 3 turns over this length (Jones et al. 1985). It has hemispherical ends and from one pole originate between 1 and 8 sheathed flagellar filaments. Although occasionally described within GSMC by some authors (Tricottet et al. 1986)), H. pylori is generally an extracellular, gastric mucus-inhabiting organism. Bacteria may attach to the epithelial cell plasma-membrane/glycocalyx complex by short (50nm) "pedestals" (Old 1986), or migrate through the gastric mucus layer by means of their flagellae.

There are compelling data to implicate H.pylori in the aetiology of DU (Rathbone et al. 1986), however, the various interpretations of such data do not conclusively prove that the organism causes DU. There are two trends of thought - either that H.pylori is simply a marker of the type of gastritis (duodenitis) that is present in DU patients, or that H.pylori plays a more direct role by damaging the duodenal mucosa and thereby promoting ulceration. Irrespective of whether H.pylori is instrumental in the genesis of DU, irradication of the organism during curative DU therapy has been shown to greatly increase the average period of remission following termination of treatment (Coghlan et al. 1987, Marshall et al. 1988a).

1.3. Treatment Of Duodenal Ulcer

Duodenal ulcers probably occur when luminal aggressive factors - acid and pepsin - overcome mucosal defense mechanisms. These may be weakened by the destructive action of H. pylori on surface mucus and mucosal enterocytes. Duodenal ulcer disease is a chronic relapsing condition. Effective therapy, therefore, should not only heal existing DU, but prevent ulcer recurrence. The hallmark of current therapy is the reduction of intragastric acidity. There are, however, a number of drugs in common use whose primary action is either to provide directly or to stimulate an improvement of natural cytoprotection. More recently, to rid the mucosa of H. pylori infection, these drugs have been administered in conjunction with a variety of antibiotics. In the present study, duodenal morphology related to five anti-ulcer drugs is examined. The mechanism of action and efficacy of these drugs will now be discussed to provide the necessary background to the experimental work that follows.

1.3.1. Acid Reducing Drugs

Gastric acidity can be reduced either by neutralising gastric juice with antacids (Peterson et al. 1977, Vergin and Kori-Lindner 1990, Zaterka et al. 1991) or by inhibiting the synthesis or secretion of HCl by blocking either the histamine (Burland et al. 1975, Aadland and Berstad 1978) or muscarinic receptors embedded in the parietal cell plasmalemma (Wolfe and Soll 1988). Parietal cell secretion can also be controlled by inhibition of parietal cell H^+/K^+-ATPase, the (proton) pump responsible for acid secretion (Wallmark 1988, Schmueli 1992).

1.3.1.1. Histamine H^2-receptor antagonist.

The company Smith Klyne and French pioneered the concept of histamine receptor blocking in the early 1970's with their drug cimetidine (Tagamet^R). This provided the "gold standard" against which other medication was judged.

Cimetidine shares the imidazole ring structure of histamine itself. It is generally given in a dose of 800mg daily for 28 days. Healing rates with cimetidine are similar to most other acid inhibitory and cytoprotective drugs on the market and range from 65% to 90% after 4 to 6 weeks of therapy (Marks 1980, Bardhan et al 1986, Lipsey et al. 1990, Marks et al. 1991, Reynolds and Schoen 1992). About half of the unhealed DU heal after a further 4 weeks of therapy (Bardhan 1984). Although rare and reversible on withdrawal of treatment, reported deleterious side effects include impotence, gynecomastia (McCarthy 1983), confusion, somnolence and dizziness (Cerra 1982). These generally occur only after high doses administered for the control of acid hypersecretory conditions and not as consequences of DU therapy.

Recurrence rates after the termination of cimetidine therapy are 50% to 70% after 6 months and 80% to 90% after 1 year (Hetzel et al. 1978, Bodemar and Walan 1978). These data are similar to the recurrence rates reported with other H^2-receptor anatagonist drugs (Hui et al. 1992) or proton pump inhibitors (Lauritsen et al 1985, Graham et al. 1992a). Maintenance therapy of 400mg per day may be given to patients predisposed to relapse. Maintenance treatment with cimetidine will prevent relapse in 60% to 85% of patients (Bianchi-Porro and Petrillo 1986). These figures compare favourably with other H^2-receptor antagonist drugs (Texter et al 1986, Penston and Wormsley 1992) and proton pump inhibitors (Shmueli and Record 1992).

1.3.1.2. Selective antimuscarinic agent.

Pirenzipine is a selective anticholinergic drug that blocks the delivery of acetylcholine to the muscarinic receptors of parietal cells, thereby inhibiting the production of HCl. Although pirenzipine is largely selective for the muscarinic receptors in the stomach, it nevertheless influences receptors in smooth muscle, heart and salivary glands causing "dry mouth" and blurred vision side effects in some patients (Feldman 1984 ,Carmine 1985). Pirenzipine is less effective than cimetidine in inhibiting basal and pentagastrin-stimulated acid secretion (Williams et al 1986). Carmine (1985) reported that pirenzipine healed DU in 70% to 87% of cases in 4 to 6 weeks. However, DU healed by pirenzipine therapy are reported to relapse less rapidly than those healed by H^2-receptor antagonists (Eichenberger et al 1982). A daily maintenance dose of 50mg to 100mg of pirenzipine is reported to provide a one year recurrence rate of 15% to 40% (Carmine 1985).

1.3.2. Mucosal Cytoprotective Drugs.

Cytoprotective drugs promote DU healing by physically providing a barrier between corrosive chyme and the luminal surface of enterocytes and/or stimulating natural mechanisms of mucosal cytoprotection (Szabo 1988). In addition, bismuth suspensions have been shown to reduce H. pylori infection (Schmueli 1992). With the exception of the prostanoids, cytoprotective drugs do not influence HCl secretion.

1.3.2.1. Sucralfate

Sucralfate (Carafate) is a sulphated disaccharide complexed with aluminium hydroxide. In acid conditions it dissociates, releasing aluminium. The negatively charged residual compound polymerises to form a viscous paste which binds avidly to the luminal surface of both damaged and normal mucosa at a pH of less than 3.0. Luminal sucralfate binds bile salts and reduces peptic activity either by the absorption of pepsin by the drug or by direct inhibition via the aluminium component of sucralfate (Samloff 1983, Graham et al. 1984). Also, the surface paste reputedly impedes the diffusion of hydrogen ions, thus providing a physical protective barrier against corrosive chyme (Rees 1991).

In addition to direct on-site or cytoprotection, sucralfate is also reported to stimulate the release of prostaglandins resulting in increased bicarbonate secretion (Shorrock et al. 1990), mucus production (Tasman-Jones et al. 1989) and/or mucosal blood flow (Szabo and Hollander 1989). Sucralfate is also said to improve the cytoprotective properties of mucus (Slomiani et al. 1986) and by binding EGF to the site of ulceration, promote the growth of epithelial cells in the vicinity of the DU, thus accelerating healing (Konturek et al. 1989).

Duodenal ulcer healing after 4 to 6 weeks of sucralfate therapy (1gm per day) is reported to be at least as good as that of cimetidine (Glise et al 1986) and in some instances superior to H^2-receptor antagonists (Lam 1991). Sucralfate has been reported to overcome the adverse effects of smoking on duodenal ulcer healing (Lam et al 1987, Lam 1989). Recurrence of DU after termination of therapy with sucralfate is reported to be less than that of cimetidine (Marks et al 1981, Lam et al 1987, Tovey et al. 1989). Maintenance doses, usually in the form of 1gm daily, are reported to reduce recurrence when compared with placebo (Liebeskind 1983). After 1 year on maintenance therapy with sucralfate, relapse rates were virtually identical to those of patients treated with H^2-receptor antagonists (Marks and Girdwood 1985, Paakkonen et al 1989, Hui et al. **(a)** 1989). Although small, there is a potential risk of aluminium absorption during extended therapy with Sucralfate (Giesing et al 1982). Although sucralfate has been reported to reduce the density of H. pylori in the gastric antrum (Hui et al. **(b)** 1989), the drug has no known antibacteriocidal properties.

1.3.2.2. Bismuth

Bismuth subnitrate, bismuth subcarbonate, bismuth subsalicylate (Pepto-Bismol) and tripotassium dicitrato bismuthate (TDB; colloidal bismuth subcitrate-CBS) are colloidal suspensions of bismuth that have been employed to promote healing of DU. In this study TDB (De-Nol) was used to promote healing. TDB has been shown to have little or no acid-neutralising effect and no anti-secretory activity (Baron et al. 1986). There is some controversy as to whether TDB decreases pepsin secretion. Flavell et al. (1965) reported no effect on secretion while more recently, Baron et al. (1986) showed a reduction in pepsin secretion. There is, however, agreement that TDB inhibits peptic activity (Roberts and Taylor 1982).

TDB, like sucralfate, precipitates in acidic conditions (optimal pH 3.0), binding to proteins in the ulcer base (Koo et al. 1982, Elder 1986) thereby forming a layer that may protect the mucosa against further acid and pepsin attack. Bismuth compounds are reported to enhance mucus glycoprotein secretion and reinforce the viscoelastic gel properties of mucus (Moshal et al. 1979, Hollander et al. 1983). In addition, TDB has been shown to increase duodenal alkali secretion (Konturek et al. 1987a) and cause accumulation of EGF in the ulcerated areas, thereby possibly promoting mucosal repair (Lambert et al. 1989). De-Nol has been shown to increase the synthesis and secretion of prostaglandins in the upper gastro-intestinal tract (Konturek et al. 1987**b**), a phenomenon that may be responsible for the cytoprotective alterations described above.

Suspensions of colloidal bismuth have proven bacteriocidal properties. Studies have shown that they effectively suppress H.pylori in both the stomach and duodenum (Goodwin et al. 1986**b**). Also, by blocking H. pylori adhesion to epithelial cells, inhibiting mucus destructive bacterial proteases and membrane destructive lipases and phospholipases, colloidal bismuth blocks some of the processes that may damage the mucosa after infection (Sarosiek et al. 1989, Czinn et al. 1990).

Colloidal bismuth is as efficacious in healing DU as H^2-receptor antagonist drugs (Lee et al 1985, Hamilton et al 1986, Ward et al 1986, Eberhardt et al 1987). Trials have shown that TDB healed up to 85% of DU that were resistant to H^2-receptor antagonist therapy (Lam et al. **(b)** 1984, Bianchi-Porro et al. 1987). The irradication of H. pylori does not appear to influence the rate of DU healing (Lambert et al. 1987) but does have a profound effect on DU relapse

after the termination of therapy. The DU relapse rate in subjects where H. pylori had been erradicated was 0% to 22% compared with 31% to 81% in those with persisting H. pylori infection (Borody et al. 1989, Rauws and Tytgat 1990).

Colloidal bismuth may be administered as a liquid or as tablets, 1 or 2 to be taken twice to four times daily. Recent studies show that normal oral doses of TDB are not overtly neurotoxic (Bierer 1990, Benet 1991). However CBS is minimally absorbed in the gastrointestinal tract and there is a slight risk of bismuth toxicity or encephalopathy even during a normal 6 week course of treatment (Hamilton et al 1983). Other side effects after taking colloidal bismuth are black staining of the teeth and discoloration of the faeces and anus.

1.3.2.3. Prostaglandin analogues

Misoprostol (Cytotec) is a 15-deoxy-15-hydroxy-16-methyl analogue of prostaglandin (PGE^1). The drug has been shown to display a moderate inhibition of both basal and food stimulated acid secretion in man (Wilson 1986, Monk and Clissold 1987). As well as having an anti-acid secretory effect, prostaglandin analogues have been shown to enhance mucosal resistance to injury in animals (Roszzkowski et al 1986, Bauer et al 1986). Endoscopic studies have shown that misoprostol prevents NSAID-induced injury to gastroduodenal mucosa in man (Stiel et al 1986, Lanza 1986). Misoprostol has a similar healing rate to the H^2-receptor antagonists (Thomson 1986, Winters 1986). However, misoprostol is reported as particularly effective in preventing the development of DU in patients taking NSAIDs (Agrawal et al 1987). Few comparative studies have been undertaken to determine the relative efficacy of the prostaglandin

analogues in preventing DU relapse. However, those that have been reported suggest that the prostaglandin analogues were less effective than the H^2-receptor antagonists in preventing ulcer recurrence (Bardhan 1987, Lauritsen et al. 1987). Side effects include crampy abdominal pain, diarrhoea, intermenstrual bleeding, menorrhagia and vaginal bleeding in premenopausal women (Shmueli and Record 1991). Prostaglandins of the E-group are contraindicated in pregnancy as they may cause partial or complete expulsion of the conceptus (Shmueli and Record 1992).

1.3.3. Summary

Irrespective of whether a drug promotes DU healing by reducing HCl secretion or protecting the mucosa, it heals a consistent 60% to 85% of patients after 4 weeks and up to 95% of patients after 6 weeks of treatment. Unfortunately, within one year of therapy, up to 90% of patients have recurrent DU. In order to prevent relapse, lower, maintenance doses of the curative drugs have been prescribed. In addition, since it was realised that persistent *H. pylori* infection was a factor that predisposed to early relapse, various strategies have been employed to reduce or erradicate *H. pylori* infection during treatment. Inclusion of one or more antibiotics with acid reduction or cytoprotective therapy, although not improving the incidence of DU healing (Massarrat et al. 1992, Mannes et al. 1992), has substantially reduced relapse rates (Lamouliatte et al. 1992, Graham et al. 1992b).

CHAPTER 2

MORPHOLOGICAL ASPECTS OF DUODENAL ULCEROGENESIS AND HEALING

Peptic ulcers are discrete breaks in the gastrointestinal mucosa. In the duodenum they are generally from 5mm to 10mm in diameter (Sun and Stempien 1971, Scheurer et al. 1977, Sonnenberg et al. 1979) and are situated in the first part of the duodenum (Oi and Sakurai 1959, Kirk 1968). Duodenal ulcers are generally surrounded by an area of inflammation, the morphological appearance of which may indicate a regressive phase of ulcerogenesis or a progressive phase of healing (Gregory et al. 1987).

This study investigates the possibility that an evaluation of juxta-DU or scar mucosal morphology may indicate the severity of individual DUs prior to treatment and the quality of healing after therapy. This may reveal why some DUs are difficult to heal and why some relapse shortly after various pharmaceutical regimens. In order to address these questions it was necessary to obtain one or more biopsies from within the area of inflammation, at a consistent and predetermined location near the margin of each DU.

2.1. Endoscopic Biopsy

Of particular importance to this study was the ability of the endoscopist to obtain biopsies from precise, predetermined positions near the edge of DU or from scars. In the past, when examination of the gastroduodenum was undertaken with rigid or semiflexible endoscopes, biopsies

could not be obtained while visualising the mucosa. Under such circumstances, biopsies were generally taken from positions that may have only approximated the areas of interest. With the advent of the new breed of flexible endoscopes (Shiner 1956, Crosby and Kugler 1957, Brandborg et al. 1959, McCarthy et al., 1964 Sebus et al. 1968), biopsy specimens can be obtained under direct guidance. Thus biopsies may be taken from a precise position within the ulcer crater or, using the width between the edges of open biopsy forceps as a guide (Sonnenberg et al. 1979), at a reproducible distance from a DU or scar.

2.1.1. Safety of the Biopsy Procedure

Prior to 1957, biopsy specimens obtained using semiflexible endoscopes were large (up to 12mm in length) and often contained the full thickness of the mucosa, a strip of muscularis mucosa and often submucosal connective tissue (Doniach and Shiner 1957). Bleeding from the site of biopsy could be profuse. Biopsies obtained using flexible endoscopes rarely exceed 3.5mm in diameter and generally only include villi, crypts and some Brunner's glands (Morrissey 1972). Bleeding is generally only slight. Whereas in the past the endoscopist could only risk a single biopsy from a patient, with the advent of the flexible endoscope it is possible to take multiple biopsies at more frequent intervals during the course of a disease or its treatment (Goldman and Antonioli 1982). Wormsley (1983) and others have shown that even in a corrosive, ulcerogenic intraluminal milieu, multiple endoscopic biopsy of the ulcerated duodenal mucosa did not create foci for new ulcers. The accumulated data suggests that the procedure is safe with complications reported in only 0,1% to 0,2% of patients (Shahmir and Schuman 1980, Gilbert et al. 1981).

2.2. Duodenitis

The best documented duodenal patho-histological condition is duodenitis. Duodenitis is often asymptomatic (Kreuning et al. 1978) but may present with symptoms similar to those of DU (Joffe et al. 1978). At endoscopy, duodenitis is characterised by focal areas of haemorrhage, mucosal swelling and erythema (Hirschowitz 1962).

2.2.1. Histology

Histologically, duodenitis is characterised by mucosal oedema and infiltration of inflammatory cells into the lamina propria (Joffe et al. 1978). There is usually a reduction in both the height and number of villi (Hasan and Ferguson 1981**a**, Hasan et al. 1981**b**) and there are often necrotic and gastric metaplastic changes in the villous and crypt epithelium (Joffe et al. 1978, Stephan et al. 1978). The degree of villous, crypt and sub-mucosal alteration has been used as a means of classifying and grading the histological severity of duodenitis (Beck et al. 1965, Whitehead et al. 1975, Stephen et al. 1978).

2.2.2. Duodenitis And Duodenal Ulcer

Duodenitis in healthy subjects is uncommon (Kreuning et al. 1978) but may occur without obvious DU (Greenlaw et al. 1980). Duodenitis is also found extensively throughout the proximal duodenum in many (Paoluzi et al. 1985), but not all patients with DU (Aronson and Norfleet 1962, Cheli 1968, Classen et al. 1970). Although not necessarily widespread throughout the ulcerated duodenum, pathohistological alterations typical of duodenitis are invariably extant in the mucosa surrounding duodenal ulcers (Cheli 1968, Tweedle and Ravenscroft 1979, Collins et al. 1990).

The presence of duodenitis near DU has led to an extended controversy about the importance of duodenitis in the genesis (Ostrow and Resnick 1959, Beck et al. 1965, Gear and Dobbins 1969, Joffe 1978) and recurrence (McCarty 1924, Judd and Nagel 1927, Ostrow and Resnic 1959) of DU. Nagal (1928) proposed that duodenitis may be a stage in the healing of duodenal ulcers while Rivers (1931) suggested that duodenitis was a precursor of duodenal ulceration. Ostrow and Resnick (1959) developed these hypotheses and proposed a pathological sequence from gastric acid hypersecretion through duodenitis to ulcer as steps in the DU diathesis. They proposed that under emotional stress or other aggravating factors, some patients who were genetically predisposed would develop inflammation of the duodenum and ultimately a DU.

Although duodenitis does not inexorably lead to an ulcer and acid hypersecretion is not a prerequisite for the development of either duodenitis or DU (Donovan et al 1975, Cheli & Asti 1976) the concept that duodenitis may be a phase in both the production and healing of DU may be correct (Joffe et al 1978, Venables et al 1980, Venables 1985). Irrespective of the role of duodenitis in ulcerogenesis, the condition often persists at the site of the scar, (Paoluzi et al. 1985). Many authors have reported that persistent duodenitis after ulcer healing may predispose to early relapse (Venables et al. 1980, Pan & Liao 1990, Pan et al. 1991).

2.2.3. Ultrastructure

Little has been reported of the ultrastructural appearance of non-ulcer related duodenitis. There are, however, a number of studies that describe the fine structural

appearance of duodenitis in the abnormal mucosa near to DU. Patrick, Denham and Forrest (1974) were the first to use transmission electron microscopy to describe the ultrastructural appearance of the epithelium near the edge of DU in man. Their most significant finding was the presence of metaplastic gastric mucus secreting cells (MSC) in the villous and crypt epithelium. Although there was some controversy as to whether MSC were derived from Brunner's glands (Florey et al. 1939, Rhodes et al. 1968, Patrick et al. 1974) or undifferentiated stem cells in the crypts of Lieberkuhn (James 1964, Gregory et al. 1982**a,b**), the presence of MSC near DU has been consistently confirmed by others (Steer 1984, Malfertheiner et al. 1985, Gregory et al. 1987, Tovey et al. 1989**a,b**).

In addition to alterations associated with gastric metaplasia there are other ulcer related, sometimes necrotic alterations to the morphology of enterocytes or their organelles. Microvilli are sometimes "clubbed", shortened and/or reduced in numbers and the glycocalyx is often reduced in thickness or absent (Pillay et al. 1977, Gregory et al. 1982**a,b,c**, Gregory et al. 1987, Tovey et al. 1989). Mitochondria are sometimes enlarged, cristae reduced in number and rough endoplasmic reticulum is generally swollen (Pillay et al. 1977, Gregory et al. 1987, Tovey et al. 1989**a**). A consistent feature in each of the ultrastructural studies was a general widening of the intercellular space between enterocytes. In addition, where gastric metaplasia is evident, there is a significant reduction or absence of goblet cells (Morrissey et al. 1983, Gregory et al. 1991).

Following various curative regimens, the scar mucosa rarely appears normal (Moshal et al. 1979, Gregory et al. 1982**a,b,c**). Enterocytes populating the scar epithelium

retain many of the morphological characteristics present in the juxta-DU mucosa, the most common being gastric metaplasia (Pillay et al. 1977, Zoli et al. 1984, Malferheiner et al. 1985).

2.3. Gastric Metaplasia In The Ulcerative Duodenum

Gastric epithelium presents in the duodenum in two situations - either over areas of heterotopic gastric body type mucosa, or as a metaplastic change in the duodenal epithelium. Gastric heterotopia, characterised by islands of fully developed fundic mucosa including parietal and chief cells is rare, only occurring in about 1% to 2% of duodenal biopsies (Shousha et al. 1983). Gastric heterotopia is considered to be congenital and not generally associated with inflammation (Wyatt et al. 1987). Gastric metaplasia, however, is much more common, presenting in as many as 64% of normal individuals (Kreuning et al. 1978, Wyatt & Rathbone 1989), usually as small foci of gastric epithelial cells on the tips of villi. It is rare in children, being present in only 5% of paediatric duodenal biopsies. The difference in the incidence of gastric metaplasia between children and adults argues against a congenital origin. Gastric metaplasia is particularly common and more extensive in patients with duodenitis and ulceration, where it may present in up to 100% of cases (Morrissey et al. 1983, Caselli et al. 1988).

Gastric metaplasia probably represents a response to injury of the duodenal mucosa. This is supported by animal experiments where gastric metaplasia developed during healing of surgically produced defects in cats (Classen et al. 1974). It also occurs as a consequence of experimentally induced hyperacidity in the duodenum of cats, pigs and

monkeys (Florey & Harding 1935, Florey et al. 1939, Gaskin et al. 1975, Natelson et al. 1977).

In man, gastric metaplasia is primarily seen in patients with a low fasting gastric juice pH (Wyatt et al. 1987) and is extensive in patients with the Zollinger-Ellison syndrome (Parrish & Rawlins 1965). It is not found in the duodenum of patients with atrophic gastritis or subjects with intestinal metaplasia, a condition that reduces the area of acid secreting gastric mucosa. Gastric metaplasia also appears to be reduced after selective vagotomy (Wyatt et al. 1987). It is not specifically a response to acid injury since it is sometimes seen in Crohn's disease and ulcerative jejunitis (Whitehead 1984). However, in the proximal duodenum, high acid load is the most frequent potential cause of mucosal injury and cause of gastric metaplasia.

2.3.1. Gastric Metaplasia In The Juxta-DU Mucosa

Although MSC in the ulcerated mucosa probably occurs consequent to high levels of acid and pepsin in the duodenal lumen (Rhodes 1964, James 1964, Hoedemaker 1970, Johansen and Hansen 1973), it has been suggested that gastric metaplasia particularily in the juxta-DU position represents a natural protective mechanism evoked by the mucosa to promote healing (Patrick et al. 1974, Gregory et al. 1982a). Those authors proposed that the secretion of copious amounts of neutral gastric mucosubstance from numerous well differentiated MSC at the periphery of DU may better protect the ulcer crater from the corrosive effects of low pH chyme than the sulphated acid-mucosubstance secreted by the relatively few goblet cells in the non-ulcerated and ulcerated non-metaplastic mucosa. While gastric mucosubstance promotes healing, the soma of metaplastic

enterocytes circumscribing a lesion may prevent further mucosal erosion and DU enlargement.

2.4. Prediction Of DU Prognosis

For many years investigators have been looking for objective criteria to predict DU healing and determine whether patients would relapse quickly or remain in remission after one or other type of drug therapy. Such information would be of particular benefit to patients with intransigent, recurring DU. Lam and Koo (1983), by determining clinical, personal, physiological and endoscopic characteristics, used discriminant analysis successfully to predict whether DU would heal after cimetidine or placebo. Sonnenberg et al. (1981), considered such factors as moderate alcohol consumption, abstinence from smoking, young age, female gender and cimetidine treatment as predictors for DU healing and longer remission.

2.4.1. Morphological Grading Systems

Semi-quantitative grading systems have been used objectively to compare the morphological state of the ulcerative mucosa before and after anti-ulcer therapy (Hasan et al. 1981b, Paoluzi et al. 1985, Pan et al. 1990). O'Brien et al. (1987), by measuring and calculating the relative length of damaged mucosa in histological sections, attempted to quantify the phenomenon of cytoprotection after various prostaglandin therapies. Their methodology was used recently to evaluate the cytoprotective properties of various anti-ulcer drugs in rats (O'Brien et al. 1990). More recently, in an attempt to determine the cause of early relapse, Pan et al. (1991) used semi-quantitative morphological criteria to assess the histological maturity of healed duodenal ulcers

after therapy with colloidal bismuth subcitrate or cimetidine.

To date, only Gregory et al. (1982**c**, 1985, 1986**a,b**) and Tovey et al. (1989**a,b**) have devised and used quantitative methods of comparative morphological analysis to investigate DU healing or remission prognosis in man. My studies form the basis of this thesis and will be described and discussed in detail later. The methodology and results obtained by Tovey and his coworkers are detailed below.

Tovey et al. (1989**a,b**) report the endoscopic and morphologic results obtained from 46 patients before and after treatment with either sucralfate (n=24) 1g qds/6wks/12wks) or cimetidine (n=22) 200mg tds and 400mg nocte/6wks/12wks). The progress of each patient was followed by endoscopy during one year of maintenance therapy (sucralfate, 1g bd: cimetidine, 400mg) and for up to three years after the termination of therapy in patients who remained in remission. There were no significant differences in the healing rates between sucralfate or cimetidine either after 6 weeks (58%:64%) or 12 weeks (88%:96%) or the relapse rates after one year on maintenance therapy (22%:25%). There was, however, a difference in the percentage of patients who relapsed within three years after the termination of treatment (36%:77%). Tovey's group investigated the possibility that this difference correlated with the morphological appearance of the juxta-scar mucosa during and after the termination of therapy.

Biopsies for light (LM) and transmission electron microscopy (TEM) were taken at endoscopy from two sites near the edge of each DU or scar before and after curative therapy and

again after one year on maintenance treatment. Two control duodenal biopsies were taken from each of 20 patients with non-ulcerative dyspepsia and with endoscopically normal duodenal mucosa. To quantify changes in mucosal morphology before and after each therapeutic regimen and to correlate such changes statistically with the incidence of healing and duration of remission, scores were awarded to various pathomorphological features at both the light and electron microscopic level. The following five pathognomonic features were noted by light microscopy: Loss of villi; loss of goblet cells; the replacement of absorptive cells with gastric metaplastic cells; the presence of erosions and inflammatory cell infiltration. Each of the five criteria was allotted a maximum score of three with zero being near normal and 3 the most severe pathology.

In addition to the objective light microscopic evaluation, a semi-quantitative appraisal was made by electron microscopy of each paired biopsy. Gastric metaplasia was classed as a negative criterion and increasing scores in the range from 1 to 5 were awarded in accordance with the ammount present in each biopsy. Pathological severity was graded from minor changes in absorptive cell morphology (1 point) through complete replacement of normal mucosal cells with MSC (4 points) to MSC plus necrosis (5 points). The higher the score the more pathologically abnormal the mucosa.

Tovey's group found LM scores to be significantly reduced after both types of therapy. There was, however, no numeric difference in scar mucosa after 6 or 12 weeks of treatment with either drug. After one year of therapy with sucralfate they reported a sustained reduction in scores, a phenomenon not evident after cimetidine treatment. There was no significant difference in the TEM scores between the three

treatment phases in the cimetidine treated patients whereas the trend for numeric improvement in mucosal pathomorphology after initial healing with sucralfate became significant after maintenance therapy.

Interestingly, although Tovey and co-workers showed a correlation between low LM and TEM scores and extension of remission in patients treated with sucralfate, they found no prognostic relationship between mucosal morphology and period of remission. This lack of correlation may have been due to the morphological key on which their scoring was based. In the present study, a new key based on different premises was created and applied to juxta-DU and scar morphology. Whereas Tovey's group studied only the possible correlations between scar morphology and duration of remission, the present study extends their contribution by, in addition, investigating possible correlations between juxta-DU morphology and healing prognosis.

CHAPTER 3

THE ORIGIN AND DESIGN OF THIS STUDY

Duodenal ulcers are prevalent in approximately 1.5% of the world's population and may affect up to 10% of people during their lifetime. Many ulcers heal spontaneously, but others require some pharmaceutical intervention. Drug therapy heals 65% to 95% of DU within 4 to 6 weeks. Some patients do not respond with one drug regimen but will heal with another. Although uncommon, patients continue to die from DU haemmorhage and perforation. After therapy many patients relapse within 6 months whereas others remain in permanent remission. Why should there be such variation in both the short and long-term outcome after therapy in patients suffering from the same disease? Is it possible that there are different prognostic classes of DU and is it possible to identify these by appraisal of juxta-DU and/or scar morphology?

The results of a fine-structural study performed by the University of Natal gastrointestinal research group (Pillay et al. 1979) showed that the duodenal mucosa did not return to normal after curative therapy. This result together with the unexplained phenomena outlined above prompted the questions:

1. Does the morphological severity of the DU prior to therapy influence the incidence of healing?
2. Does the morphological quality of healing after curative therapy influence the duration of remission?

The present study was primarily designed to address these two questions. It was extended to investigate the possibility that a morphological appraisal of the juxta-DU and scar mucosa may enable prediction of DU healing and duration of remission. If it is possible to determine in advance the probability of DU healing with a particular regimen, then such information would have a profound effect on the treatment of DU. Intransigent DU might be identified and early surgical intervention could be considered.

3.1. Outline of Study

3.1.1. Historical Perspectives

I was a member of a research group investigating many aspects of peptic ulceration including the possibility that differences in duodenal ulcerative mucosal morphology prior to and/or after treatment may indicate DU healing and remission prognoses. Of special interest to the group was the possibility that selective drug therapy may influence the morphological quality of healing and thereby extend the period of remission.

The experimental work reported in this thesis was performed over a period of 6 years. The individual studies were undertaken in 2 phases:

1. **Preliminary:** Normal and ulcerative mucosal characteristics were identified, optimal position for endoscopic biopsy was located and the morphological changes that occur during and at the end of DU healing were determined.

2. **Experimental:** Biopsies were obtained from the juxta-DU and scar mucosa before and at predetermined times after different therapeutic regimens.

In the experimental phase it became obvious that using standard observational techniques, very little difference was discernable both before and after treatment within and between the various groups of specimens studied. It therefore appeared that the best approach would be to quantify mucosal morphology and then correlate the morphological data with incidence of healing and duration of remission before and after the different types of curative therapy.

Based on the sum of morphological data and the known outcome from two long-term drug trials, a morphological key was devised and a morphological index arrived at. This index enabled good correlations to be made between juxta-DU and scar morphology and healing and remission prognoses. It also showed that drug specific prediction of DU healing and remission prognoses were possible.

3.1.2. Thesis Profile

This study describes, in sequence, the methodology and qualitative morphological results of the preliminary and experimental studies and the rationale behind the formulation of the morphological index. Then retrospectively all prognostic correlates between morphology and DU healing and remission are examined. Discriminant analysis is employed to identify morphological criteria associated with healing and non-healing and extended remission and relapse in patients treated with cimetidine. The discriminant formulae are then used to predict DU healing in patients to be treated with cimetidine in a further study. Having shown that some DU are more likely to heal than others, the discriminant formulae are used as a means of compensating for any disproportion of prognostically good or bad DU in groups of patients enrolled for comparative drug studies.

PART II

CHAPTER 4

SUBJECTS AND METHODS

The work was undertaken in two phases. First, preliminary studies established the morphology of duodenal mucosa from healthy volunteers, located an optimal position to take biopsies from the ulcerated mucosa, and determined the sequence of morphological events that occurred during DU healing. The experimental second phase can be subdivided into the following sections.

a) Mucosal biopsies were evaluated before and after various curative regimens to determine whether there was a collective association between the morphological appearance of juxta-DU and/or scar mucosa and the incidence of DU healing and/or duration of remission.
b) A numerical morphological index was created to evaluate data determined from (a) above critically.
c) Discriminant analysis was employed to weight morphological criteria so as to best separate specimens from patients treated with cimetidine according to whether they: healed or did not heal; remained in remission for more or less than six months.
d) Using the discriminant formulae derived from (c) above, the possibility that morphological analysis could predict DU prognosis after cimetidine therapy was investigated.
e) The prognostic formulae derived from the cimetidine data were employed as standards with which to compare the relative efficacy of different types of drug therapy.
f) The morphological and numeric data were critically evaluated to determine whether different drugs may preferentially heal particular classes of DU and influence the duration of remission.

This chapter outlines the general criteria for patient selection or exclusion and describes the endoscopic, light and electron microscopical procedures employed. The preliminary studies and comparative drug studies are described. The morphometric methodology is detailed and statistical procedures tabulated. The rationale and methodology pertaining to the creation of the morphological index and prediction of prognoses are described in chapters 6 & 7.

4.1. Control of Studies, Consent and Patient Criteria

All specimens were obtained from the Gastrointestinal Unit, Department of Medicine, University of Natal, Durban. All experimental work and drug trials were initiated and monitored by Professors MG Moshal and Professor AE Simjee. Biopsies were obtained at endoscopy under the direction of Dr JM Spitaels.

Protocols for each drug study were approved by the ethics committee of the Medical School, University of Natal. Informed consent was obtained from each subject prior to endoscopy. Criteria for acceptance as a normal volunteer were a clinical history void of any gastrointestinal disease and a normal mucosa as determined by an experienced endoscopist. Patients were included if they had a clearly defined DU in the duodenal cap. Duodenal ulcer subjects required an ulcer of at least 5mm to qualify for inclusion.

Exclusions: Patients were excluded if they were under 21 years of age, pregnant, lactating or were considering pregnancy. Other exclusions included linear ulcers, malignancy of any type, recent significant alcoholism, Zollinger-Ellison syndrome, inflammatory bowel disease, acute pancreatitis and any other medical condition that was

considered sufficiently serious to interfere with the conduct of the study. Patients whose condition deteriorated during the study were withdrawn and excluded from analysis.

Each duodenal ulcer patient was enrolled in a double-blind randomised drug trial. Randomisation was achieved by random number tables and by treating patients with drugs identified by number only. The numeric code for the type of therapy being administered to each patient was controlled by the company supplying the drugs and only broken during the trial should a patient's condition deteriorate. After completion of the trial and the morphological analysis, the code was broken and the patients placed into therapy groups.

4.2. Endoscopy

After fasting for a minimum of 12 hours, patients suspected of suffering from duodenal ulcer disease were prepared for endoscopic examination. Prior to the procedure, no drugs were administered as pre-medication other than a 4% lignocaine (xylerone - Astra) for light pharyngeal analgesia. Endoscopy was performed using either an Olympus GIF-D2, GIF-K2 or GIF-Q endoscope and a standard endoscopy technique. The endoscopic appearance of the duodenal cap and the position within it of any DU or scar was recorded before and after therapy (Appendix A). The endoscopic severity of individual lesions was estimated by awarding a score (0-4) to various semi-quantitative parameters associated with mucosal macro-pathology (Table IA). After therapy, the degree and extent of healing was determined by reference to previously described endoscopic parameters (Table IB). Using the open biopsy forceps as a reference length (Sonnenberg et al. 1979), biopsies of the villous mucosa were obtained from a predetermined position near the edge of the ulcer crater. A minimum of 2 and a maximum of 4 biopsies were taken from each subject.

Key To Estimation Of DU Severity Prior To Treatment

4 Large (>20mm), deep DU - often more than one
3 DU with length or diameter of 15-20mm
2 DU with length or diameter of 10-15mm
1 DU with length or diameter of 6-10mm
0 DU with diameter <6mm.

Table IA: Endoscopic determinants of DU severity

Key To Estimating The Degree Of Healing After Therapy

0 New DU (worse)
1 Increase in crater size (worse)
2 Decrease in crater size (improvement)
3 Ragged scar (improvement - near complete healing)
4 Clear scar

Table IB: Endoscopic determinants of DU healing

4.3. Specimen Preparation

The complete biopsy was immediately immersed in a cooled (8C) 0,2M sodium cacodylate buffered, glutaraldehyde (4%) paraformaldehyde (5%) mixture at pH 7,4 (Karnovsky 1965). Within 10 minutes of excision, paired biopsies were examined with a dissecting microscope to establish the presence of villi. If present in both biopsies, one specimen was prepared for light microscopy while the other was reduced to 1mm cubes for transmission electron microscopy, ensuring that some surface epithelium was present in each cube. If the surface epithelium was present in only one specimen, the biopsy was bisected in such a manner as to ensure that surface epithelium was present in both segments. In each case, specimens were fixed for a further one hour at 8C prior to being processed for light and transmission electron microscopy.

The specimens for light microscopy were processed in accordance with well proven schedules (Method 1 - Appendix

B). In brief, after appropriate fixation specimens were dehydrated through graded ethanols, cleared in two changes of xylene and impregnated with three changes of paraffin wax. The specimens were orientated to ensure a longitudinal appraisal of villous morphology. Sections of 4um were cut on a Cambridge rotary microtome and ribbons of sections picked up on cleaned glass slides. A minimum of 4 slides was made from each specimen. The sections were dewaxed and each slide was stained with either haematoxylin and eosin (H&E), periodic acid-Sciff (PAS) for neutral mucins, PAS + Alcian Blue for neutral and acidic mucins and Southgates mucicarmine for acid mucins PAS (Methods 2 to 5 - Appendix B). Colour and black-and-white photomicrographs were taken of representative areas of interest using a Nikon "Optiphot" light microscope.

After one hour fixation, tissue for TEM was washed in 0,2M cacodylate buffer prior to being post-fixed/stained with 1% osmium tetroxide in 0,2M cacodylate buffer. The tissue was then rewashed in 0,2M cacodylate buffer prior to dehydration through graded ethanols, cleared with propylene oxide and embedded in Araldite epoxy resin (Glauert et al. 1956) in plastic moulds. All stages of the dehydrating procedures and early infiltration of resin was at room temperature. The impregnation of resin was at 50C and the Araldite was allowed to polymerise over 24 to 48 hours at 60C (Method 6 - Appendix B).

Using a Reichert "Ultracut" ultramicrotome, sections were cut with glass knives (Latta and Hartman 1950) onto a clean water bath. Using a fine glass rod, 1um sections were placed on pre-cleaned glass slides and stained with warm 1% aqueous alkaline toluidine blue for 10 seconds (Trump et al. 1961) prior to being examined with a Nikon "Optiphot" light photomicroscope. Using the microscopic appearance of the

tissue as a guide, where necessary, blocks were reorientated to facilitate the transverse sectioning of the villous mucosa. The blocks were recut for light microscopy and areas of interest photographed and trimmed for ultramicrotomy. Ultrathin sections with silver/grey interference colors (60nm to 80nm) were cut from the selected areas and mounted on uncoated, 200 mesh copper grids prior to being double stained with 1% uranyl acetate in 50% ethanol and Reynolds lead citrate (Reynolds 1963). The sections were examined with a Zeiss EM10B transmission electron microscope and electronmicrographs taken of regions of interest.

4.4. Preliminary Studies

4.4.1. Normal Villous Morphology

The light and TEM appearance of the normal human duodenum has been described by many authors. However, fine structural variations may occur as a result of differing methodology or by similar methodology in different hands. It was therefore important at the outset of this study to establish the morphological appearance of the normal duodenum using local endoscopic and preparatory procedures. Here, control samples were obtained by endoscopy from normal mucosa in the first part of the duodenum in five healthy volunteers.

4.4.2. Optimal Position of Biopsy

The variability of mucosal morphology at different distances from individual DU would preclude comparisons between juxta-DU morphology of individual lesions unless biopsies were made from a morphologically controlled, optimal and pre-determined distance from the ulcer's edge. The purpose of this procedure was to determine a position near to DU where the mucosa was abnormal but not necrotic.

To determine the optimal position for biopsy, four patients with active untreated DU in the first part of the duodenum were investigated. Each had a DU approximately 5mm in diameter. In two patients, endoscopic biopsies were taken within 3mm of the edge and at 4, 8 and 12mm from the edge of each DU. In another two patients, endoscopic biopsies were taken at 3, 4, 8 and 20mm from the border of each DU.

4.4.3. Sequence of DU Healing

The objective of this part of the study was to determine the sequence of morphological events that took place during the process of DU healing. Five patients with DU were selected for treatment with 100ml of 5% aqueous De-Nol (tripotassium dicitrato bismuthate (TDB) 4 times daily for 6 weeks. Biopsies were taken from within 8mm of the margin of DU or from scars prior to and then 1 hour, 1 week and 6 weeks after commencement of treatment. To record the spectrum of change possible during healing, further juxta-DU biopsies were taken at random from 10 patients whose DU were in various stages of healing during treatment with other drugs.

4.5. Patients, Protocols and Drug Studies.

In each study, pairs of endoscopic biopsies were taken from a carefully determined position within 8mm of the edge of the DU prior to therapy or from similar positions near the scar at the termination of successful treatment. All specimens were prepared for light and transmission electron microscopy as described in 4.4. above. All endoscopic and morphologic pre- and post-therapy information from patients treated with each of the drugs was recorded (see Appendix 3) and the data collated and compared using various statistical methods (see 4.7. below).

4.5.1. Sucralfate and Cimetidine

Patients with endoscopically diagnosed DU were allocated randomly to treatment with either sucralfate (4 grams/day) or cimetidine (2 X 500mg/day) for 6 weeks to promote healing. The study was continued until 10 patients were healed with each drug, confirmed endoscopically. Biopsies were taken from near the edge of DU or scars prior to and after curative therapy. Further biopsies were made from scars 13, 26, 39 and 52 weeks after treatment in patients who remained in remission for up to one year.

4.5.2. Cimetidine, Low Dose Misoprostol and Higher Dose Misoprostol

Patients with endoscopically diagnosed DU were randomly allocated to treatment with either Cimetidine (2 X 300mg/day), Misoprostol (2 X 50ug/day - low dose) or Misoprostol (2 X 200 ug/day - high dose) for 4 weeks to promote healing. The study continued until there were at least 6 endoscopically healed patients with each drug. Biopsies were taken from near the edge of DU or scars prior to and after therapy. Further biopsies were made from near the edge of the scar at approximately 26 weeks after treatment in patients who remained in remission for the period of the study (6 months).

4.5.3. Cimetidine and Pirenzipine

Twenty patients with endoscopically confirmed DU were randomly allocated to treatment with either Pirenzipine (2 X 50mg/day) or Cimetidine (2 X 400mg/day) for 4 weeks to promote healing. Paired mucosal biopsies were made at endoscopy from within 8mm of the edge of each DU prior to drug therapy.

4.6. Morphological Analysis

4.6.1. Morphometry

Histological slides were examined by light microscopy and areas of interest were displayed on a computer monitor via a video camera interfaced with the microscope. After appropriate calibration, measurements of villous length and width, cell size and distribution could be made. The VIDS image analyser was particularly useful in determining villous goblet cell distributions in normal epithelium and near DU and/or scars before and after curative therapy. Analysis entailed measuring the length of surface epithelium in a field of view and counting the goblet cells therein. Goblet cell numbers were expressed as goblet cells/100um of surface mucosa (GC/100um). Goblet cells were identified and distinguished from metaplastic gastric mucus secreting cells by the PAS/Alcian blue staining technique. The image analyser was also used to determine the size of organelles within electron micrographs. The calibrated accuracy of the system was $\pm$3%.

4.6.2. Qualitative Morphological Analysis

In order to describe the appearance of the juxta-DU and scar mucosa in individual and groups of biopsies, pre- and post-therapy specimens were assigned to one of the following morphological classes: **MA** (severe metaplasia); **MB** (moderate metaplasia); **NM** (degenerative non-metaplasia); **ND** (non-degenerative non-metaplasia). Details of the rationale for morphological classification are described in chapter 5.

4.6.3. Semi-quantitative Morphological Analysis

To determine whether the endoscopic appearance of DU influenced healing prognosis, the endoscopic severity of DU (**SL:4-0** see Table I) was correlated with the incidence of healing.

To determine whether juxta-DU or scar mucosal morphology influenced DU healing and/or remission prognosis, the collective morphologic appearance of juxta-DU specimens was correlated with healing, and scar specimens were correlated with the duration of remission. Intergroup comparisons of morphological data were made using:

1. The nonparametric two-sample proportions test, to test whether the population proportions of two groups were significantly different.
2. The Chi-square test, to test independence between row and column variables. In this study, the test was used to compare up to four paired columns of data (MA;MB;NM;ND).

4.6.4. Quantitative Morphological Analysis

Based on the data reported in chapter 5, a morphological index was devised that describes numerically the morphological appearance of the ulcerative mucosa (for details of rationale and method of constructing the morphological index, see chapter 6). Each specimen was re-evaluated and awarded a morphological score.

Statistical methods were applied to the numeric data to establish whether:

a) Juxta-DU morphology influenced healing prognosis.
b) Scar morphology influenced the duration of remission.

In addition, the numeric data was analysed in order to:
c) Investigate the relationship between the type of juxta DU mucosal morphology and the endoscopic severity of individual lesions.
d) Investigate the relationship between juxta-DU mucosal morphology and the duration of remission.
e) Characterise the appearance of scar mucosa at the termination of treatment and during the period of remission.
f) Predict DU healing and duration of remission with cimetidine therapy.
g) Compare the relative efficacy of various drugs.

Statistical methods employed were:

1. The nonparametric two-sample proportions test.
2. The Chi-square test.
3. The Mann-Whitney two sample test to test the difference between the means of two independent groups.
4. The nonparametric Wilcoxon Matched Pairs Test to test the difference between paired groups.
5. Regression analysis to plot the regressions derived from juxta-DU morphology and percentage healing correlations.
6. Discriminant analysis was used to find an equation from numeric endoscopic and morphological variables that could predict DU healing and extension of remission in patients treated with cimetidine.

PART III: RESULTS

CHAPTER 5: MORPHOLOGICAL ASPECTS

As mentioned previously, the studies described in Chapter 4 were undertaken over six years. Morphological results from earlier studies were updated with new data as it was collected. Reinterpretation of old data in the light of new evidence caused concepts to change and new avenues of investigation to be opened. The following results, therefore, rather than focussing on the morphological information determined from individual studies as it was collected, combines the data from each of the studies, and in retrospect uses the information to describe the general appearance of the mucosa before, at the end of, and at predetermined periods of time after curative therapy. Where interesting morphological phenomena were observed, they are described in detail.

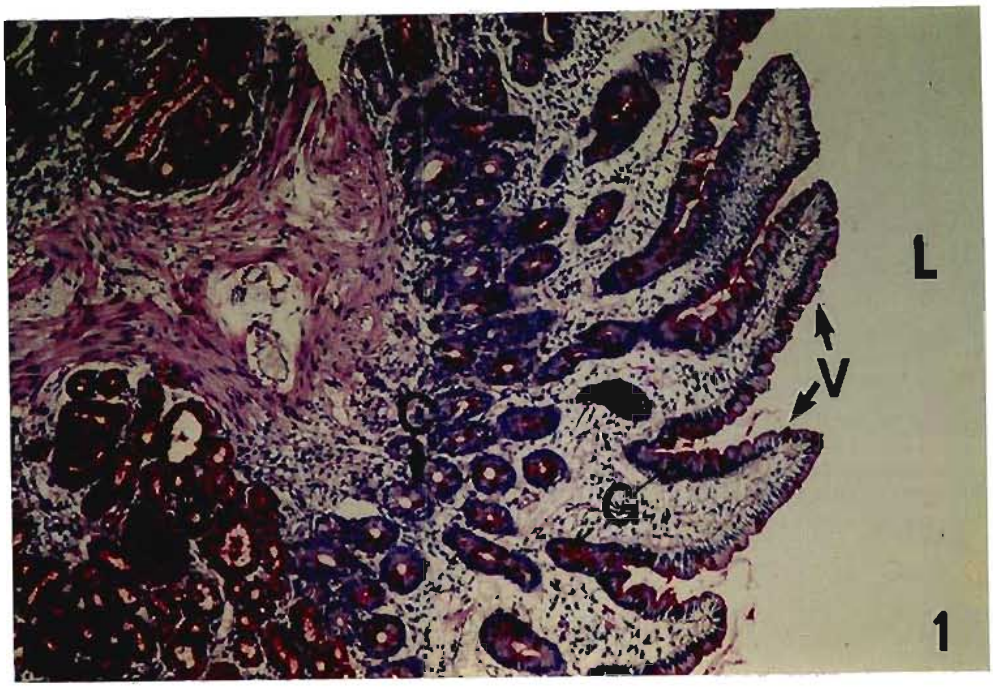
Although cognisance was taken of all cell types and stroma through the full thickness of the duodenal mucosa and submucosa, this study was primarily concerned with the morphological appearance of the villous epithelium. However, in order that the morphology of cells present in ulcerative tissue should not be confused with the normal morphology of cells populating the crypts of Lieberkuhn and Brunner's glands, the morphology of these cells in normal tissue is described.

5.1. Normal Duodenal Mucosa

5.1.1. Light Microscopy

The biopsies generally contained the full thickness of the mucosa together with a substantial number of Brunner's glands (Plate 1). Finger-shaped villi ranged in length from 250μm to 450μm and from 80μm to 300μm in thickness. The epithelium was well preserved, saw-toothed in appearance and populated by goblet and columnar absorptive cells in a ratio of approximately 1:5 (Plates 2 to 4; Figure 36). Goblet cells were 10μm to 12μm in width, up to 26μm in length and contained a single round/oval nucleus near the base of each cell (Plates 3 and 4). Absorptive cells were 6μm to 8μm in width and ranged in length from 16μm near the tips of villi to approximately 26μm near the base (Plate 4). Each cell had an apical brush border and contained a single elongated nucleus located towards its base. Examination of toluidine blue stained, resin embedded sections showed that the brush-border varied in thickness over the length of each villus from approximately 0.6μm near the tip (Plate 5) to 1.5μm near the base (Plate 6). Occasional cells near the tip of villi appeared necrotic and some appeared to be in the process of exfoliation (Plate 7).

The crypts of Lieberkuhn were populated with columnar and goblet cells in varying phases of differentiation, Paneth cells and very occasional enteroendocrine cells (Plates 8 to 10). Paneth and immature goblet cells were most prevalent near the base of crypts (Plate 8) while enteroendocrine and more well developed goblet and immature absorptive cells were more prevalent in the mid-crypt regions (Plate 9). Undifferentiated stem cells, some undergoing mitosis, were found in the lower/mid-portions of the crypt (Plate 10). All the above cell types were similar to those described by Leeson (1988 pp435-453).



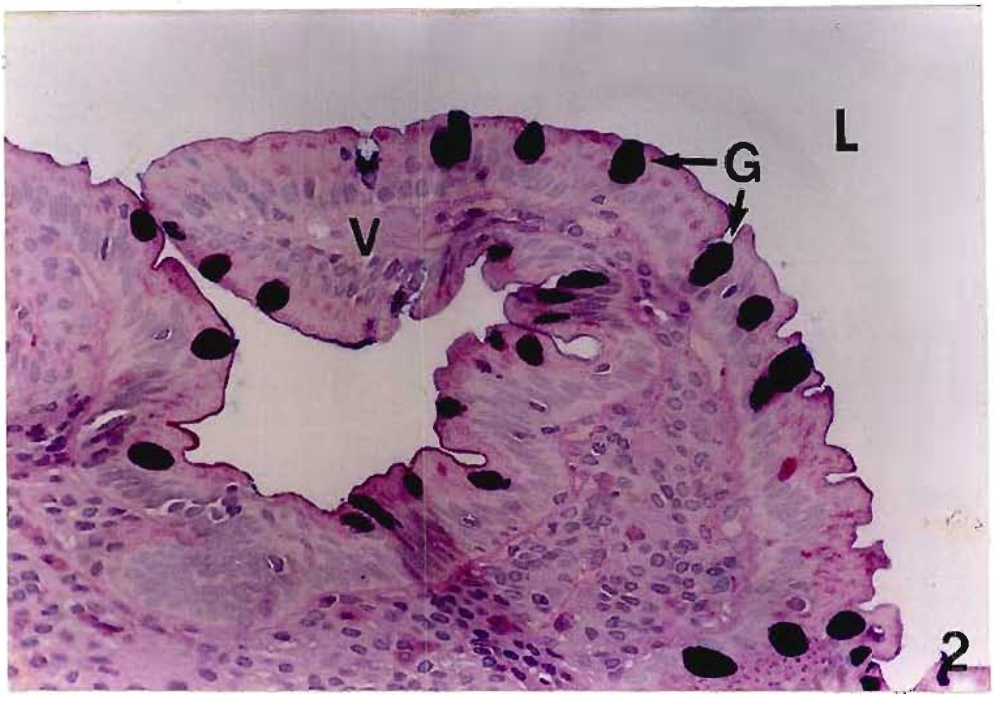
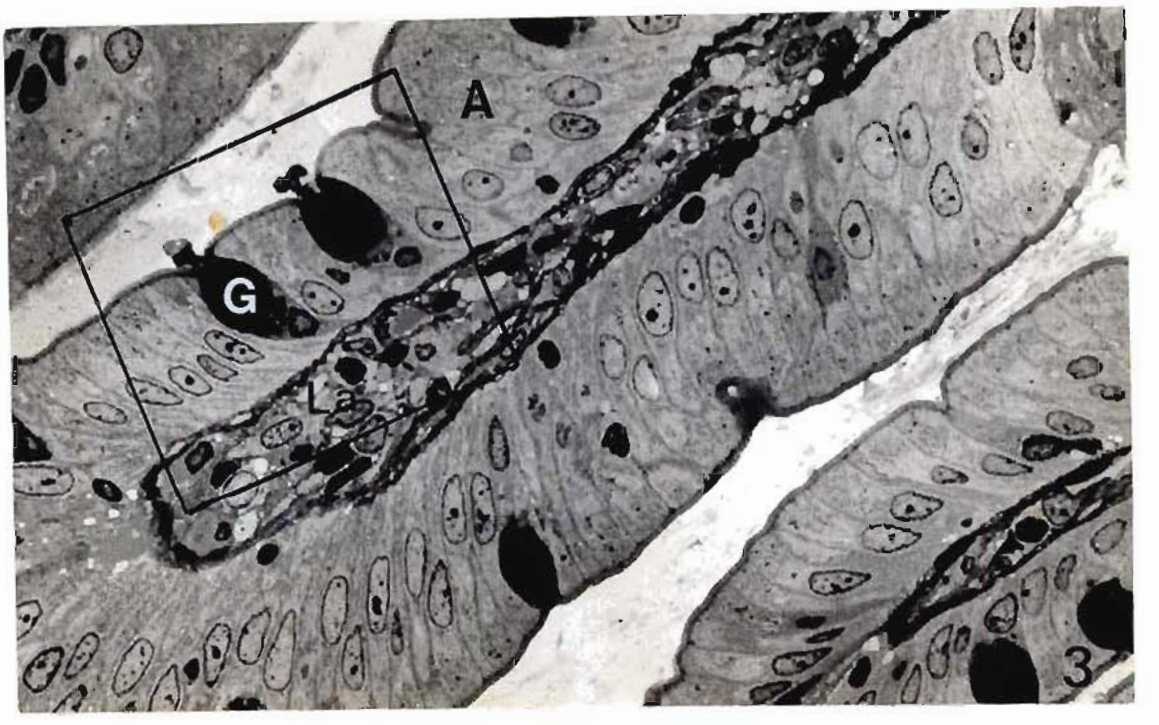


Plate 1: Light micrograph (LM). Section through the full thickness of the normal mucosa showing finger shaped villi (V), crypts of Lieberkuhn (C) and Brunner's glands (B). The villi are populated with mucicarmine positive goblet cells (G) and absorptive cells. Southgates mucicarmine stain: Magnification X 130.

Plate 2: LM of normal villus detailing alcian blue positive goblet cells. Alcian blue/PAS stain: Magnification X 450.



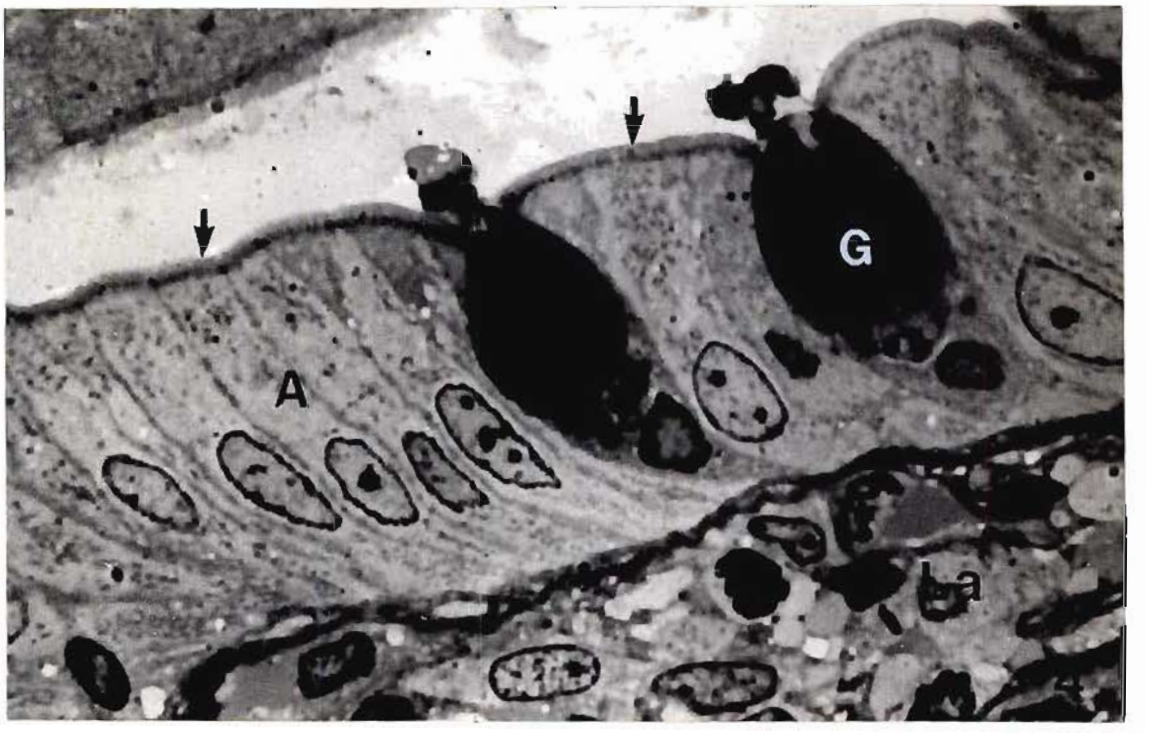


Plate 3: LM showing absorptive cells (A) and actively secreting goblet cells populating the epithelium of normal villi. La = Lamina propria. Toluidine blue stain. Magnification X 760.

Plate 4: LM detailing area marked in Figure 3. Note the well defined brush border (arrowed) projecting from the absorptive cells and mucus being secreted into the lumen by the goblet cells. Magnification X 1800.

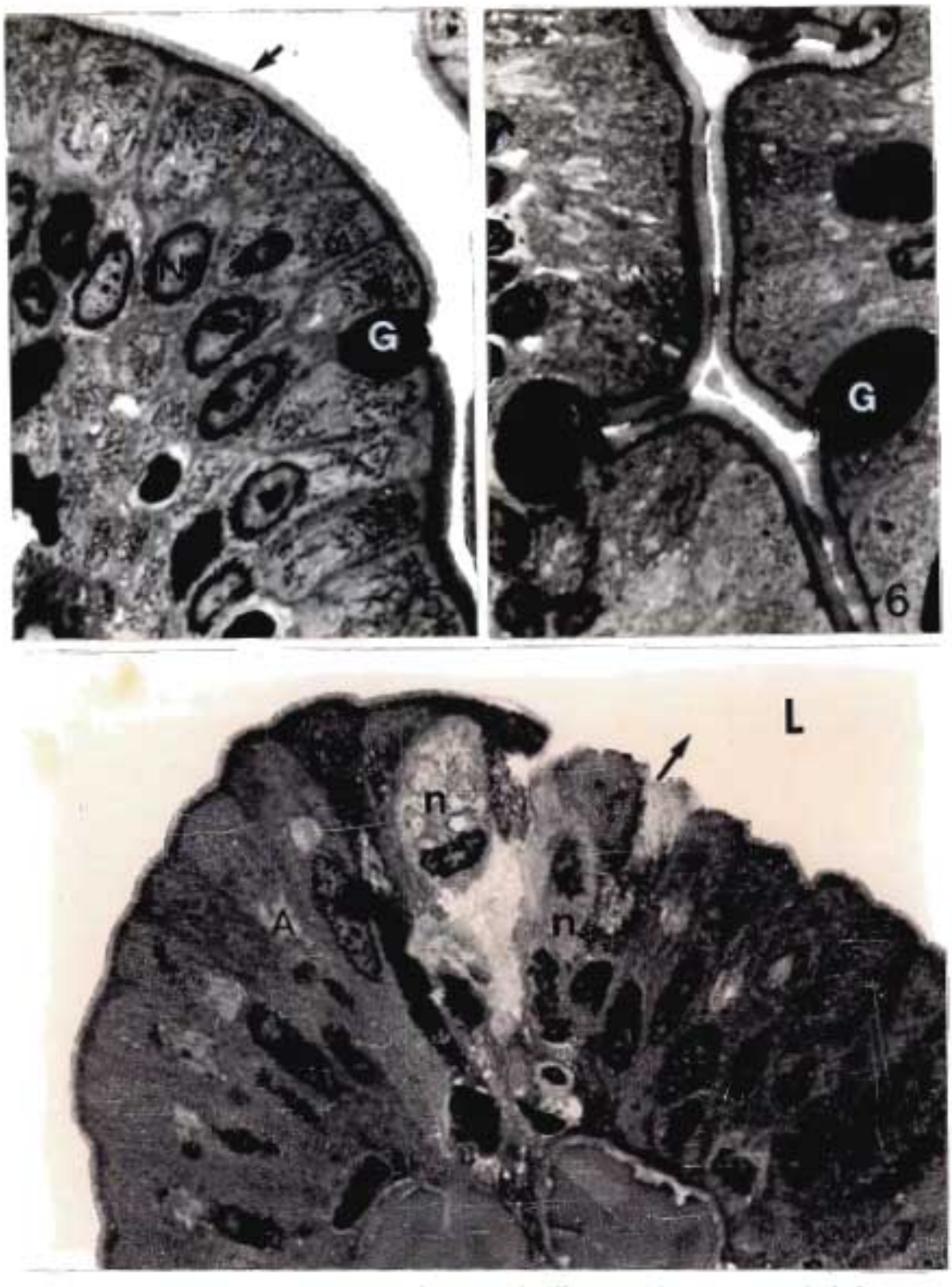
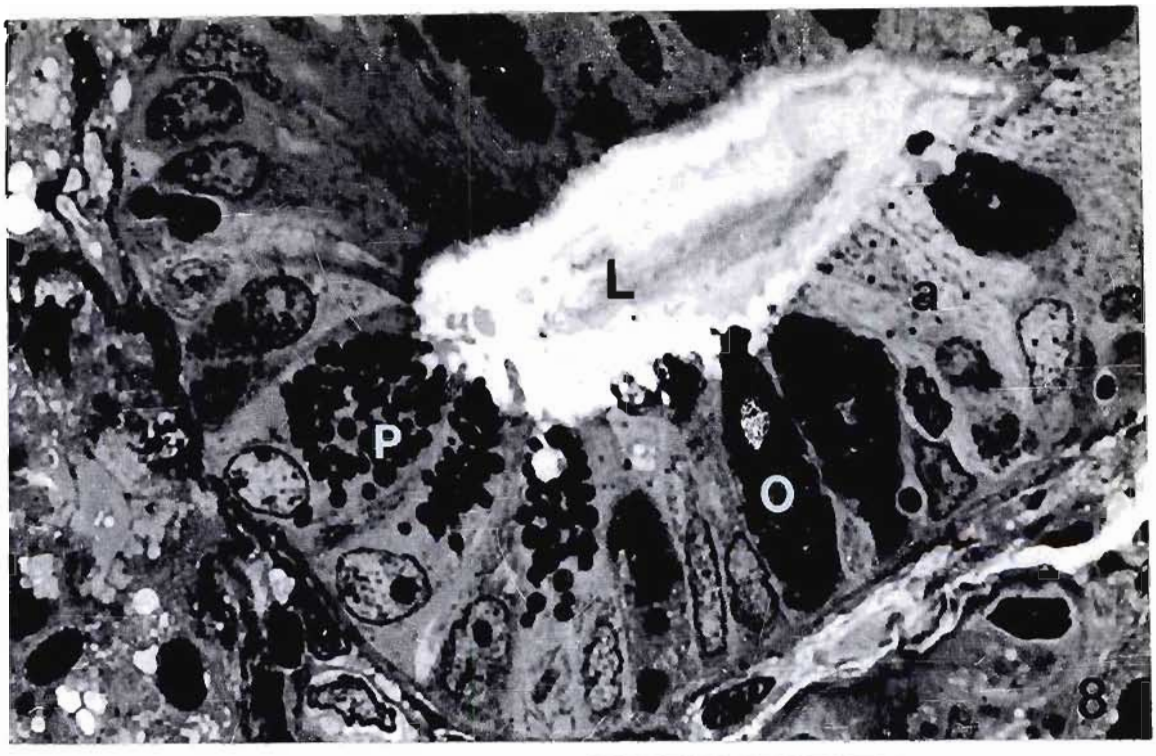
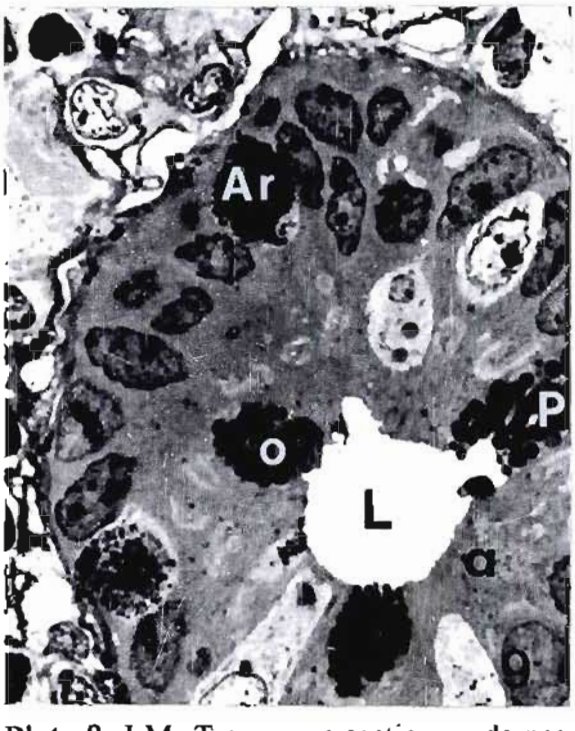


Plate 5: LM of area near to the tip of a normal villus. The brush border is 0.6um in thickness in this region (arrowed). Toluidine blue stain. Magnification X 2100.

Plate 6: LM of area near to the base of a normal villus. The brush border is up to 1.5um in thickness in this region. Toluidine blue. Magnification X 2100.

Plate 7: LM of area at the tip of a normal villus. Occasional necrotic cells(n) are in the process of exfoliation. Toluidine blue. Magnification X 1900.





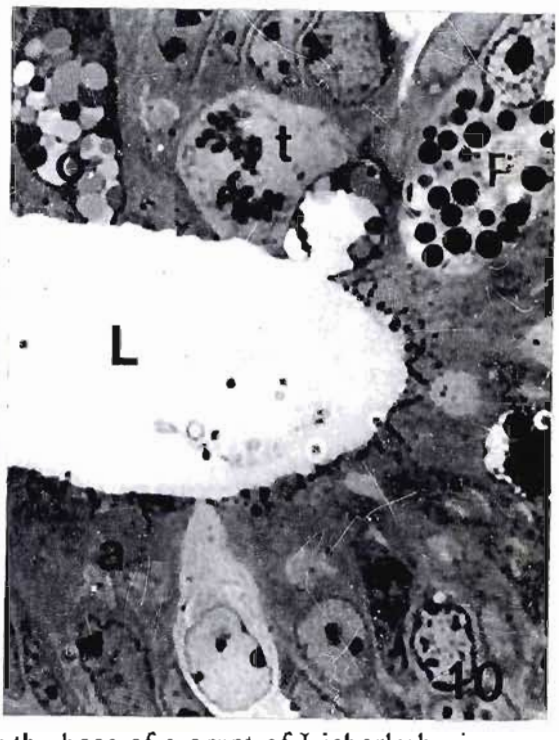


Plate 8: LM: Transverse section made near the base of a crypt of Lieberkuhn in a normal specimen. Numerous Paneth cells (P) are located in this region of the crypt. Also present are immature goblet (o) and absorptive cells (a). Toluidine blue stain. Magnification X 2100.

Plate 9: LM: Transverse section through a normal crypt showing the presence of enteroendocrine cells (Ar). Toluidine blue. Magnification X 1900.

Plate 10: LM: Oblique section through the germinal region of a normal crypt showing a mitotic cell (t), a Paneth cell, and immature goblet and absorptive cells. Toluidine blue. Magnification X 2100.

5.1.2. Electron Microscopy

The villous epithelium was comprised of a monolayer of columnar absorptive and secretory goblet cells attached by hemidesmosomes to a basal lamina. Occasional cells conforming to descriptions of thelio-lymphocytes (Shiner 1983 pp35-38) were present between enterocytes (Plate 11).

Absorptive cells (Plates 11 and 12) contained a single, elongated oval nucleus enclosed by two tri-laminar membranes. Small stacks of Golgi dictyosomes were distributed throughout the supra-nuclear cytoplasm. Elongated mitochondria with well formed cristae, although present throughout the cytosol, were often aggregated near nuclei. Short strands of rough and smooth endoplasmic reticulum (RER and SER respectively) and osmiophilic ribosomes and occasional lysosomes were distributed throughout the cytosol. Occasional multivesicular bodies were present beneath the terminal web (Plate 13).

The apical surface of absorptive cells had a regular layer of microvilli (MV) projecting into the lumen (Plates 11 to 15). Microvilli were bound by a trilaminar plasmalemma and ranged from 0.6μm to 1,5μm in length and from 0,09μm to 0,1μm in diameter. The MV were closely packed, each having a filamentous core that extended into the apical cytoplasm of the enterocyte. Here, cores from neighbouring MV joined in a network of interlacing fibres to form the terminal web. The terminal web was distinct and clear of any cytoplasmic organelles except for a few, small pinocytic vesicles of varying electron densities. The glycocalyx emanating from the outer lamella of the MV plasmalemma appeared as a continuous filamentous coat ranging in thickness from 60nm on the short MV of cells near the tips of villi (Plate 14), to 300nm on the longer MV of cells near the villous base (Plate 15).

Absorptive cells were connected laterally by three types of junctions (Plate 16). The tight junctions (zonular occludens) immediately below the MV were formed by fusion of the outer lamellae of adjacent plasmalemmae. These apparently sealed the intercellular spaces from the luminal environment. Beneath the tight junction, the membranes diverge to a distance of 15nm to 20nm to form the gap junction (zonular adherens). There was an electron density of the cytoplasm lateral to the membranes in this region. The third and most prominent lateral cell junctions were the desmosomes (macula adherens). These appeared as dense plaques composed of 4 membrane leaflets and a gap surrounded by an electron-dense granular material. Below the desmosomes, the lateral cell membranes were seen as parallel interdigitating folds which were divided by spaces of varying width (Plate 17). Absorptive cells were connected by hemidesmosomes to a thin basal lamina (Plate 18).

Goblet cells, aptly named for their goblet shape, were characterised by large numbers of amorphous, moderately electron dense, membrane bound mucous droplets in the apical portion of each cell (Plates 19 and 20). In the paranuclear position, the cells contained a few rounded mitochondria (Plate 19) and numerous lengths of dilated cisternae of RER (Plate 19 - inset). Droplets containing a single non-membrane bound osmiophilic inclusion were often present in the Golgi region. These appeared to coalesce to form larger granules. The more mature mucus droplets near the apex of the cell were less electron-dense and did not contain an osmiophilic inclusion. In some instances, mucus droplets fused prior to exocytosis (Plate 20). In others, intact mucus droplets were discharged into the lumen where the droplet membrane lysed and the mucosubstance became less electron-dense prior to merging with the luminal mucus (Plate 20).

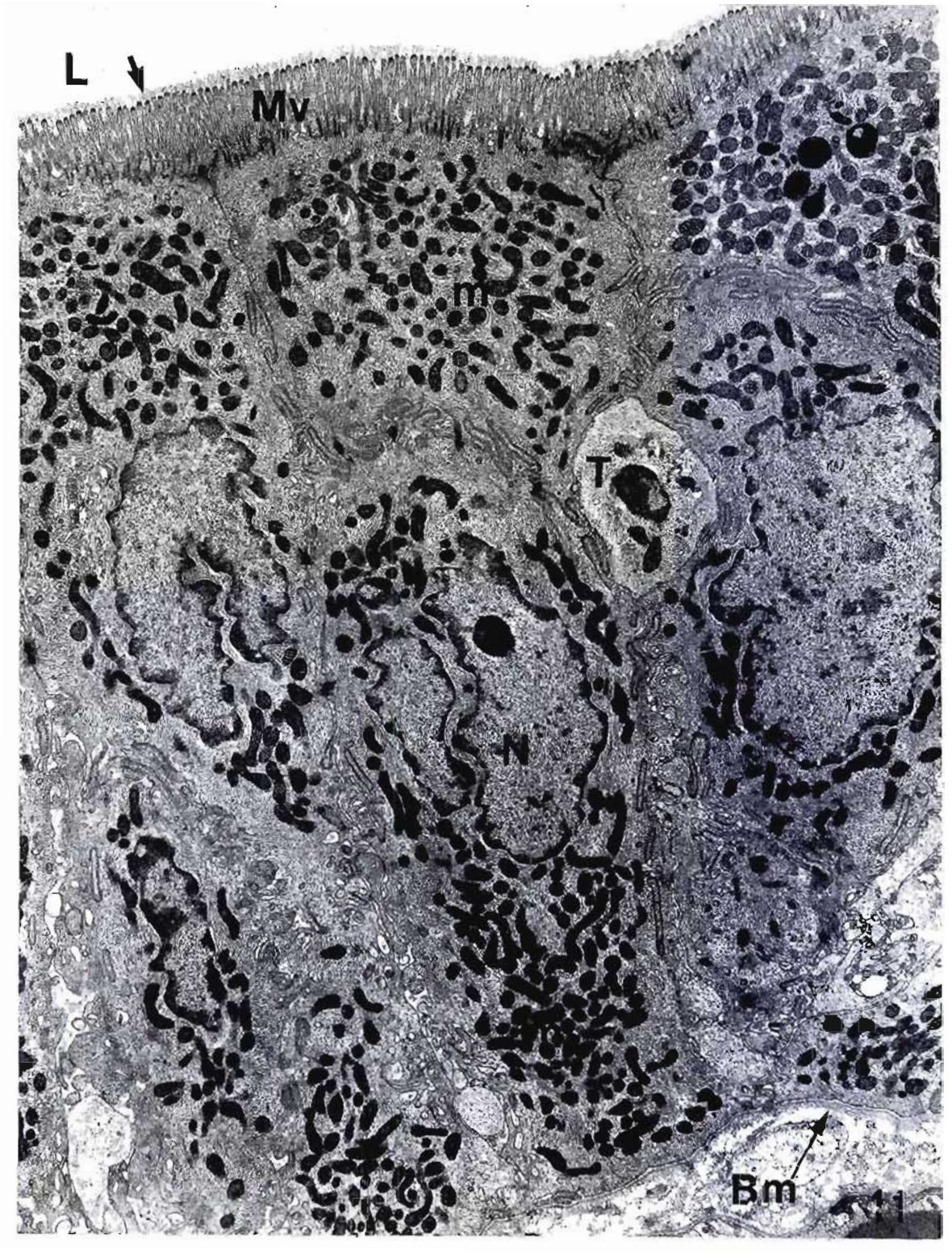


Plate 11: Transmission electron micrograph (EM) of normal absorptive cells. Aggregates of elongated mitochondria (m) are present in both the sub and supranuclear positions. Numerous microvilli (Mv) project into the lumen (L). A well formed glycocalyx (arrowed) emanates from the surface of Mv. A theliolymphocyte (T) is present. Magnification X 10500.

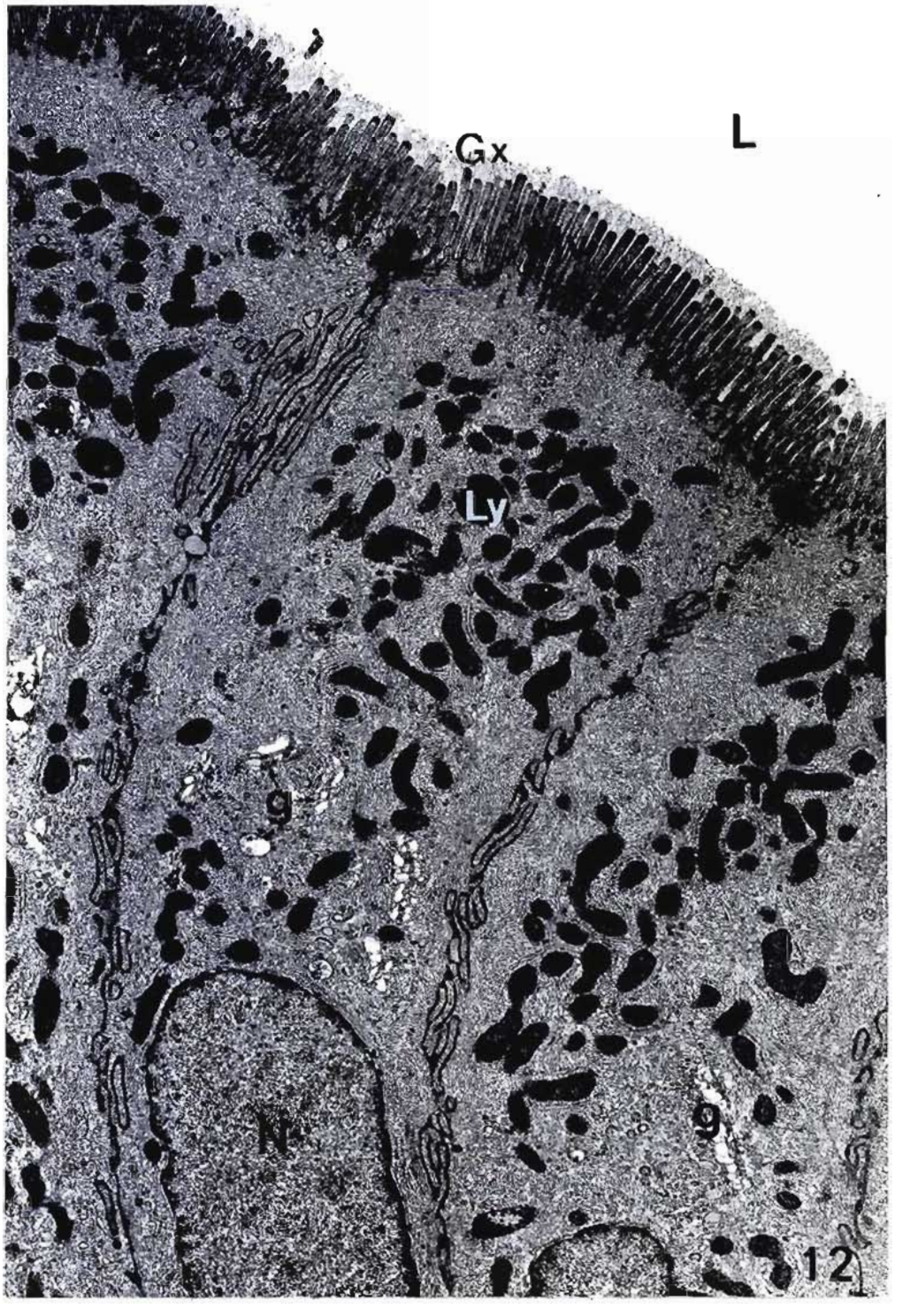


Plate 12: EM of normal absorptive cell. The dictyosomes of the golgi apparatus (g) are situated in the supranuclear position. Occasional lysosomes (Ly) are present near the apex of the cell. The terminal web formed by the osmiophilic "rootlets" of microvilli is clearly demonstrated in this micrograph. Note the interdigitating lateral cell membranes. Magnification X 17000.

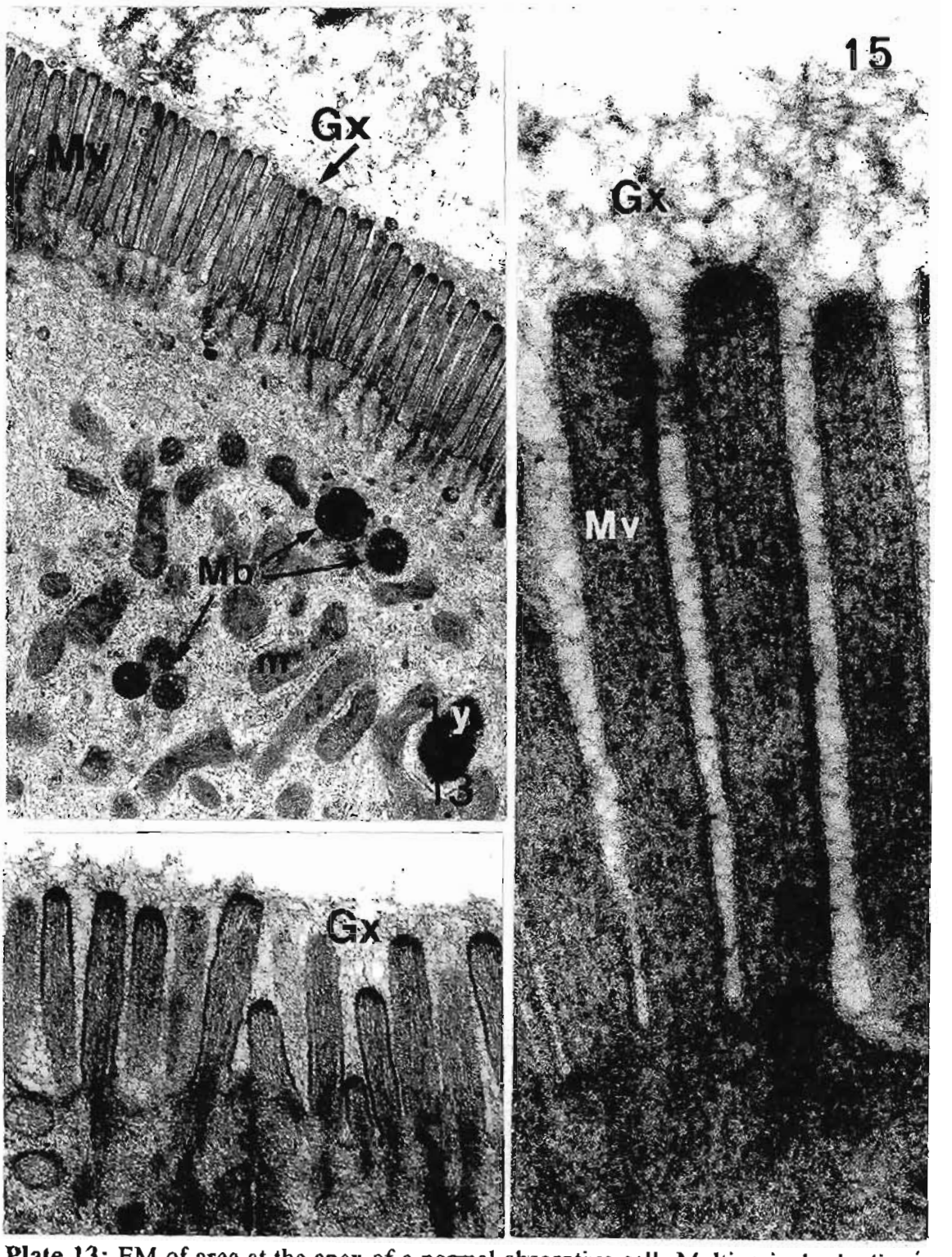


Plate 13: EM of area at the apex of a normal absorptive cell. Multivesicular bodies (Mb) are often present in this region. Magnification X 20000.

Plate 14: EM: Detail of microvilli projecting from an enterocyte near the tip of a villus. The Mv are 0.6um in length and surmounted by a Gx that averages 60nm in thickness. Magnification X 60000.

Plate 15: EM: High magnification of villi showing tri-laminar plasmalemma and longitudinal filaments that comprise the core of each Mv. These Mv project from enterocytes closer to the villous base and have a Gx that is up to 250nm in thickness. Magnification X 150000.

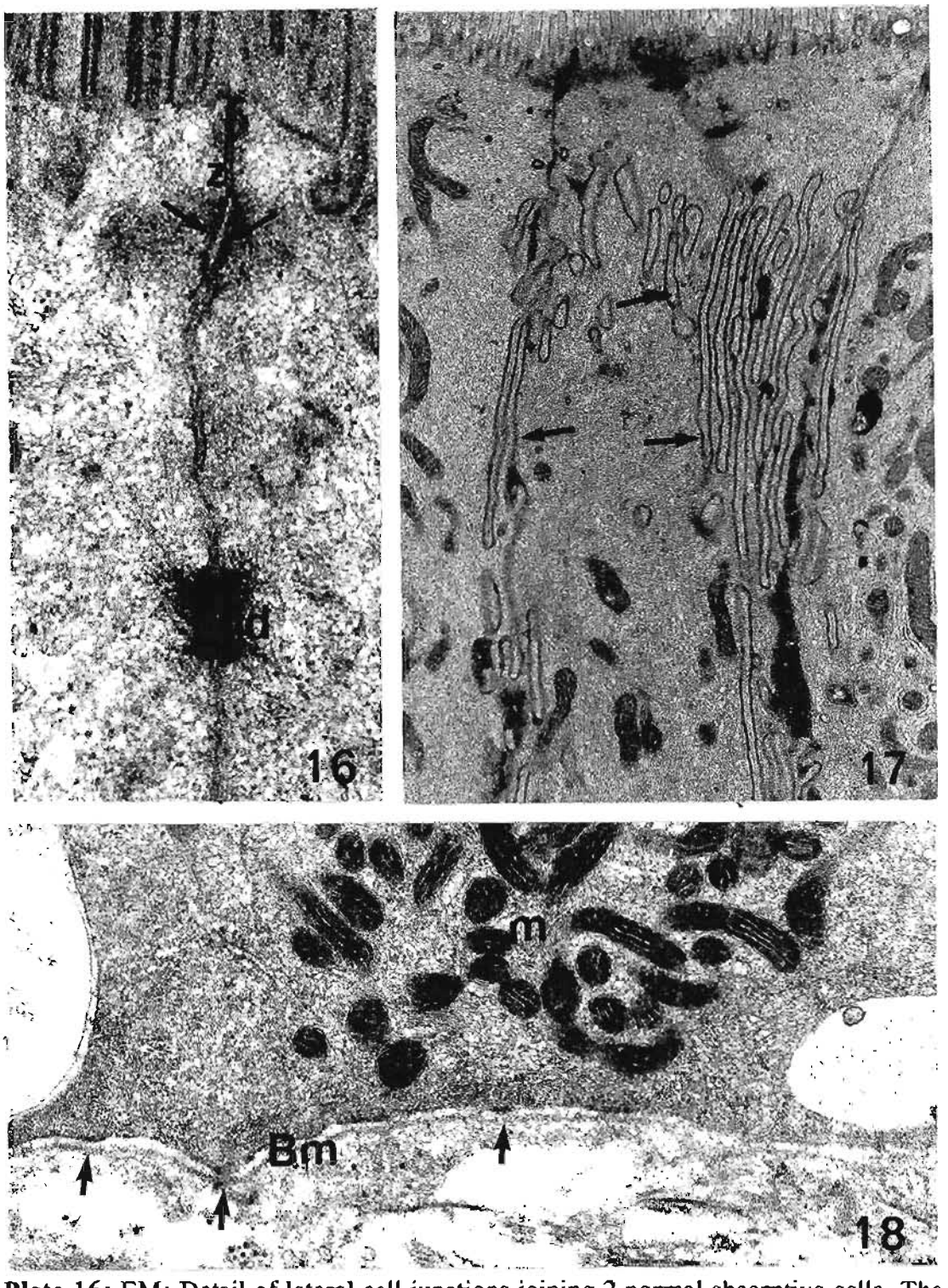


Plate 16: EM: Detail of lateral cell junctions joining 2 normal absorptive cells. The zonular occludens (z) join the cells at the lumen. The plasmalemmae at the zonular adherens (arrowed) are separated by a gap of 20nm. Numerous desmosomes (d) are present along the length of each cell. Magnification X 58000.

Plate 17: EM: Normal absorptive cells. Interdigitating lateral cell membranes. Magnification X 20000.

Plate 18: EM: Normal absorptive cell attached to the basement membrane (b) by hemidesmosomes (arrowed). Magnification X 23000.

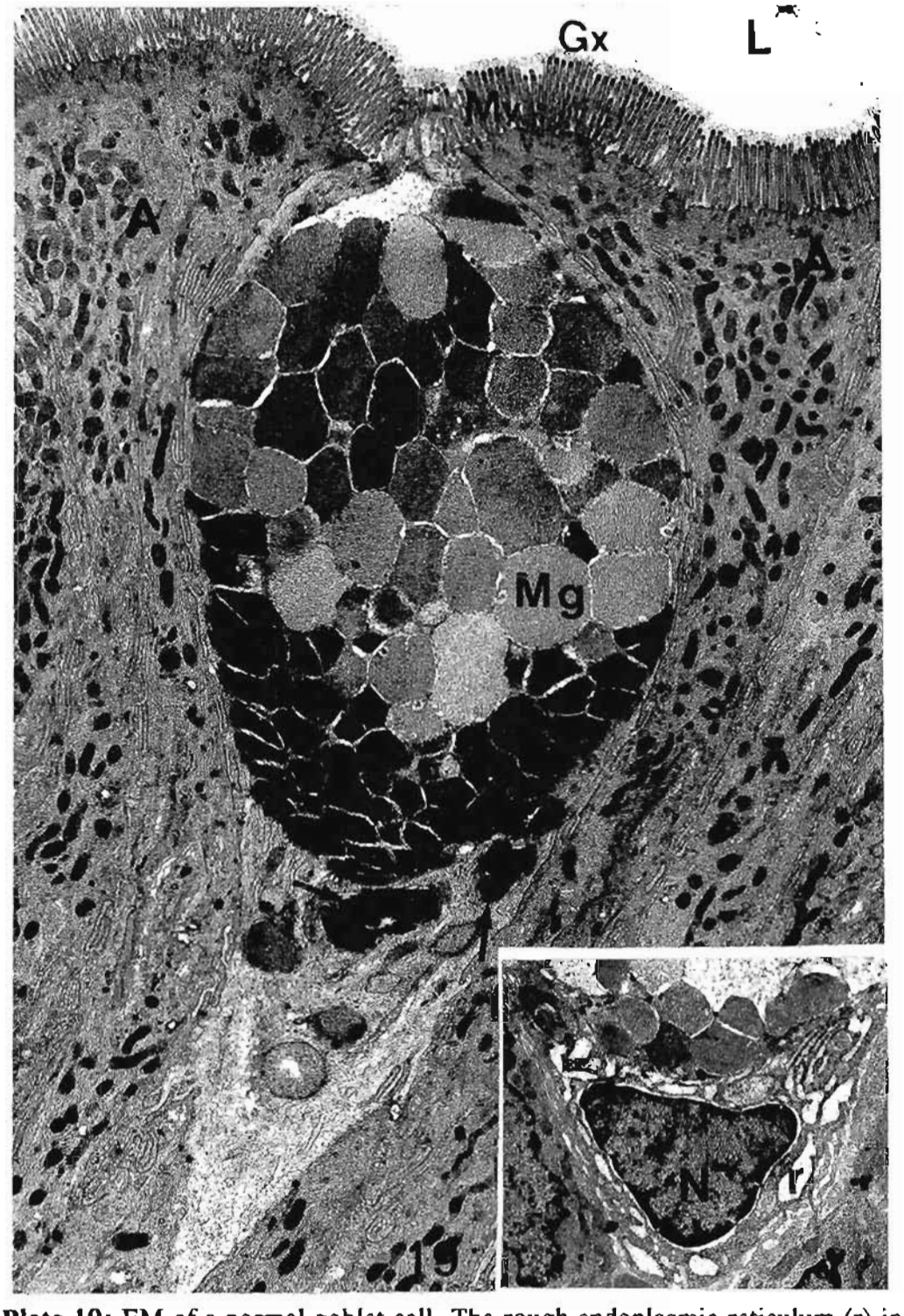


Plate 19: EM of a normal goblet cell. The rough endoplasmic reticulum (r) in the vicinity of nuclei is often dilated in these secretory cells (inset). Note the osmiophilic inclusions in the mucus granules in the region of the golgi apparatus (arrowed). The granules near the apex of the cell do not contain osmiophilic inclusions. Magnification X 8300: Inset: Magnification X 9000.

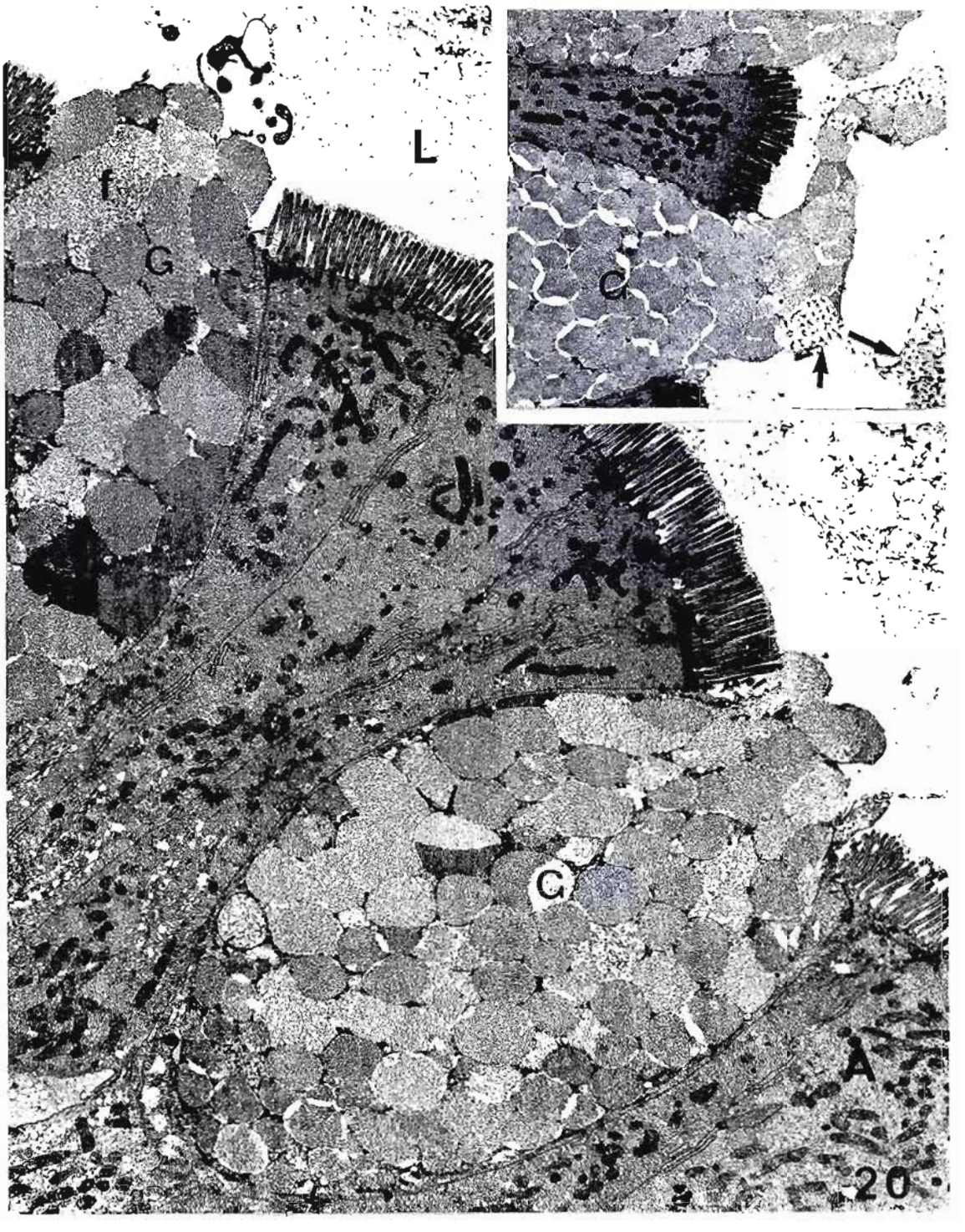


Plate 20: EM: Actively secreting goblet cells in normal mucosa. Prior to exocytosis, in some instances mucodroplets have fused (f) while in others, the droplets are exocytosed intact. After granule membrane lysis, the mucosubstance becomes stippled prior to merging with the luminal mucus (arrowed - inset).
Magnification X 8300; Inset: Magnification X 4500.

In normal specimens, the epithelium of the crypt was primarily populated by precursor cells of villous enterocytes (Plates 21 to 25). The undifferentiated absorptive cells each contained a single elongated basal nucleus, were up to 21μm in length and tapered from 3μm to 5μm in width at the base to approximately 2μm at the apex. The cells generally contained a few small granules of varying electron densities within their cytosol (Plates 21 and 22). The apex of each cell had a number of short (0.5μm to 0.8μm) microvilli projecting into the lumen from which emanated a thin, discontinuous glycocalyx.

Paneth cells were primarily situated near the base of crypts. These cells contained a single, round basal nucleus, were up to 22μm in length and approximately 7μm in width (Plate 21). Paneth cells were characterised by the presence of numerous large, membrane-bound, osmiophylic granules within the supranuclear cytoplasm. No microvilli were seen projecting from the surface of these cells.

Enteroendocrine cells were distributed throughout the length of the crypt. They each contained a single, round central/basally situated nucleus, were up to 22μm in length and approximately 7μm in width at their widest point (Plates 21 and 22). These cells were characterised by the numerous small granules of varying electron densities situated in the sub-nuclear cytosol. In some cases, enteroendocrine cells had numerous densely packed, apical microvilli that ranged in length from 1.2μm to 1.5μm. No obvious glycocalyx emanated from these microvilli (Plate 21).

Immature goblet cells were distributed throughout the mid/upper regions of each crypt of Lieberkuhn (Plate 22).

Most were similar in size and appearance to goblet cells on villi. In keeping with earlier descriptions (Ham and Cormack 1979 p.682), approximately 50% of immature goblet cells contained mucus droplets within which was a single, dense osmiophilic inclusion.

The germinal regions of each crypt contained numerous "stem" cells. These cells contained a single, round/oval nucleus, were up to 18um in length and ranged from 4μm to 7μm in diameter (Plate 23). Stem cells were generally devoid of secretory material, mitochondria were sparse and there were only occasional strands of RER and SER in a ribosome rich cytosol. Stem cells undergoing mitosis were rounded, up to 10μm in diameter and had short, sparse microvilli on their luminal surface (Plates 24 and 25). These cells were devoid of secretory material and had an abundance of ribosomes in the cytosol.

The columnar acinar cells of the Brunner's glands had basal oval nuclei, were up to 6μm in width and 12μm in length. Numerous pale staining secretory granules, each frequently containing a non membrane-bound, osmiophilic inclusion, were situated in the supranuclear position (Plate 26). The cells bordering the ducts of the glands of Brunner were cuboidal to columnar in shape, approximately 10μm in width and 14μm in length (Plate 27). They contained a large, round basal nucleus and were typified by the presence of small aggregates of granules at their apices. Short (0.3μm) microvilli projected into the lumen.

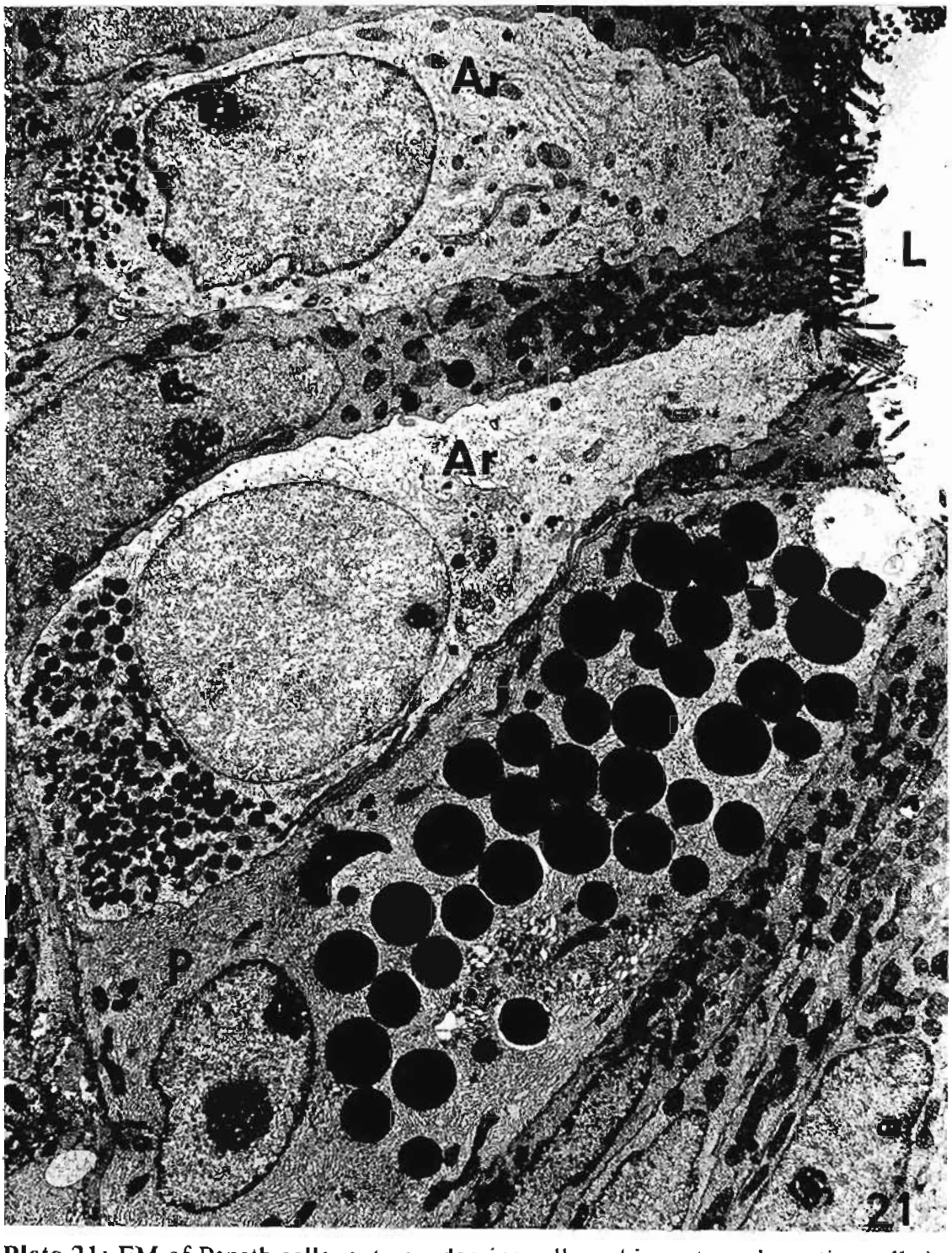
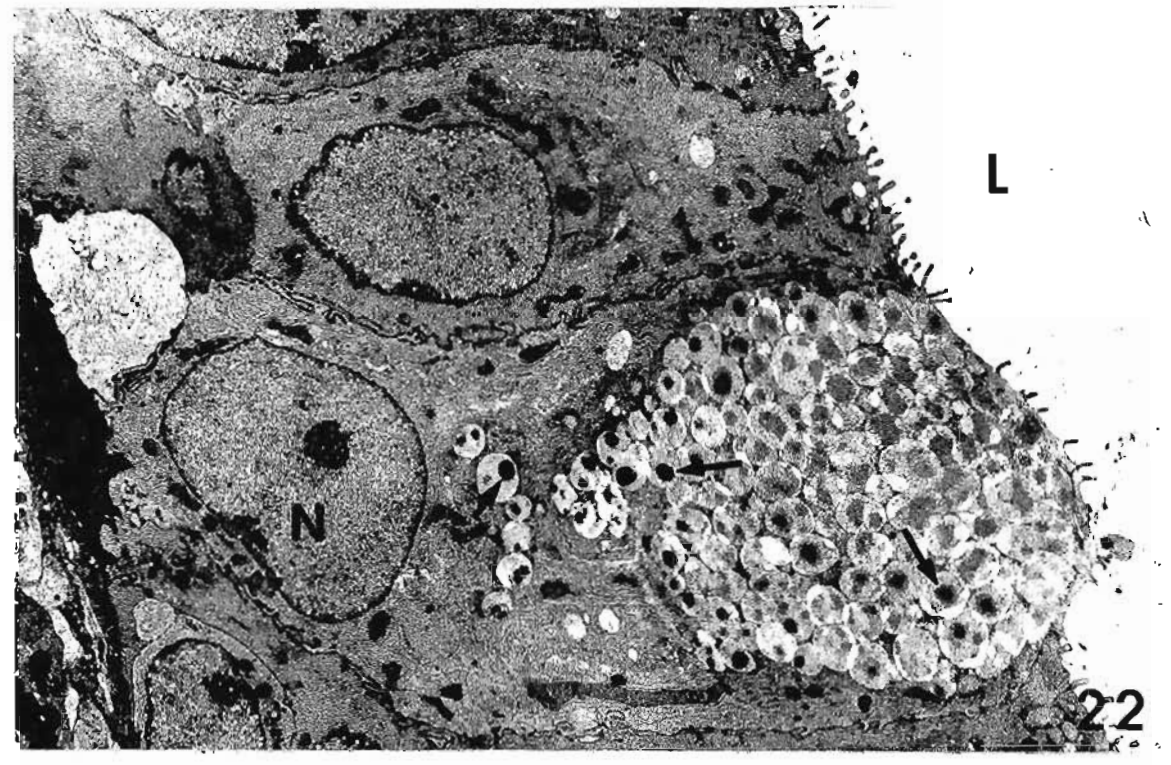


Plate 21: EM of Paneth cells, enteroendocrine cells and immature absorptive cells in the normal crypts of Lieberkuhn. Paneth cells are characterised by their osmiophilic granules in the supranuclear position. Enteroendocrine cells have less electron dense granules in the subnuclear region. Immature absorptive cells contain occasional osmiophilic droplets throughout their cytosol (arrowed). Note the numerous microvilli projecting from enteroendocrine cells.
Magnification X 8000.



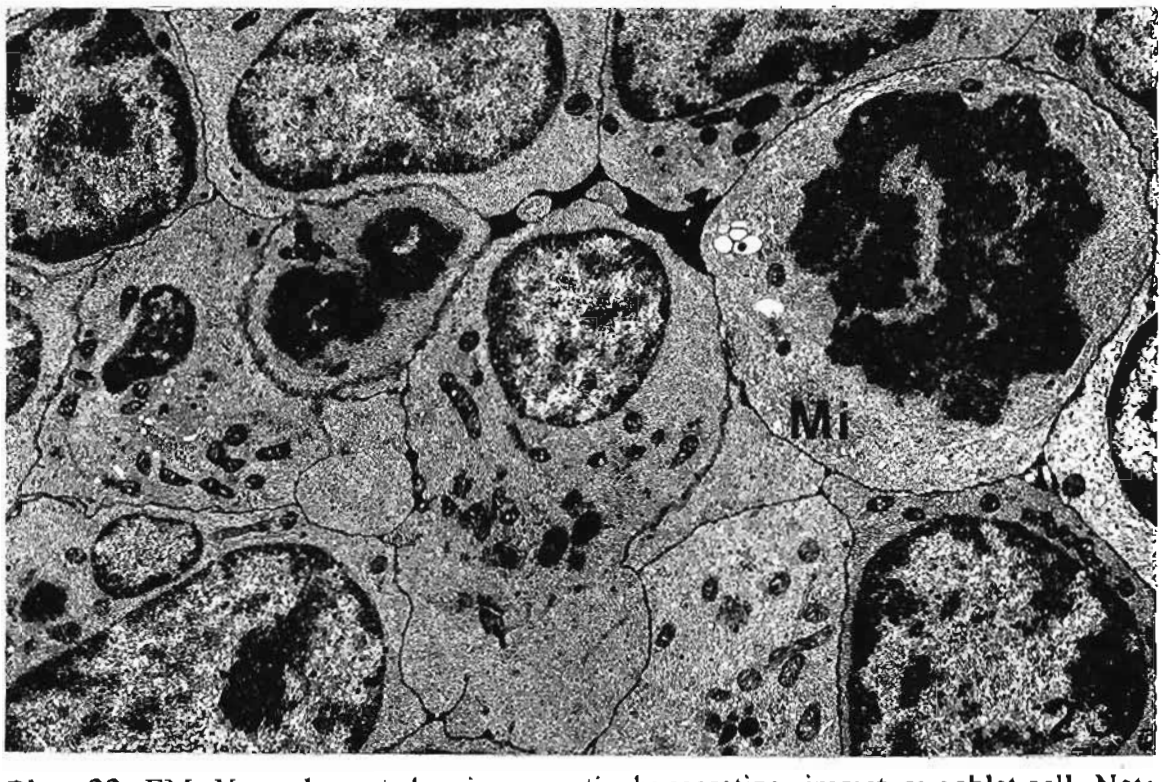


Plate 22: EM: Normal crypt showing an actively secreting, immature goblet cell. Note mucus granules within which is a single osmiophilic inclusion (arrowed). Magnification X 9000.

Plate 23: EM: Oblique section through the germinal region of a normal crypt. The stem cells are tightly packed together. A cell is undergoing mitosis (Mi). Magnification X 9000.

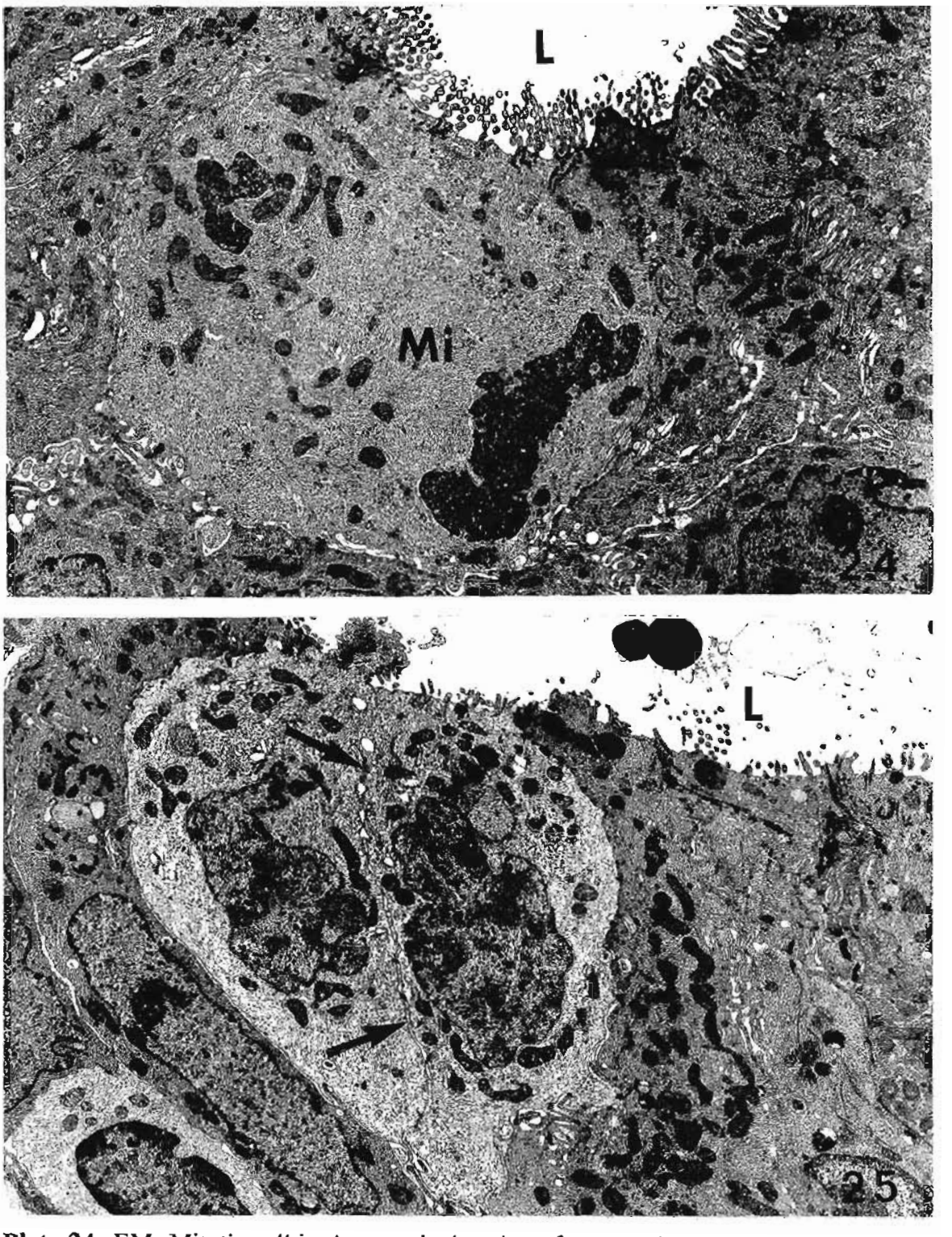


Plate 24: EM: Mitotic cell in the germinal region of a normal crypt. Magnification X 10000.

Plate 25: EM: A stem cell undergoing division in the germinal region of a normal crypt. Note membrane forming between the daughter cells (arrowed). Magnification X 10000

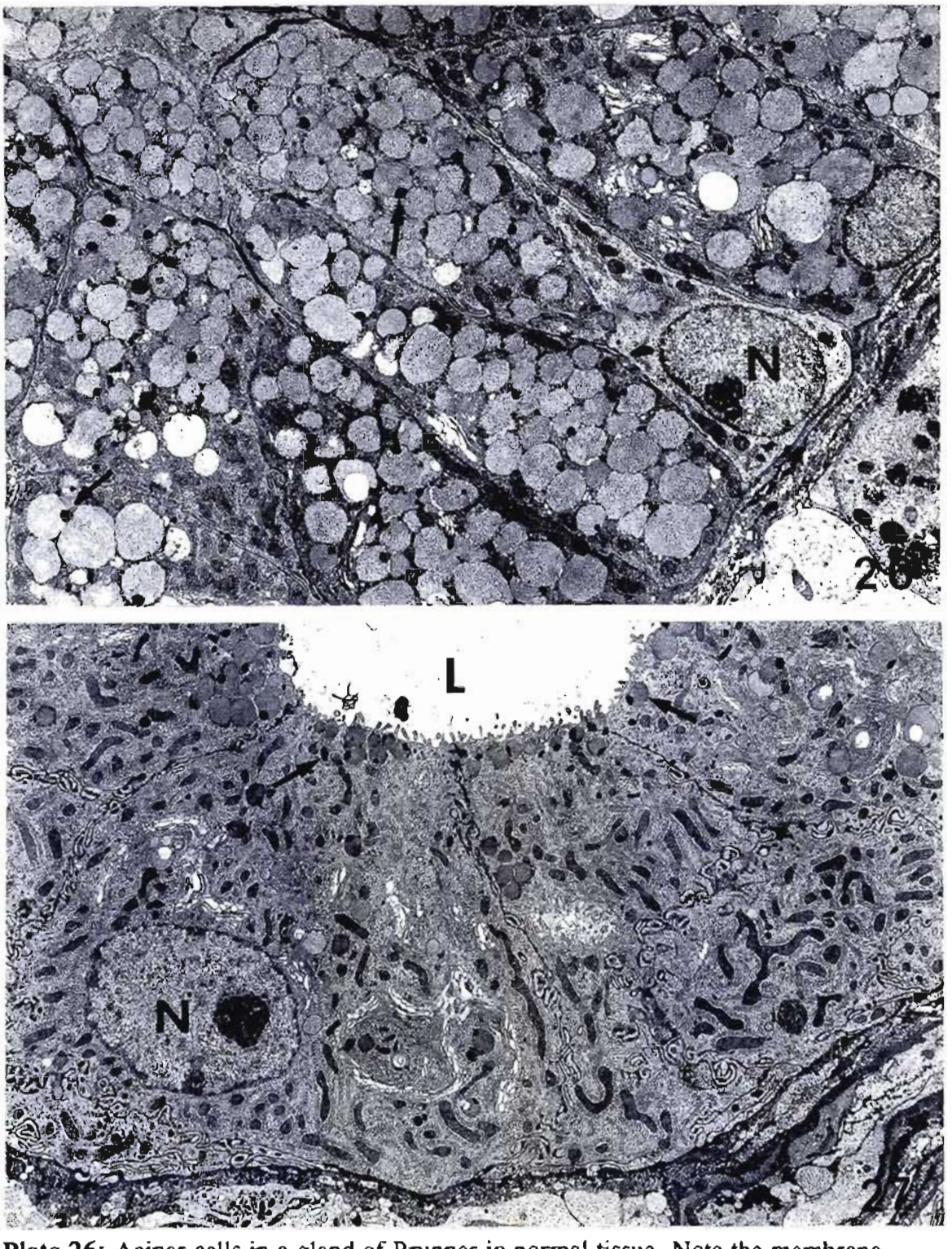


Plate 26: Acinar cells in a gland of Brunner in normal tissue. Note the membrane-bound osmiophilic inclusions within most mucus droplets. Magnification X 8000.

Plate 27: Cuboidal cells lining the duct of a Brunner's gland. Note the reduced numbers of secretory droplets near the apex of each cell. Magnification X 5500.

5.2. Determination of Optimal Biopsy Position

Based on similarity of morphology, the biopsies were grouped as follows: Position A - the edge and up to 3mm from the DU; postion B - 4mm to 8mm from the DU; position C - 12mm to 50mm from the DU. A summary of the variations in mucosal morphology at increasing distances from the edge of DU is described in Figure 1.

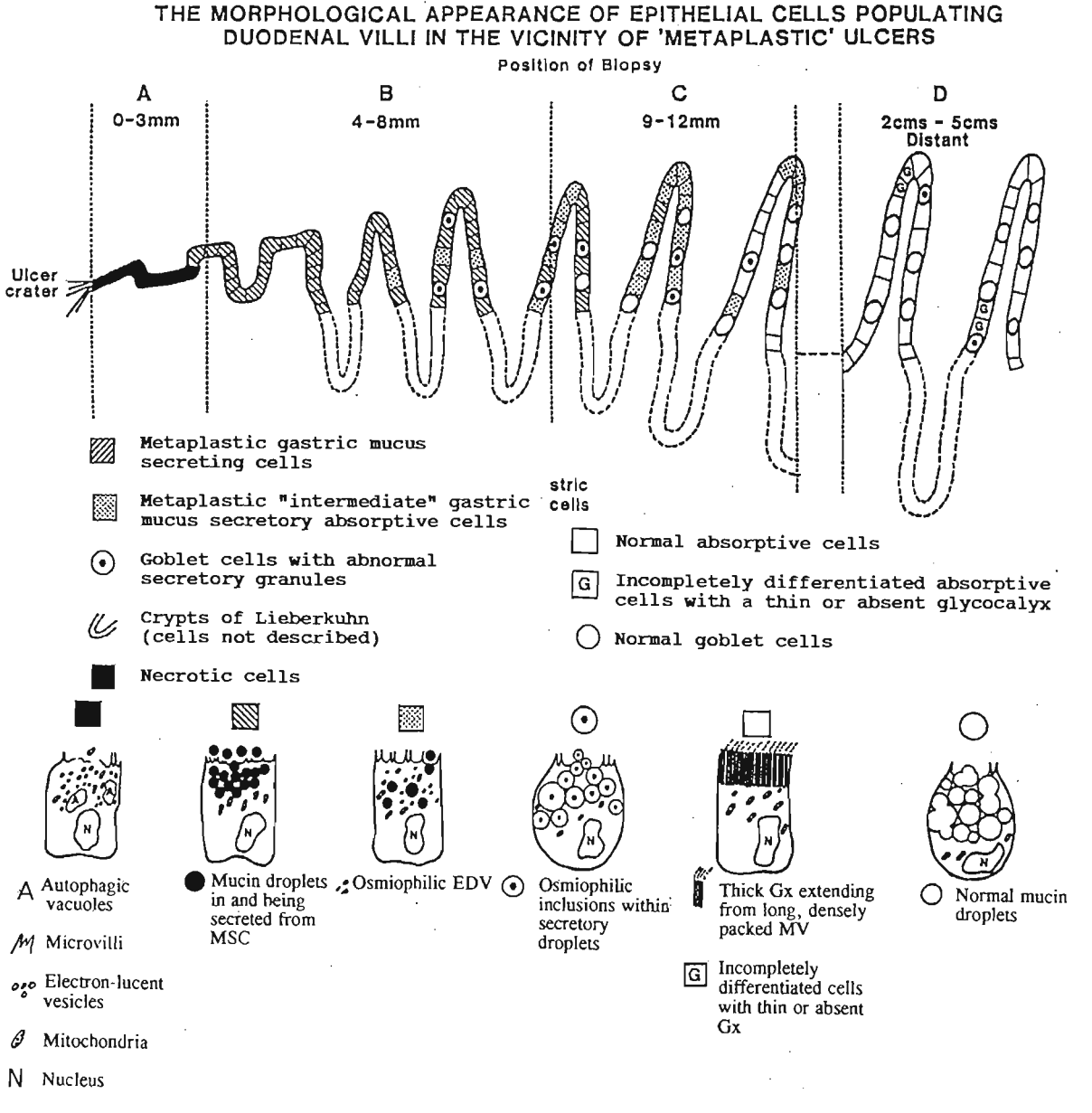


Figure 1: The morphological appearance of epithelial cells populating duodenal villi in the vicinity of DU surrounded by metaplastic mucosa.

Position A: In biopsies from within 3mm of the DU, villi were absent and the epithelium was populated with necrotic and vacuolated cells (Plates 28 to 30). The mucosa in biopsies from the edge of DU was exclusively necrotic and had been invaded by numerous inflammatory cells (Plate 28). The larger part of the epithelium contained vacuolated cells and cells within which were large inclusion bodies (Plate 29). Groups of vacuolated cells appeared to have fragmented from the epithelium into the lumen (Plate 30).

Position B: All specimens 4-8mm from the edge of the DU crater had atrophic villi or undulating mucosal surfaces and were populated with metaplastic columnar mucus secreting cells (MSC) similar to gastric epithelial cells in various phases of secretory activity and/or stages of metaplastic differentiation (see 5.8.1. p.138). Two specimens were exclusively populated with well differentiated MSC (Plate 31). Four specimens, while primarily populated with MSC in all stages of metaplastic differentiation, had patches of apparently normal absorptive and goblet cells on some villi (Plate 32). The absorptive cells, however, did not have a brush border (Plate 33). By electron microscopy, the apices of these cells were seen to contain numerous vesicles and occasional secretory droplets (Plate 34). The Golgi apparatus was active and the RER dilated. Mitochondria were small, sparse, electron lucent and rounded and the microvilli, where present, were short.

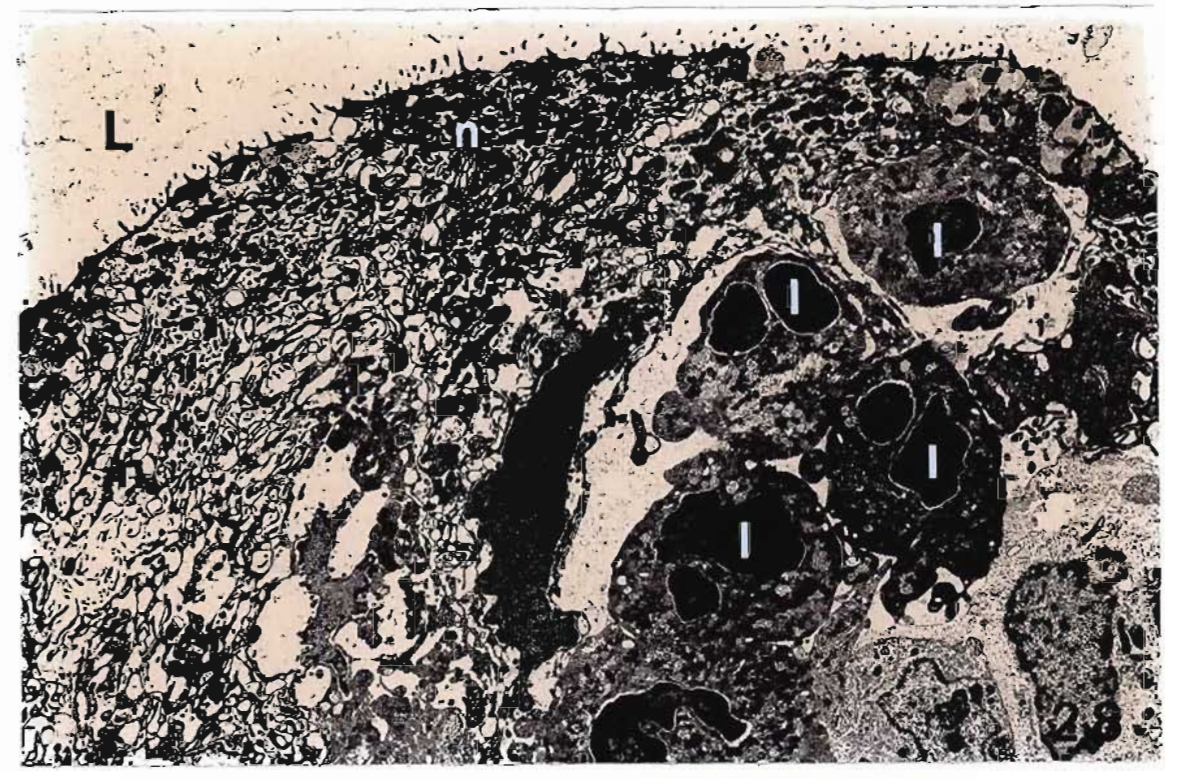
Positions C and D: The villi were atrophic in three specimens 9-12mm from the edge of the crater and normal in one (5cm from edge). The majority of cells populating the mucosa were morphologically normal. Occasional patches of well and/or partially differentiated MSC were seen in one specimen at 12mm and one at 5cm from the DU. The remaining

specimens had villi of normal length and were populated with absorptive and goblet cells of normal appearance.

The morphological evidence, especially that obtained from position B, suggests a sequential transformation in structure and secretory activity of absorptive cells to MSC and perhaps vice-versa. That is, there is a concommitant reduction in the length and number of MV for absorption as cells show signs of increasing secretory activity (Plates 31 - 34). The variation in mucus content may indicate phases in the synthesis storage and secretion of mucus and/or indicate various stages in the development of MSC. The latter premise is examined in more detail in 5.8. below.

It is interesting to note that the degree of metaplastic differentiation decreased with increasing distance from the DU crater. N.b. Near the DU (position B), the number of well differentiated MSC and cells in an intermediate stage of metaplastic differentiation were higher than those in positions C and D. As MSC are thought to occur as a protective response to low luminal pH (see 2.3. p.35), the presence of fully differentiated MSC near the crater suggested that the DU crater was either a focus of acid attack and/or these cells had developed to protect the damaged mucosa. These and other premises are discussed in depth in Chapter 8.9 (pp.230-237).

To summarise, within 3mm of the ulcer crater, the enterocytes were frequently necrotic. At 12mm from the edge of each DU the mucosa was essentially normal. The region that showed the most consistent changes were at 4mm and 8mm from the DU. It was therefore decided to obtain biopsies for subsequent studies, 4-8mm from the edge of the crater.



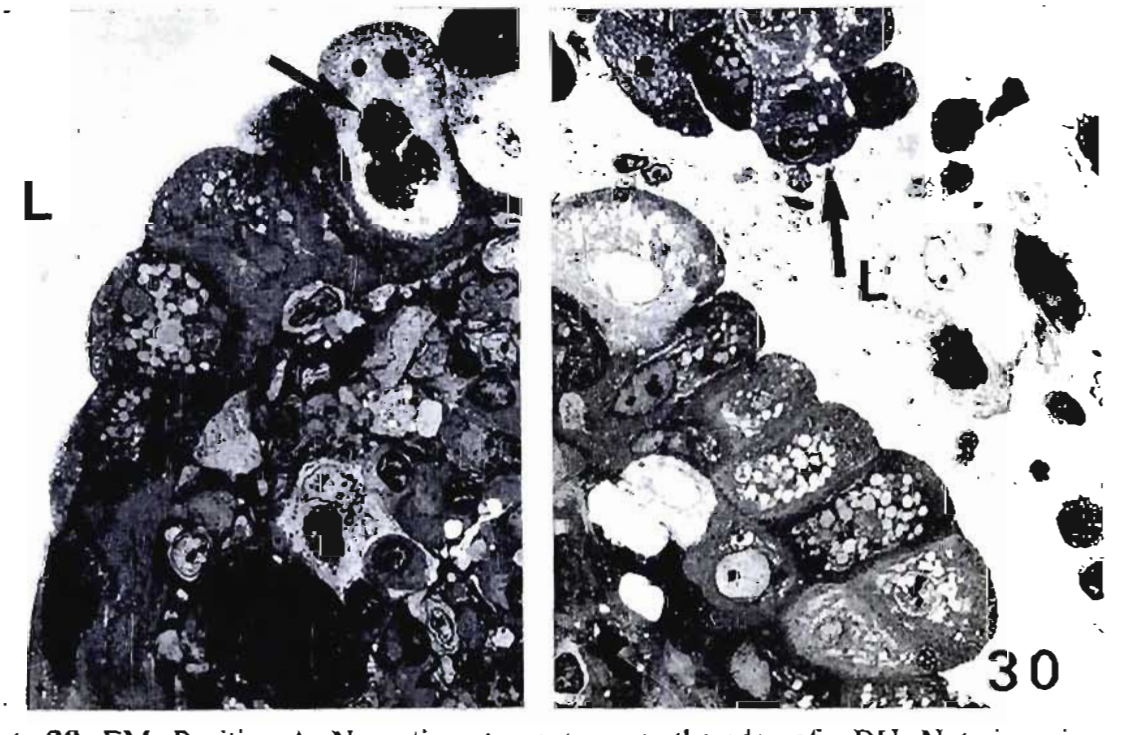
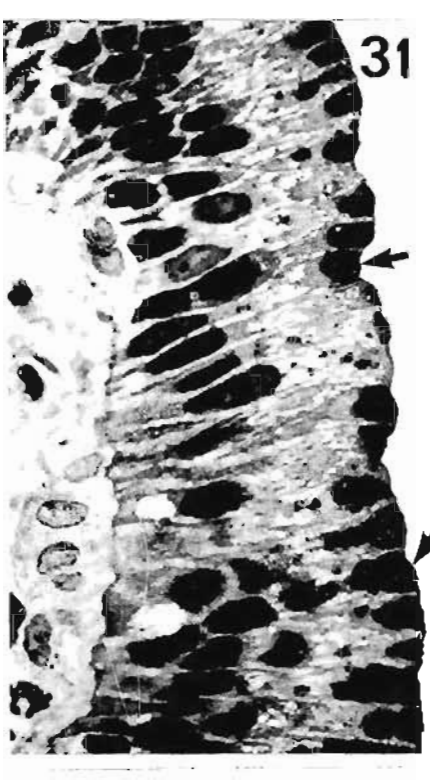
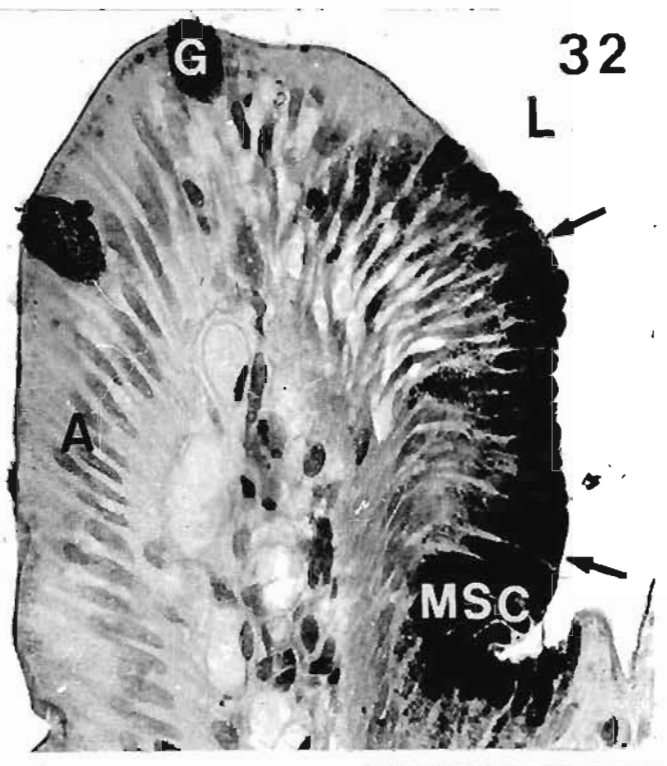


Plate 28: EM: Position A: Necrotic enterocytes near the edge of a DU. Note invasion of inflammatory cells (I) into the mucosa. Magnification X 4000.

Plate 29: LM: Position A: Less necrotic area within 3mm of the edge of a DU. The enterocytes are vacuolated and contain large inclusion bodies. Toluidine blue stain. Magnification X 1500.

Plate 30: LM: Position A: Vacuolated cells fragmenting from the epithelium. Toluidine blue stain. Magnification X 1500.





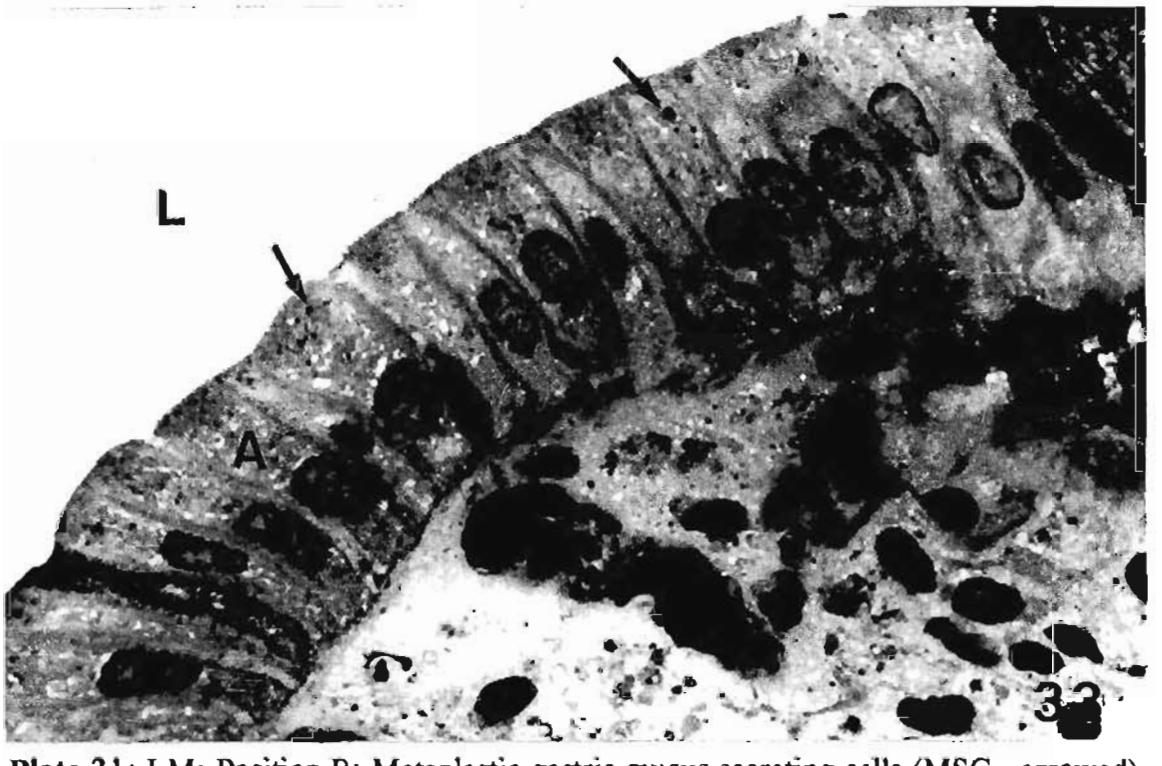


Plate 31: LM: Position B: Metaplastic gastric mucus secreting cells (MSC - arrowed) lining the surface of a villus. Toluidine blue stain. Magnification X 750.

Plate 32: LM: Position B: MSC in a region of a villus. The remainder of the villus appears normal and contains goblet and absorptive cells. PAS stain. Magnification X 800.

Plate 33: LM: Position B: Detail of apparently normal absorptive cells in non-metaplastic areas. Note absence of brush border and inclusions near the apex of each cell (arrowed). Toluidine blue stain. Magnification X 1800.

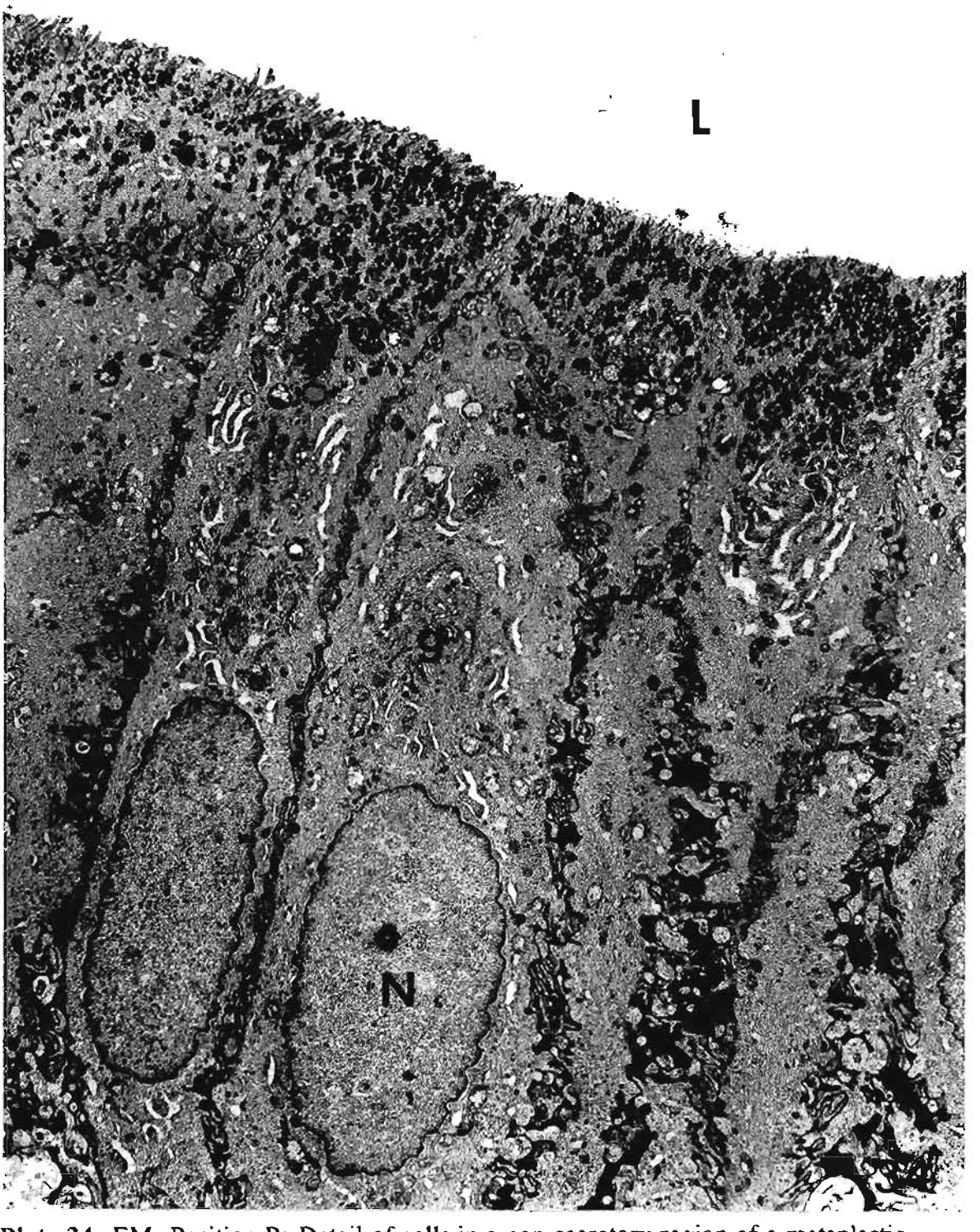


Plate 34: EM: Position B: Detail of cells in a non-secretory region of a metaplastic mucosa. The cells contain numerous electron dense vesicles at their apices. Microvilli are short, sparse or have been eroded from the surface. The RER is dilated. Magnification X 10000.

5.3. Ulcerated Duodenal Mucosa - Prior to Drug Therapy

5.3.1. Endoscopic Appearance of the Duodenal Mucosa

A total of 168 paired biopsies from 84 patients were evaluated by both light and electron microscopy prior to therapy with either cimetidine, sucralfate, misoprostol or pirenzipine. Figure 2 shows that biopsies were taken from within 8mm of 23 large, deep DU with a severity level (SL) determined by endoscopy of 4; 9 from DU with a SL of 3; 13 from DU with a SL of 2; 18 from DU with a SL of 1 and 21 from small DU (SL-0). Forty-five DU were graded by endoscopy as "severe" (SL:4;3;2) and 39 "moderate" (SL:1;0).

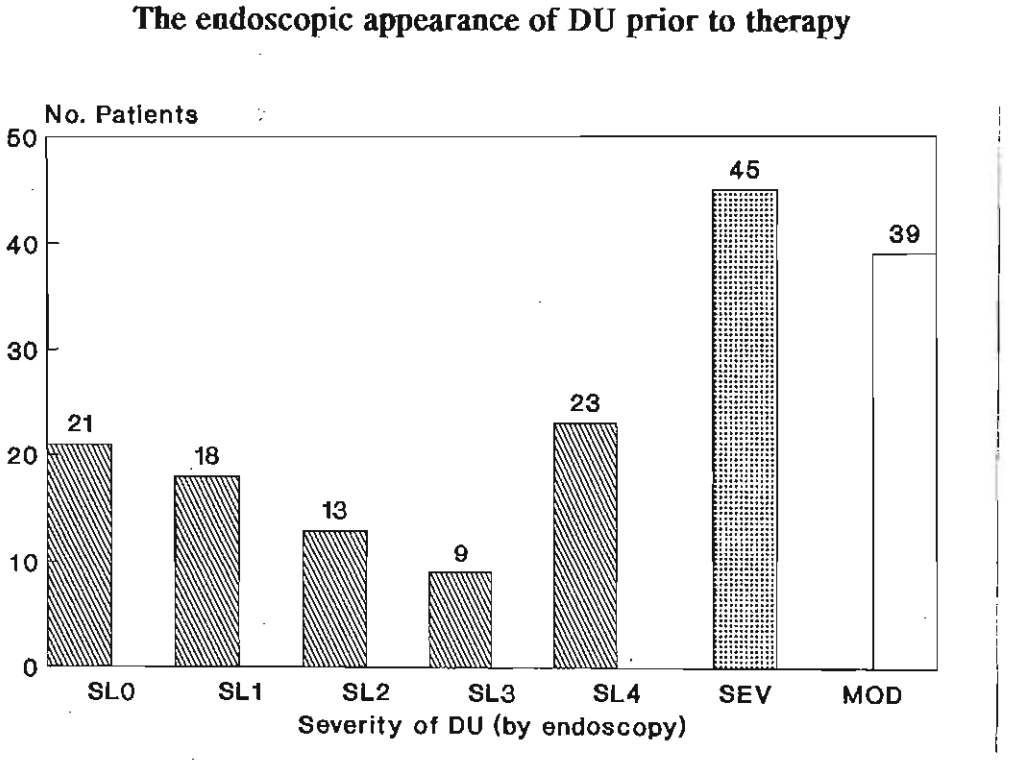


Figure 2: The appearance of 84 DU as determined by endoscopy. The endoscopic severity (SL) of each lesion is graded as per Table 1 (SL:0-4). There were 45 lesions graded as **SEV**ere (SL:4;3;2) and 39 graded as **MOD**erate (SL:1;0).

5.3.2. Light Microscopy

On the basis of the shape of villi, distribution of cell types and presence of PAS+ mucosubstance contained within villous epithelial cells, the 84 pre-therapy specimens prepared for histological evaluation were divided into two groups: those that showed evidence of gastric metaplasia (Group 1) and those that did not (Group 2) (Table II). Sixty one specimens (73%) exhibited varying degrees of gastric metaplasia and 23 (27%) appeared non-metaplastic.

Group 1: Metaplastic specimens ranged from those exclusively populated with well differentiated MSC, to those where MSC were limited to a small area on one or more villi (Plates 35 to 42). Where the mucosa was exclusively populated with well differentiated MSC, villi were generally atrophic (Plates 35 to 38). In some cases, MSC exclusively populated the epithelium that lined both the villi and the crypts of Lieberkuhn. (Plates 36 to 38). In all specimens exclusively populated with well differentiated MSC, large quantities of mucus was secreted into the duodenal lumen (Plate 38).

Where the mucosa was not exclusively populated with well differentiated MSC, the epithelium contained columnar cells in all phases of secretory activity and stages of gastric metaplastic differentiation (see 5.2. p77). As in position B (p76), cells in intermediate stages of metaplastic differentiation were characterised by a thin or absent brush border and contained varying quantities of secretory droplets near their apices (Plates 39-41). These cells were interspersed with varying numbers of goblet cells (Plates 39-42). A few specimens categorised as metaplastic were essentially normal histologically with only small areas of metaplasia on a few villi (Plate 42).

Table II

P	PATIENT DATA EM	GI.No	D	H±	ESL SL	S/M	DH	HISTO PT	TT
1	898	20079	S	+	3	S	-	1	1
2	928	20772	S	+	0	M	-	1	2
3	1061	22399	S	+	4	S	-	1	2
4	1068	22519	S	+	4	S	-	1	1
5	1097	22727	S	+	0	M	-	1	1
6	1146	23530	S	+	4	S	-	1	2
7	875	19543	S	+	0	M	-	2	1
8	894	20009	S	+	0	M	-	1	2
9	901	20171	S	+	4	S	-	2	2
10	1077	22667	S	+	0	M	-	2	1
11	897	20054	C1	+	0	M	-	1	1
12	906	20281	C1	+	4	S	-	1	1
13	967	21233	C1	+	4	S	-	1	1
14	1095	22724	C1	+	1	M	-	1	2
15	889	19840	C1	+	1	M	-	2	2
16	893	20007	C1	+	0	M	-	1	2
17	902	20195	C1	+	2	S	-	1	1
18	968	21234	C1	+	3	S	-	1	2
19	1123	23246	C1	+	0	M	-	1	1
20	1147	23531	C1	+	3	S	-	1	ns
21	1447	27886	C3	+	1	M	-	1	-
22	1446	27882	C3	+	2	S	-	1	-
23	1448	27890	C3	-	2	S	1	1	-
24	1450	27903	C3	-	1	M	0	1	-
25	1488	29105	C3	-	4	S	3	1	-
26	1494	29294	C3	-	0	M	0	2	-
27	1495	29300	C3	+	0	M	-	1	-
28	1500	29396	C3	+	2	S	-	1	-
29	1502	29429	C3	+	2	S	-	2	-
30	1443	27757	P	-	4	S	1	2	-
31	1462	28071	P	-	3	S	1	2	-
32	1466	28419	P	-	3	S	3	1	-
33	1481	28762	P	+	3	S	-	1	-
34	1482	28835	P	+	0	M	-	2	-
35	1534	29042	P	-	4	S	3	2	-
36	1489	29174	P	-	1	M	1	1	-
37	1492	29248	P	-	2	S	1	2	-
38	1493	29250	P	-	2	S	1	1	-
39	1501	29428	P	+	3	S	-	1	-
40	1225	24657	HM	+	0	M	-	1	1
41	1318	25551	HM	+	1	M	-	1	1
42	1357	26013	HM	+	1	M	-	2	1
43	1391	26429	HM	+	0	M	-	1	1
44	1396	26510	HM	+	1	M	-	1	2
45	1384	26379	HM	+	2	S	-	1	1
46	1385	26329	HM	+	0	M	-	1	1
47	1389	26412	HM	-	2	S	0	2	-
48	1248	24769	HM	-	2	S	1	1	-
49	1241	24709	HM	-	4	S	0	2	-
50	1370	26130	HM	-	4	S	0	2	-
51	1244	24692	LM	+	2	S	-	1	1
52	1280	25053	LM	+	0	M	-	1	1
53	1286	25080	LM	+	4	S	-	1	2
54	1336	25855	LM	+	0	M	-	1	2

PATIENT DATA					ESL			HISTO	
P	EM	GI.No	D	H±	SL	S/M	DH	PT	TT
55	1354	25995	LM	+	1	M	-	1	1
56	1363	26055	LM	+	0	M	-	1	2
57	1367	26107	LM	+	1	M	-	2	2
58	1390	26413	LM	+	3	S	-	1	1
59	1223	24189	LM	-	2	S	2	1	-
60	1359	26031	LM	-	0	M	0	2	-
61	1245	24722	LM	-	2	S	0	2	-
62	1224	24653	C2	+	0	M	-	1	-
63	1264	24916	C2	+	1	M	-	1	1
64	1298	25201	C2	+	1	M	-	2	2
65	1310	25391	C2	+	0	M	-	1	2
66	1320	25564	C2	+	1	M	-	1	1
67	1379	26232	C2	+	1	M	-	1	1
68	878	19684	C1	-	4	S	0	1	-
69	899	20115	C1	-	4	S	1	1	-
70	1271	24965	C2	-	4	S	1	1	-
71	1395	26508	C2	-	0	M	2	2	-
72	1358	26016	C2	-	4	S	1	1	-
73	1078	22693	C1	-	4	S	1	2	-
74	879	19685	C1	-	1	M	1	2	-
75	877	19538	S	-	4	S	0	1	-
76	1096	22726	S	-	4	S	1	1	-
77	869	19608	S	-	4	S	0	1	-
78	939	20957	S	-	1	M	2	1	-
79	890	19829	S	-	3	S	0	1	-
80	882	19749	S	-	4	S	1	1	-
81	880	19701	C1	-	4	S	1	1	-
82	1335	26013	HM	-	4	S	2	1	-
83	1350	25941	C2	-	1	M	2	1	-
84	1373	26018	C2	+	4	S	2	2	-

Table II: Summary of subject, endoscopic, histologic and healing data.

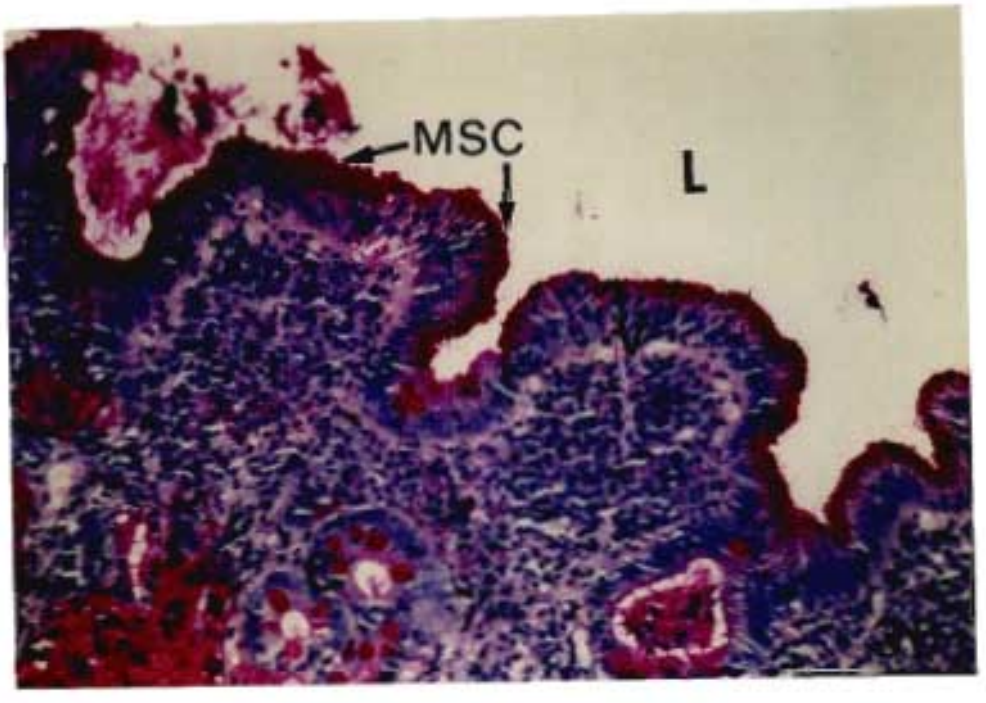
KEY

P = Thesis patient number
EM = Electron Microscope Unit specimen number.
GI = Gastrointestinal Unit patient number.
D = Type of drug therapy. S = Sucralfate; C(1,2,3) Cimetidine (3 regimens); P = Pirenzipine, HM = High-dose Misoprostol; LM = Low=dose Misoprostol.
H± = States whether the patient healed (+) or did not heal (-)
ESL = Severity of DU as estimated by endoscopy.
SL = Sevrity level (grades 0-4)
S/M = Severe (SL 2-4): Moderate (SL 0-1).
DH = Endoscopic state of the juxta-DU mucosa in patients who had not healed at the termination of therapy.
HISTO = Histological Group: 1 = Metaplastic; 2 = Non-metaplastic
PT = Morphological appearance of mucosa before therapy.
TT = Morphological appearance of mucosa after curative therapy. the trial.
2 = Biopsies included for morphological analysis of Goblet Cell Numbers (GC/100um).
# = Patient did not arrive at GI Unit for biopsy.
ns = No specimen received by EM Unit

There appeared to be a relationship between length of villi and degree of mucosal metaplastic differentiation. Where the mucosa was populated with numerous fully differentiated MSC, villi were short and stubby (Plates 35-38). Where normal absorptive and goblet cells predominated and metaplastic patches were few and often far between, villi were generally longer (Plate 42).

Goblet cells were present in some metaplastic specimens. There appeared to be a relationship between the degree of mucosal metaplastic differentiation and goblet cell numbers. A mucosa primarily populated with well differentiated MSC had few goblet cells, whereas one having numerous cells in intermediate stages of metaplastic differentiation (moderately metaplastic mucosa) had a greater number of goblet cells. Where there were large areas of non-metaplasia (as in Plate 32), goblet cell numbers were near normal.

Group 2: Non-metaplastic epithelia covered both normal and atrophic villi, i.e. there were often areas where villi apparently had been eroded from the mucosa (Plate 43). The brush border projecting from absorptive cells ranged in thickness from thin to normal (Plate 44). The cytoplasm was often vacuolated and as in metaplastic tissue, many cells contained osmiophilic inclusions. These cells differed from those in metaplastic mucosa in that they often contained a single pyknotic nucleus. In many instances, inflammatory cells had invaded the mucosa from the lamina propria. A morphometric analysis of non-metaplastic epithelium from both normal and atrophic villi revealed a significant increase ($p=<0,01$) in the number of goblet cells in these specimens, i.e. 2GC/100μm in normal tissue and 3,7GC/100μm in ulcerative mucosa (Figure 36).



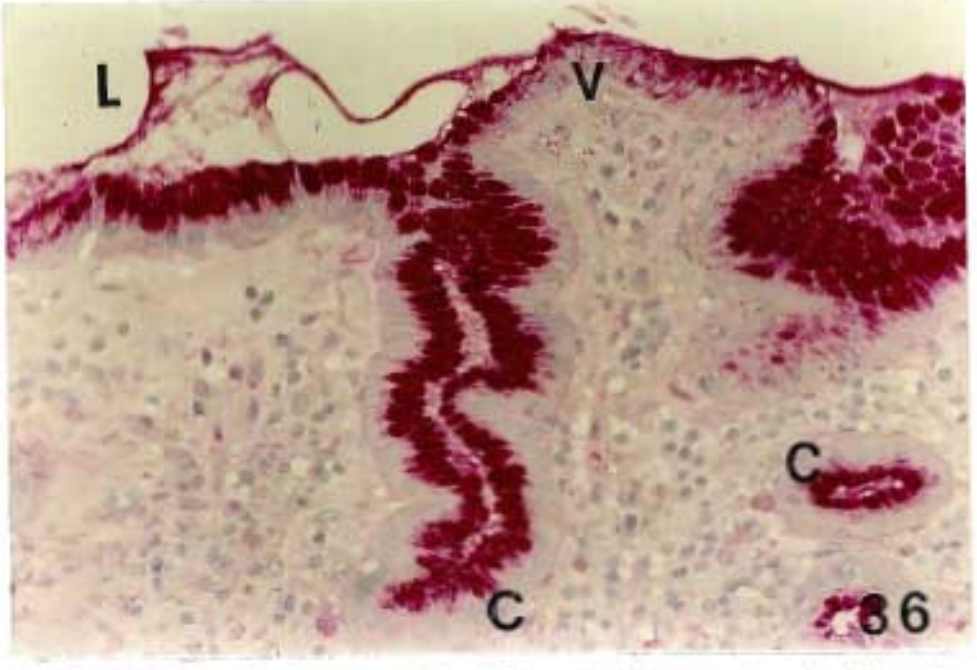
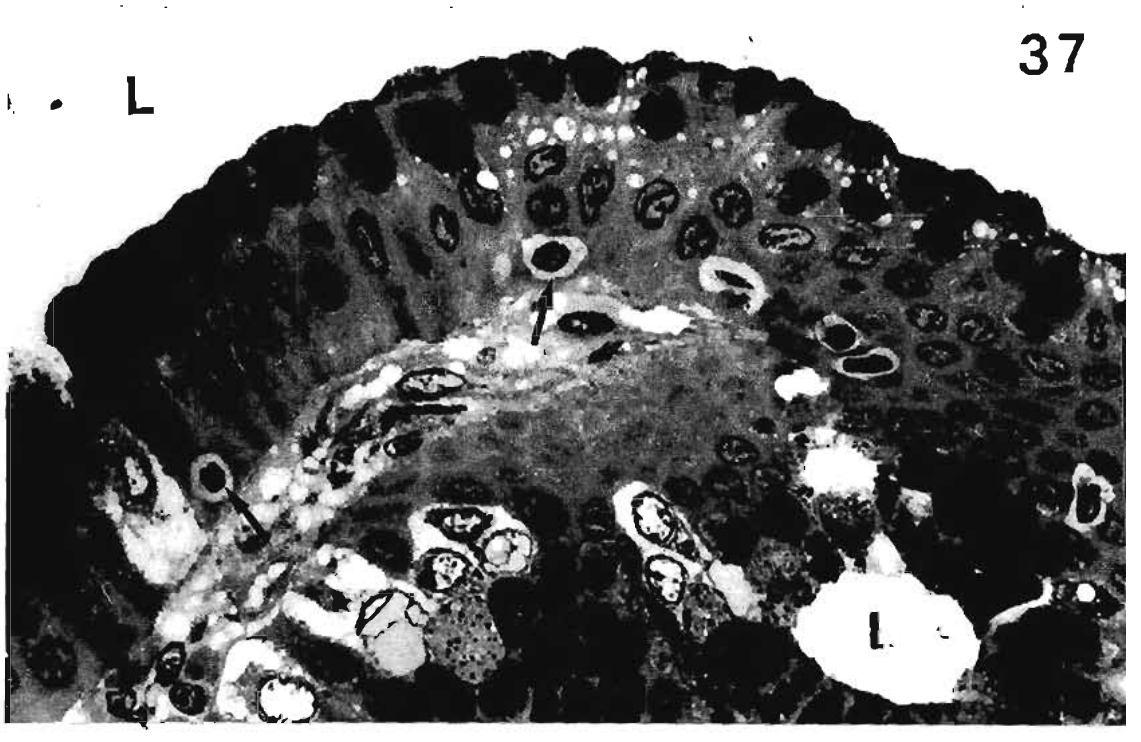


Plate 35: LM: Metaplastic: Atrophic villi exclusively populated with well differentiated MSC. Southgates mucicarmine stain. Magnification X 350.

Plate 36: LM: Metaplastic: Atrophic villi exclusively populated with well differentiated MSC. The MSC extend deep into the crypts of Lieberkuhn. PAS/Alcian blue stain. Magnification X 350.



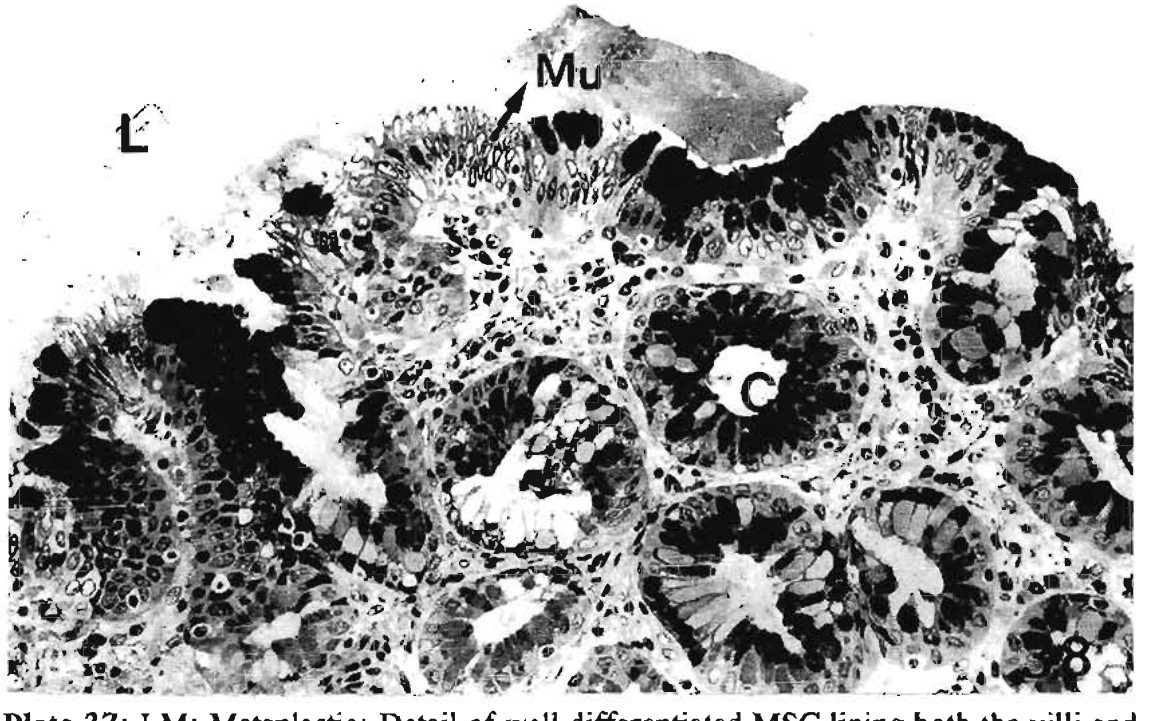
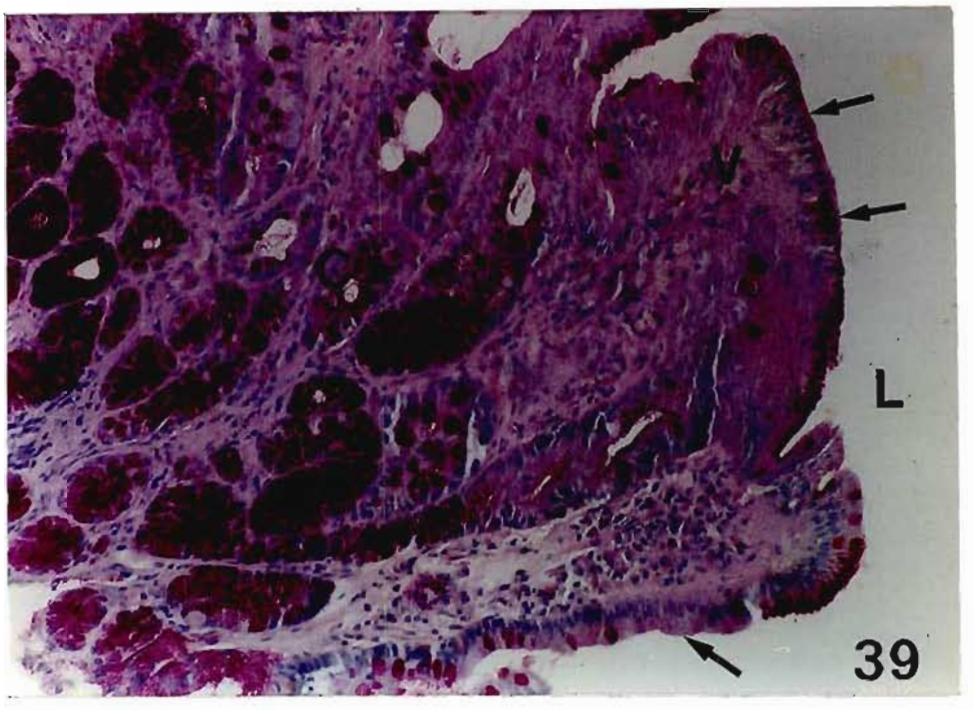


Plate 37: LM: Metaplastic: Detail of well differentiated MSC lining both the villi and the crypts of Lieberkuhn. Note the presence of inflammatory cells within the mucosa (arrowed). Toluidine blue stain. Magnification X 1200.

Plate 38: LM: Metaplastic: MSC secreting large quantities of mucosubstance into the lumen. Southgates mucicarmine stain. Magnification X 400.



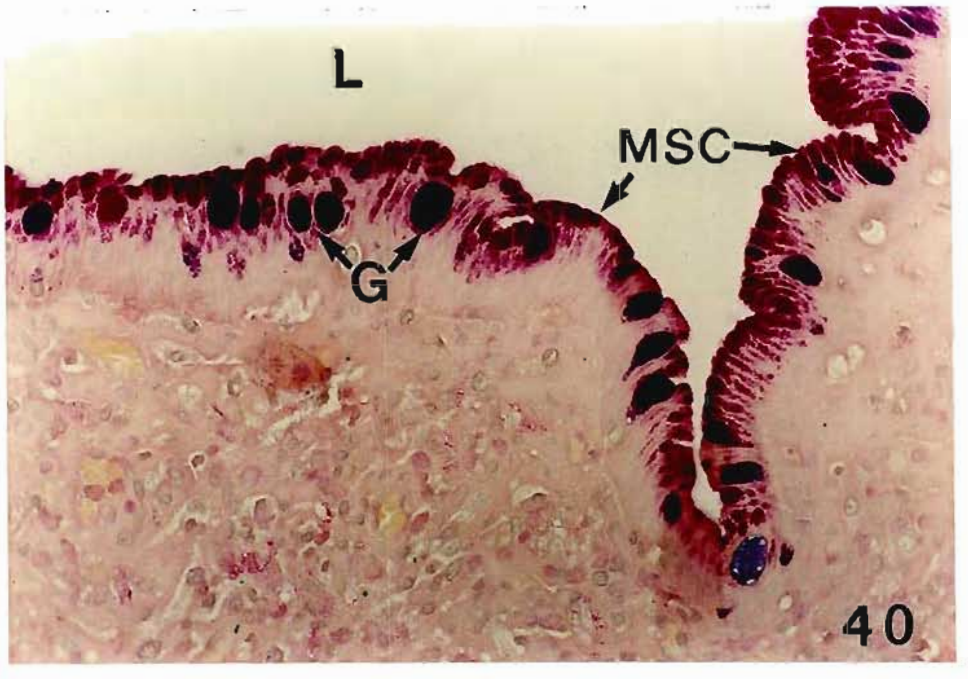
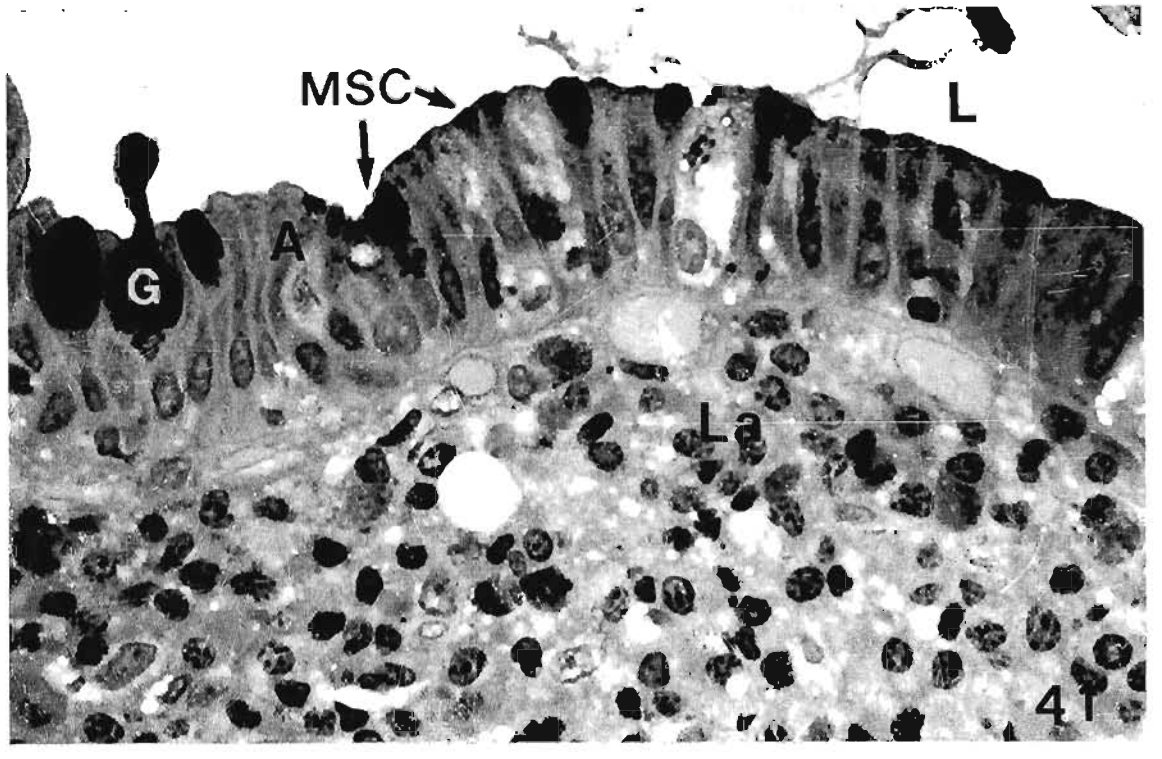


Plate 39: LM: Metaplastic: Atrophic villi with MSC in various phases of metaplastic differentiation. There are areas of apparently normal mucosa populated with goblet and absorptive cells (arrowed). The crypts are not exclusively populated with MSC. PAS stain. Magnification X 250.

Plate 40: LM: Metaplastic: Detail of epithelium populated with MSC interspersed with goblet cells. Alcian blue/PAS stain. Magnification X 450.



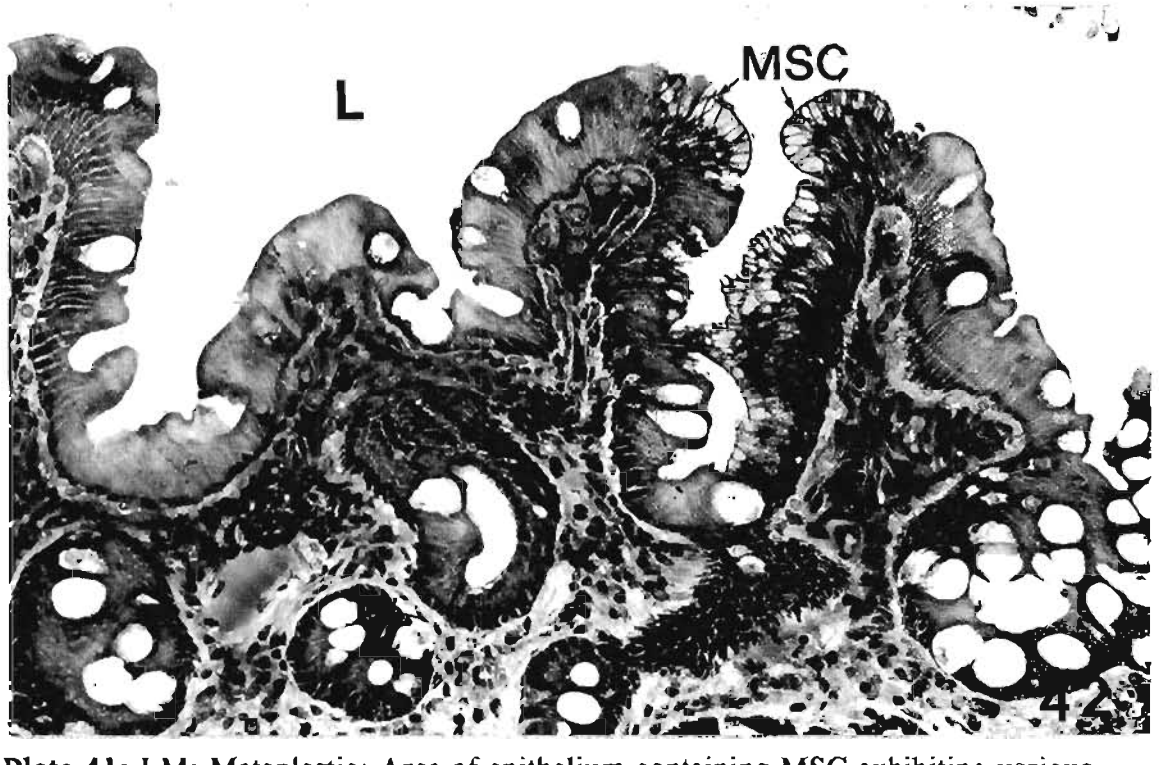
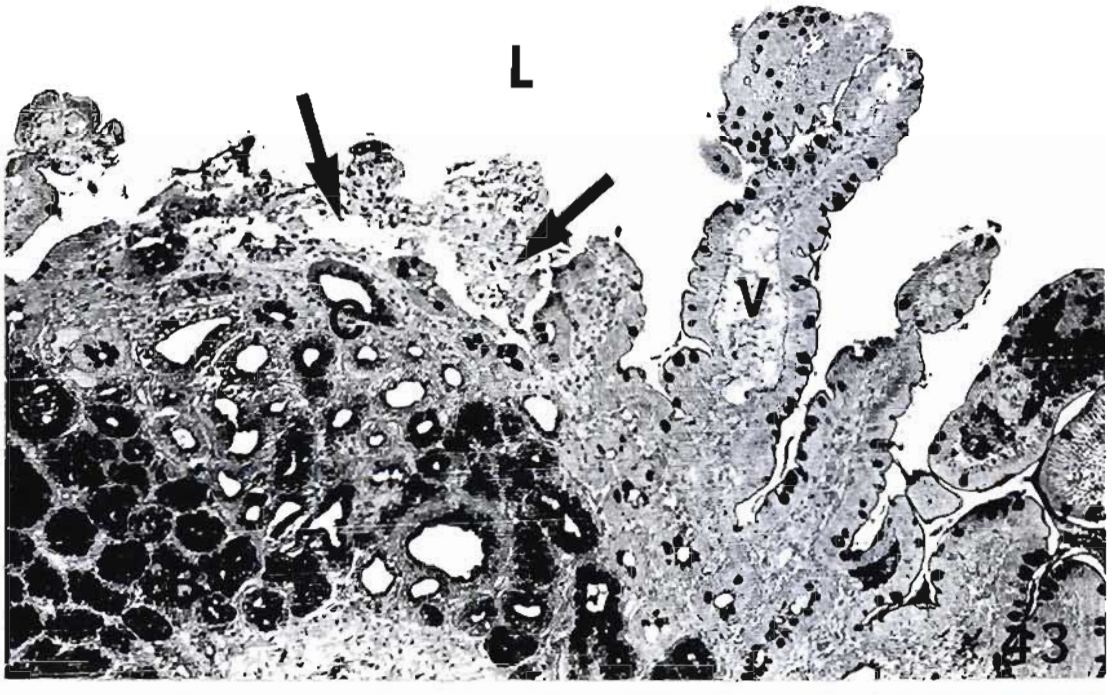


Plate 41: LM: Metaplastic: Area of epithelium containing MSC exhibiting various degrees of secretory activity and in various syages of metaplastic differentiation. Actively secreting goblet cells are present. Toluidine blue stain. Magnification X 1000.

Plate 42: LM: Metaplastic: Patches of metaplastic cells on normal shaped villi. Alcian blue/PAS. Magnification X 450.



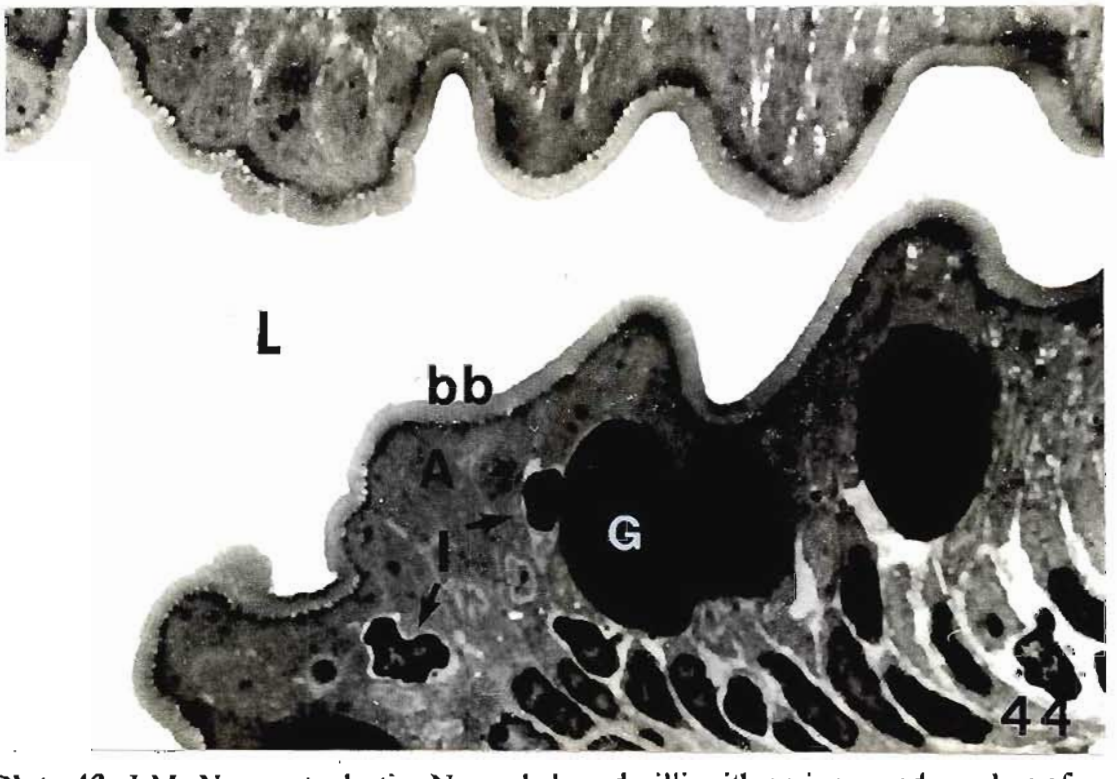


Plate 43: LM: Non-metaplastic: Normal shaped villi with an increased number of goblet cells. Note erosion of villi in some areas (arrowed). Southgates mucicarmine stain. Magnification X 150.

Plate 44: LM: Non-metaplastic: The mucosa has been invaded by numerous inflammatory cells. Many absorptive cells are vacuolated. However, the brush border is of normal thickness. Toluidine blue. Magnification X 2000.

5.3.3. Electron Microscopy

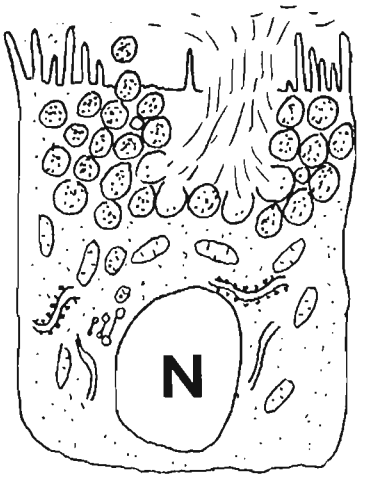
The fine-structure of epithelial cells populating the ulcerative mucosa, although variable, could be divided into 7 morphological types (a-f). Such division was based on the presence and ultrastructural appearance of mucus droplets, lysosomes, electron-dense granules (EDV), Golgi apparatus mitochondria, SER and RER. In addition, the thickness of the glycocalyx and the length and relative number of microvilli, if present, were taken into consideration (Figure 3).

It is important to note that only for descriptive convenience are cells categorised as "morphological types". It is postulated that the variations in cell morphology probably indicate different stages in the metaplastic differentiation of MSC, maturation of goblet cells and/or degeneration of absorptive cells (p.77). Nb: morphological cell types a); b); dii) some di) and e) are probably different stages in the differentiation of gastric metaplastic cells, type c) cells are probably immature goblet cells (type f) and most type di) cells are degenerating absorptive cells. For further details regarding these postulates consult 5.8. (pp.138-144).

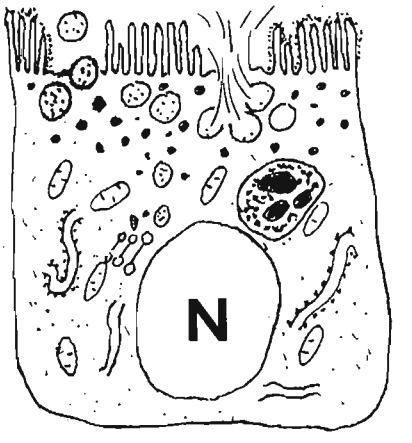
Cell type a): Fully differentiated MSC: Cells at this stage exhibited a range of the morphological characteristics of the surface mucus secreting cells normally found in the gastric antrum (Plate 45). The cells were of a columnar type, up to 7μm wide and 22μm long with a single elongated oval, basally situated nucleus. Sparse microvilli were from 0.4μm to 0.8μm in length and had a thin (approximately 40nm) glycocalyx projecting from their surface. In the immediate supranuclear cytoplasm was a well developed Golgi complex surrounded by many secretory vesicles. Between the Golgi complex and the luminally situated store of secretory granules were occasional mitochondria and cisternae of RER.

Figure 3:

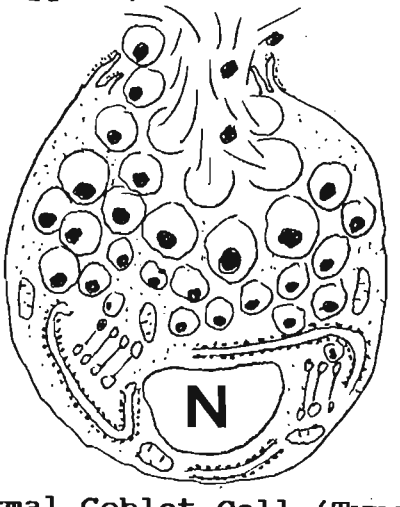
VARIATIONS IN THE ULTRASTRUCTURE OF EPITHELIAL CELLS POPULATING THE ULCERATIVE DUODENAL VILLOUS MUCOSA
Schematic Diagrams of 7 Primary Cell Types



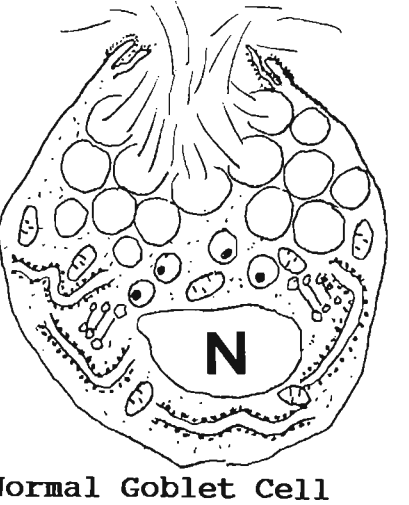
Well Differentiated Metaplastic Gastric Mucus Secreting Cell MSC (Type a)



MSC In An "Intermediate" Phase of Metaplastic Differentiation(Type b)



Abnormal Goblet Cell (Type c)



Normal Goblet Cell (Type f)

mitochondria

rough endoplasmic reticulum

Phagocytic vacuole

golgi cisternae

normal goblet cell mucodroplets

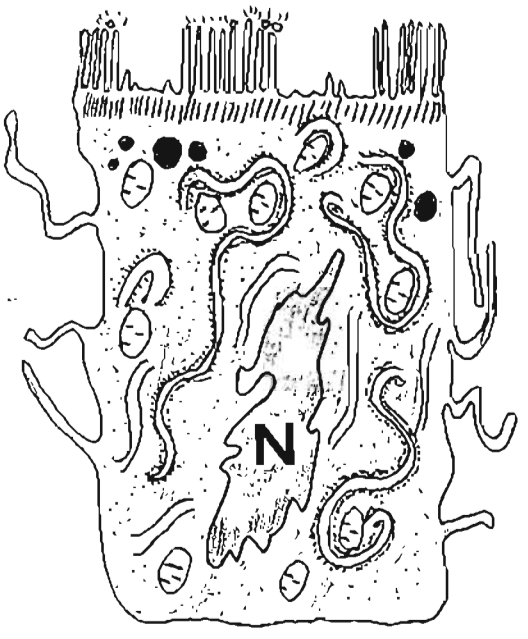
abnormal goblet cell mucodroplets

gastric mucus

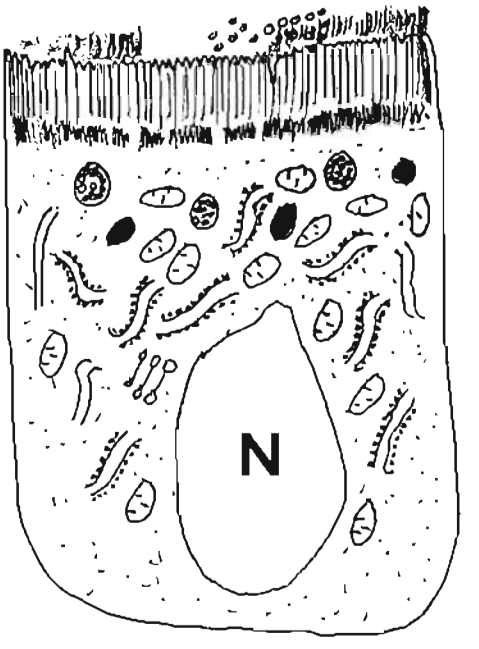
electron dense vesicles

nucleus N

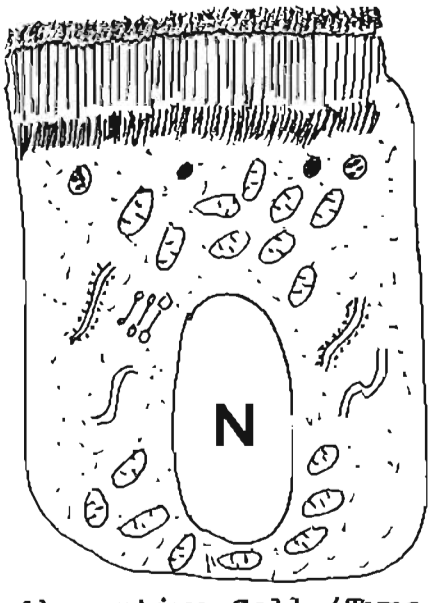
microvilli



Non-metaplastic Abnormal
Absorptive Cell (Type di)



Non-metaplastic Abnormal
Absorptive Cell (Type dii)



Absorptive Cell (Type e)

dilated rough endoplasmic reticulum

microvilli with normal glycocalyx

microvilli with fragmented, discontinuous or no glycocalyx

multivesicular body

glycocalyceal bodies

lysosomes

The cells were in various phases of mucus synthesis and secretion. Cells in an intermediate phase of mucosecretion were characterised by a sparcity of apical mucus granules and the presence of numerous small subplasmalemmal osmiophilic vesicles (Plate 46). In the pre-secretory and secretory phases, the apical store of mucus granules were generally electron dense and of various sizes and shapes and occupied most of the supranuclear space (Plates 45 to 47). During exocytosis the mucus droplets appeared to have fused within the cell body and the less electron-dense mucosubstance spread laterally over adjacent cells (Plate 47). In some areas, the act of mucus secretion appeared to have been synchronised, with many cells secreting large quantities of mucosubstance into the lumen simultaneously (Plate 48). Some cells, however, appeared to have released intact droplets of mucosubstance into the lumen (Plate 49).

Numerous inflammatory cells had invaded the metaplastic mucosa (Plate 50). Many metaplastic cells contained large phagocytic vacuoles (Plate 51). These appeared to contain the remnants of inflammatory cells. In well differentiated metaplastic specimens, MSC extended into the crypts of Lieberkuhn as far as the germinal region. Here some mitotic stem cells contained secretory granules towards the lumen (Plate 52). Many well differentiated gastric metaplastic cells lining the mid/upper regions of the crypt commonly extruded membrane bound spheres of cytoplasm into the lumen (Plate 53).

Cell type b): Partially differentiated MSC: These columnar cells were up to 6.5μm in width and 22μm in length and contained a single elongated oval, basally situated nucleus. The cells were characterised by the presence of single or small groups of secretory droplets, variable numbers of small electron-dense vesicles (EDV) and lysosmes in the

apical region (Plates 54 to 58). Microvilli ranged in length from 0.4μm to 0.6μm and were generally more numerous than those projecting from well differentiated MSC. A glycocalyx ranging from 20nm to 40nm in thickness followed the contour of each microvillus. The Golgi apparatus was well developed as was the RER (Plate 54). Electron lucent droplets containing an osmiophilic inclusion were present in some cells (Plate 54).

There appeared to be a correlation between numbers of secretory granules, number and size of EDV and lysosomes and length and number of MV. Cells with more numerous secretory granules generally had few EDV and longer MV with a more pronounced glycocalyx (Plate 54). Cells with numerous EDV and lysosomes often had no secretory droplets and generally had shorter more numerous MV and a thinner, sometimes absent Gx (Plates 55 and 56). Although not as well endowed with secretory material as the more fully differentiated MSC, these cells were seen to exocytose mucosubstance either as discrete granules (Plate 57) or en-mass from apical aggregates of mucosubstance (Plate 58).

Numerous inflammatory cells had infiltrated the "moderately" metaplastic mucosa (Plate 59). Many type b) cells appeared to have phagocytosed inflammatory cells and were contained within the cytoplasm as large supranuclear phagosomes (Plates 59 and 60). Very occasionally, mitotic cells containing EDV were present on villi (Plate 61).

Cell type c): Abnormal goblet cells: These cells populated the patially metaplastic mucosa and were found in association with type a) and b) cells. Type c) cells were similar in size and had the general appearance and apparent secretory function of goblet cells found in normal tissue

(Plates 62 to 65). They were characterised by a dense osmiophilic inclusion within most mucus granules (Plates 62-64). Whereas in normal tissue, the osmiophilic inclusion was absent in the mature granule, in these cells the inclusion remained within droplets prior to and during secretion (Plate 64). Immature goblet cells in the crypts of Lieberkuhn of metaplastic specimens also contained pale stained mucus droplets within which were the osmiophilic inclusions that characterised type c) cells (Plate 65). The cells were, however, much more slender (approximately 6μm wide) than the more mature cells on the villus.

Cell type d): Abnormal absorptive cells: These enterocytes were characterised by the following features: the general appearance of absorptive cells; an absence of secretory granules; a thinned or absent glycocalyx. Type d) cells could be further divided into 2 sub-types i) Vacuolated - where the SER and RER were severely dilated giving the appearance of cytoplasmic vacuolation and ii) Non-vacuolated - where SER and RER dilatation was minimal or absent.

d(i) Vacuolated cells In the most severe cases, vacuolated cells were characterised by a condensed, electron dense cytoplasm, within which were whorls of RER with dilated cisternae (Plate 66). These often enveloped swollen mitochondria many of whom had disrupted cristae (Plate 67). The cells were separated by large intercellular spaces within which were convolutions of lateral pseudopodia. The cells were of normal length but rarely exceeded 5um in width. Severely vacuolated cells had a crenated, basally situated nucleus and the Golgi apparatus and SER were extensively dilated. The microvilli were sparse and rarely exceeded 0.7μm in length. In many cells, microvilli had fused and cytoplasmic excrescences extended up to 3μm into the lumen (Plate 67). Most very vacuolated cells had no discernible glycocalyx. When present, however, it was thin

and fragmented (Plate 68).

There were varying degrees of degeneration and vacuolation within cells classified as di). In some cases, the cytoplasm was less condensed and the cells were closely apposed (Plate 69). In these cells, although the SER was often extensively dilated, nuclei were less crenated and mitochondria less swollen. Microvilli were short and sparse or were absent from the cell surface (Plates 69 and 70). In some instances, microvillous rootlets in the subplasmalemmal cytoplasm were all that remained of microvilli (Plate 71). In no instance did a well developed glycocalyx project from the microvilli of these cells (Plate 72). In the least severe cases, mitochondria were abundant and microvilli were normal or near normal in both numbers and length (Plate 73). The glycocalyx, however, while present on some cells was fragmented or absent on others (Plates 73 and 74). While most minimally degenerative cells were associated with non-metaplastic epithelia, a few cells categorised as di) but containing occasional EDV (Plate 71), were present in association with "types" dii) and b) cells in metaplastic tissue.

Where villi were populated with vacuolated absorptive cells, vacuolation often extended to the immature and germinal cells in the crypts of Lieberkuhn (Plate 75). It is interesting to note, however, that when goblet cells were present, other than exhibiting mild dilation of RER and Golgi cisternae, the cells appeared morphologically and functionally normal (Plate 76).

d(ii) Non-vacuolated cells The cytosol and the fine structure of organelles in these cells was generally normal. Occasional necrotic cells whose appearance mimicked di)

cells were present near "type" dii) cells (Plate 77). These, however, were probably cells that had died as a consequence of normal villous cell turnover. Many "type" dii) cells contained phagosomes (Plate 78), lysosomes and quite large numbers of multivesicular bodies (Plate 79) and EDV (Plates 77 and 81). The most striking abnormalities were associated with the cell surface. In many instances, MV were long (1μm) and densely packed but the glycocalyx was thin or absent (Plate 79). In others, the glycocalyx was "whispy" and had been invaded by numerous glycocalyceal bodies (Plate 80). In some cases, up to six microvilli had fused into blebs that had ballooned as far as 5μm into the lumen (Plate 81). These cells differed from most minimally degenerate type di) cells in that many contained occasional EDV (Plate 81).

Normal absorptive (**cell type e**) and goblet (**cell type f**) occurred either singly or in small clusters in some Group 1 and 2 specimens (Plate 82).

It is important to note that between metaplastic (cell types a) b) dii and some di) and normal absorptive cell type e), secretory (c and f) and non-metaplastic absorptive cells (di and e) there were many intermediate morphological variations. The preceding descriptions serve only to outline the main morphological characteristics of the stages of metaplastic differentiation, phase of goblet cell maturation or degree of absorptive cell degeneration in the ulcerative specimens studied.

5.3.3.1. Paired Biopsies

There was general conformity in the morphological appearance of both biopsies from each patient. There were, however, differences which could not be discerned by light microscopy only. On occasion, a fully metaplastic specimen was paired with a biopsy exhibiting only partial metaplasia by electron microscopy. On other occasions, a histologically non-metaplastic specimen was paired with a specimen exhibiting partial metaplasia by electron microscopy. Nb. the greater resolution afforded by the electron microscope was better able to detect metaplastic changes in such tissue.

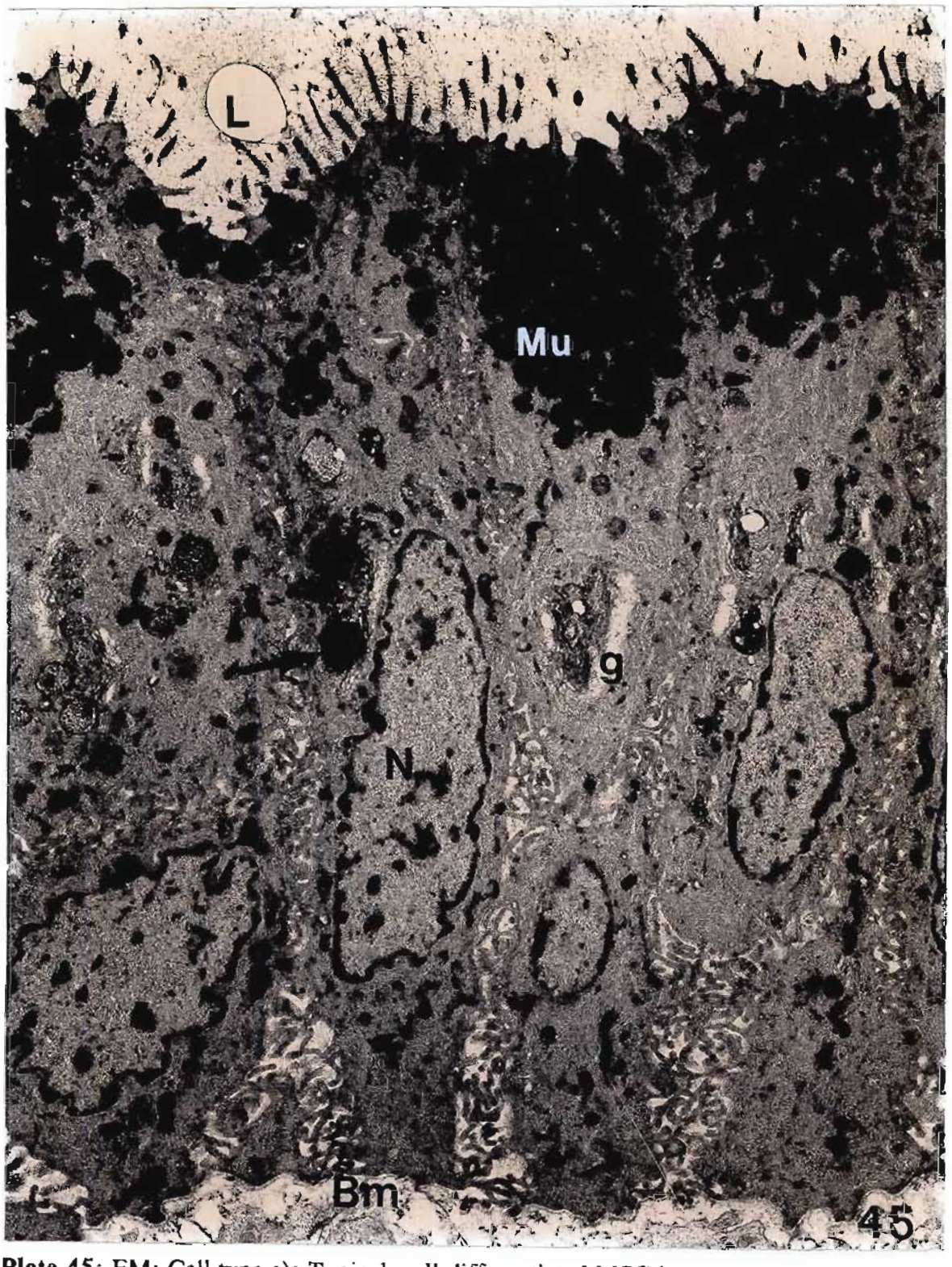


Plate 45: EM: Cell type a): Typical well differentiated MSC in a mucosa exclusively populated with MSC. Note the aggregate of osmiophilic mucus granules forming the mucus body at the apex of each cell (Mu). The presence of immature mucus granules in the golgi region (arrowed) suggests that one cell is actively synthesising mucosubstance - perhaps to replenish its store of mucus after secretion. Magnification X 9500.

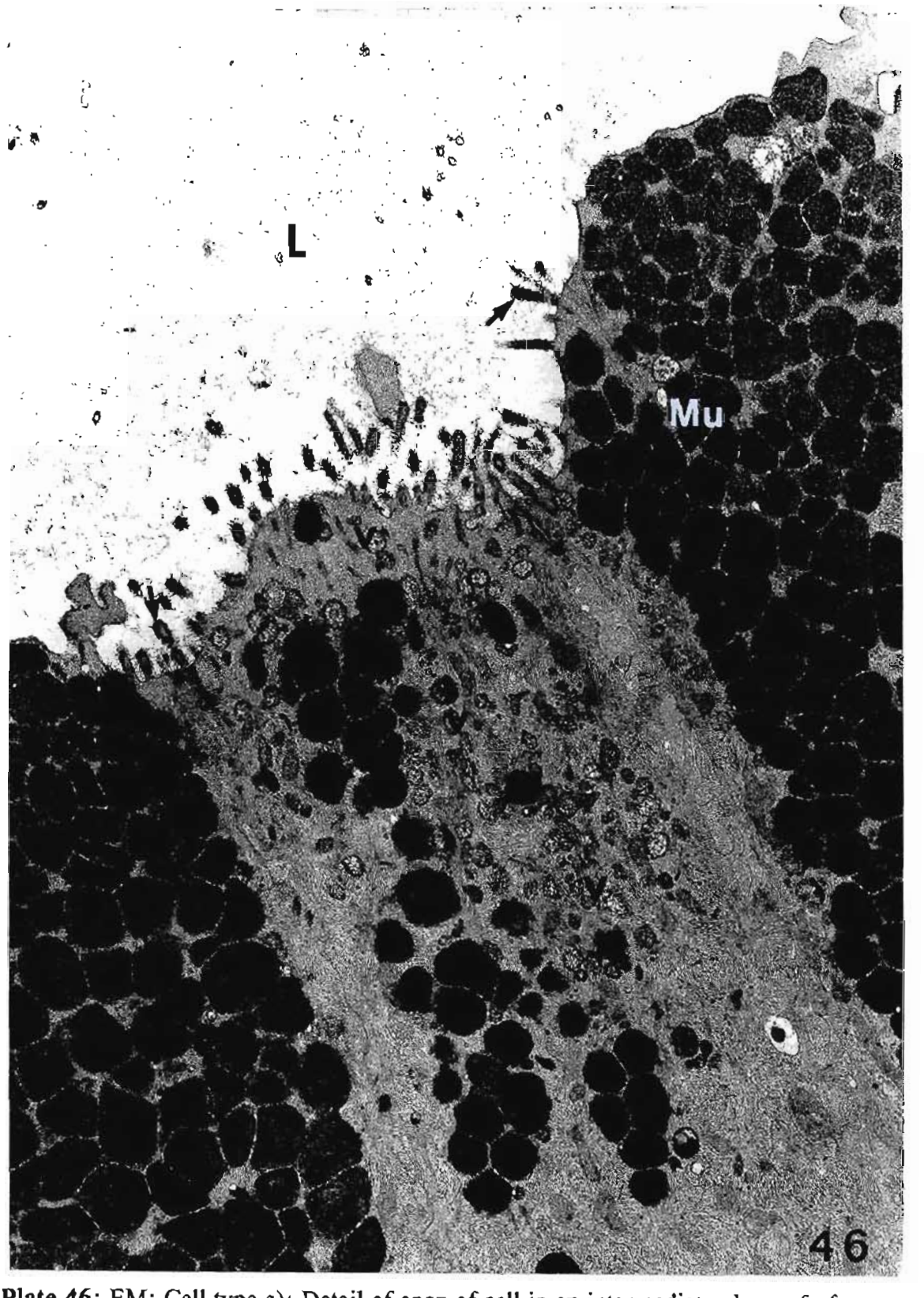


Plate 46: EM: Cell type a): Detail of apex of cell in an intermediate phase of of mucus secretion. Note the reduced number of mucus droplets and presence of numerous osmiophilic vesicles (v). Magnification X 15000.

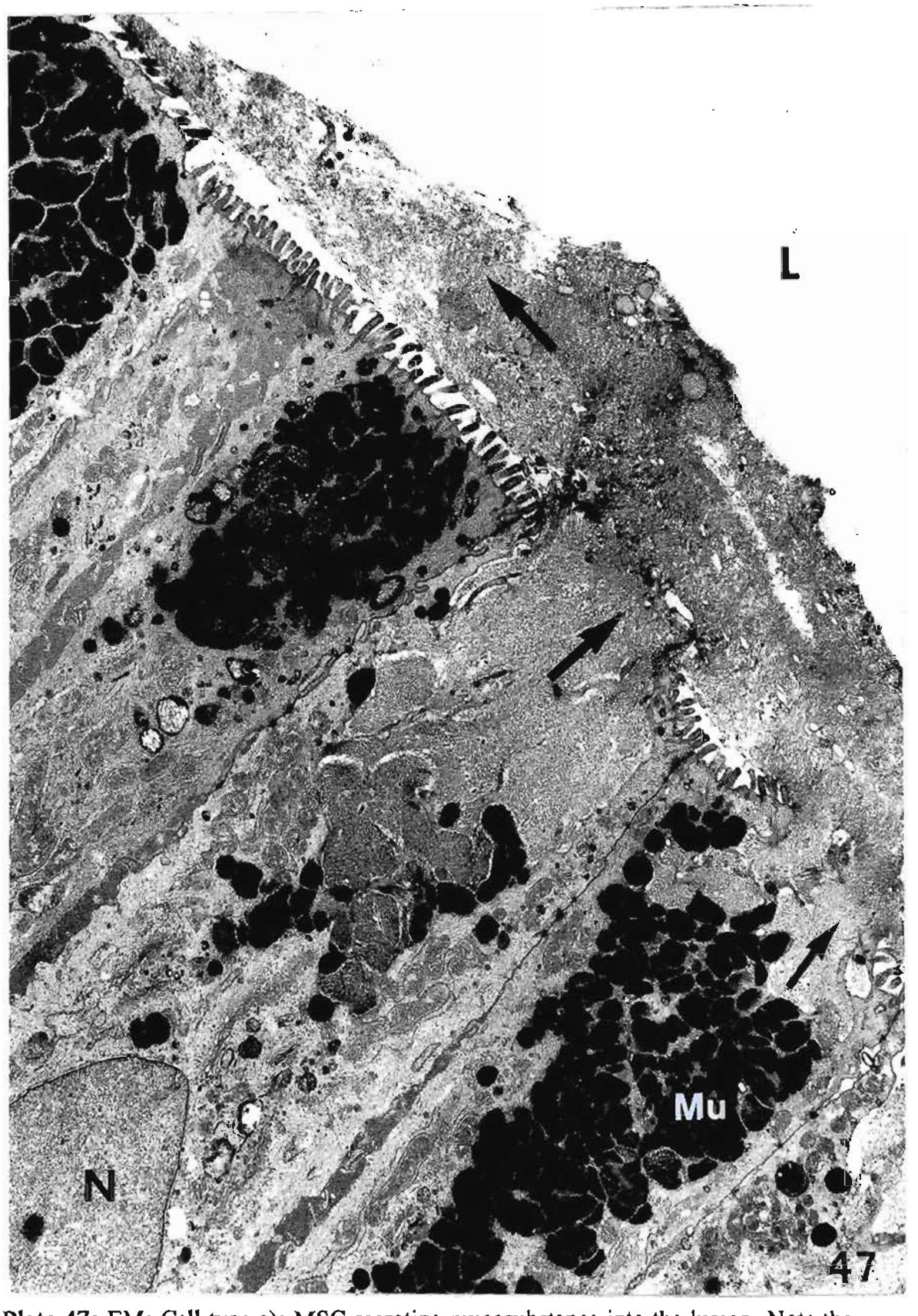


Plate 47: EM: Cell type a): MSC secreting mucosubstance into the lumen. Note the fusion of granules within the mucus body prior to exocytosis. The mucus is spreading laterally over adjacent cells. Magnification X 10000.

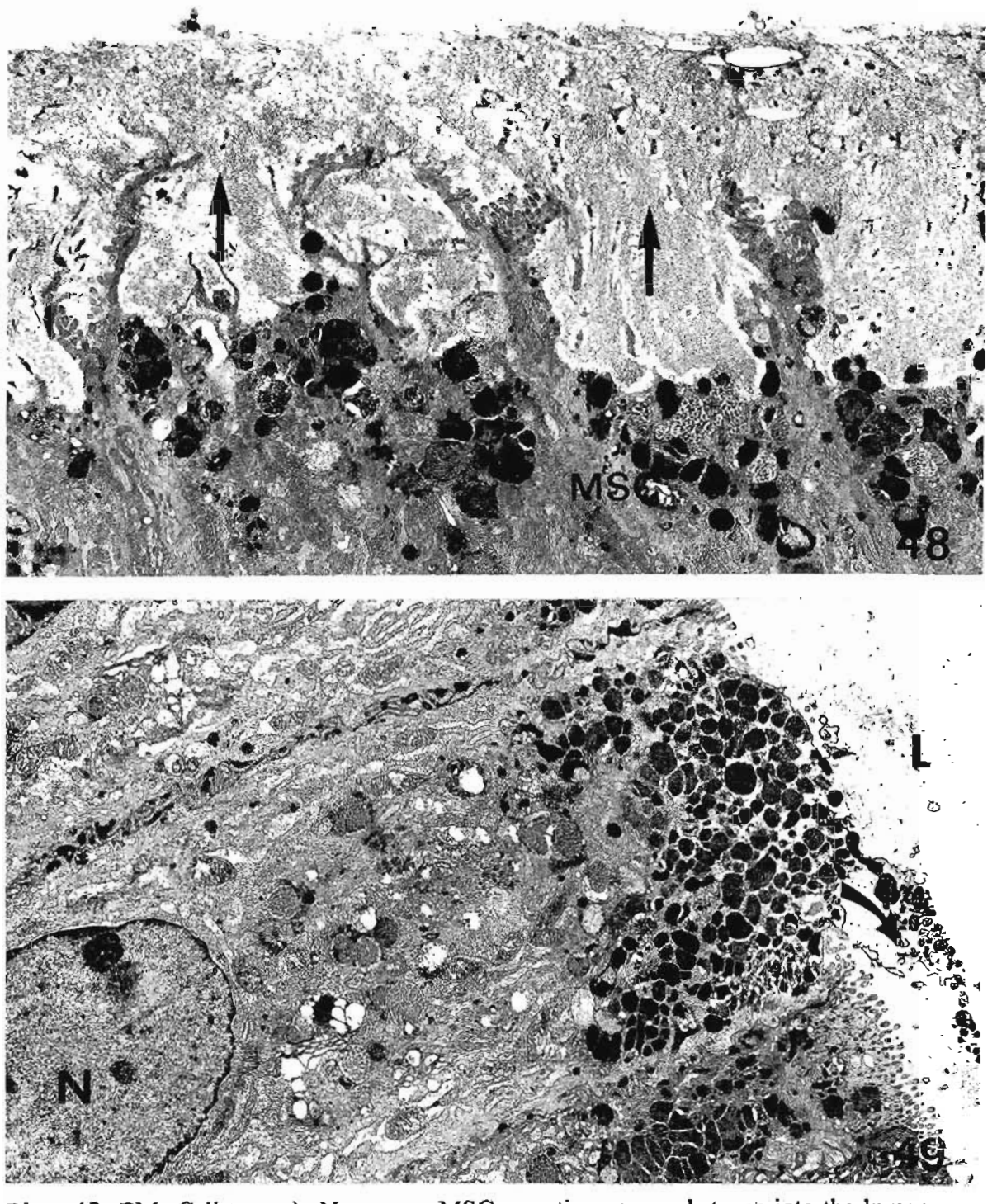
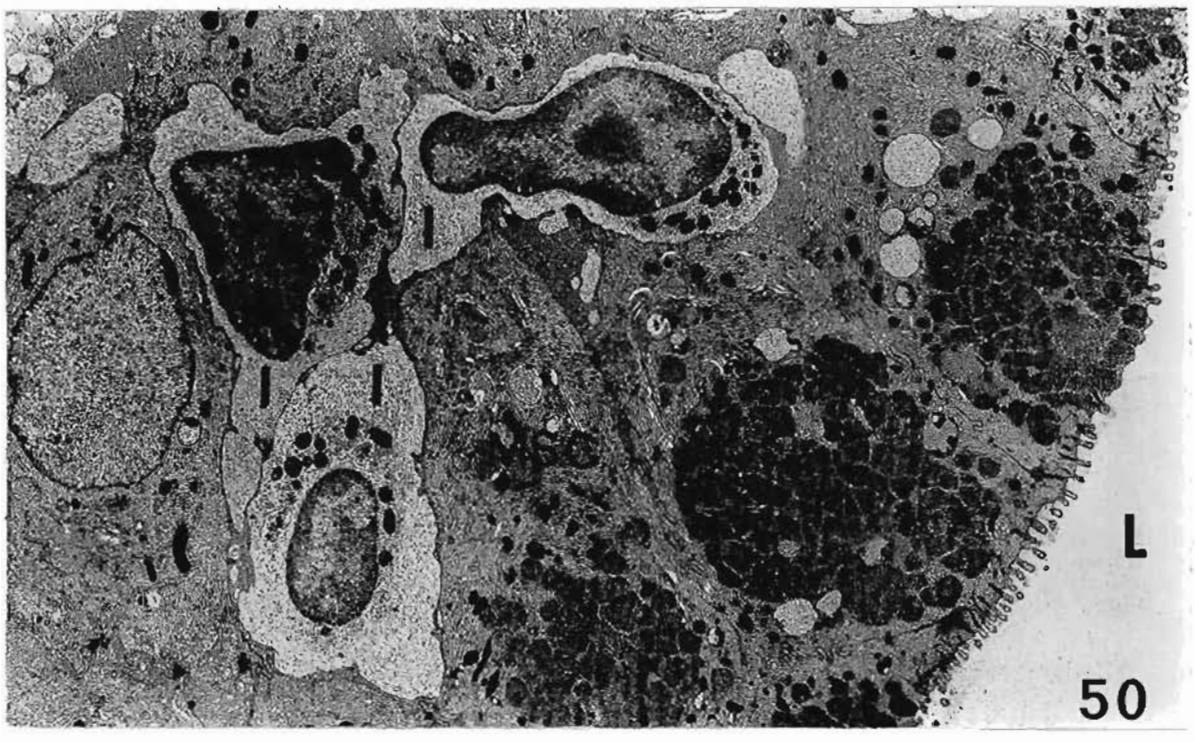


Plate 48: EM: Cell type a): Numerous MSC secreting mucosubstance into the lumen (arrowed). Note fusion of mucosubstance in the apex of the cell during exocytosis. Magnification X 7500.

Plate 49: EM: Cell type a): MSC exocytosing intact mucus droplets into the lumen. Magnification X 10000.



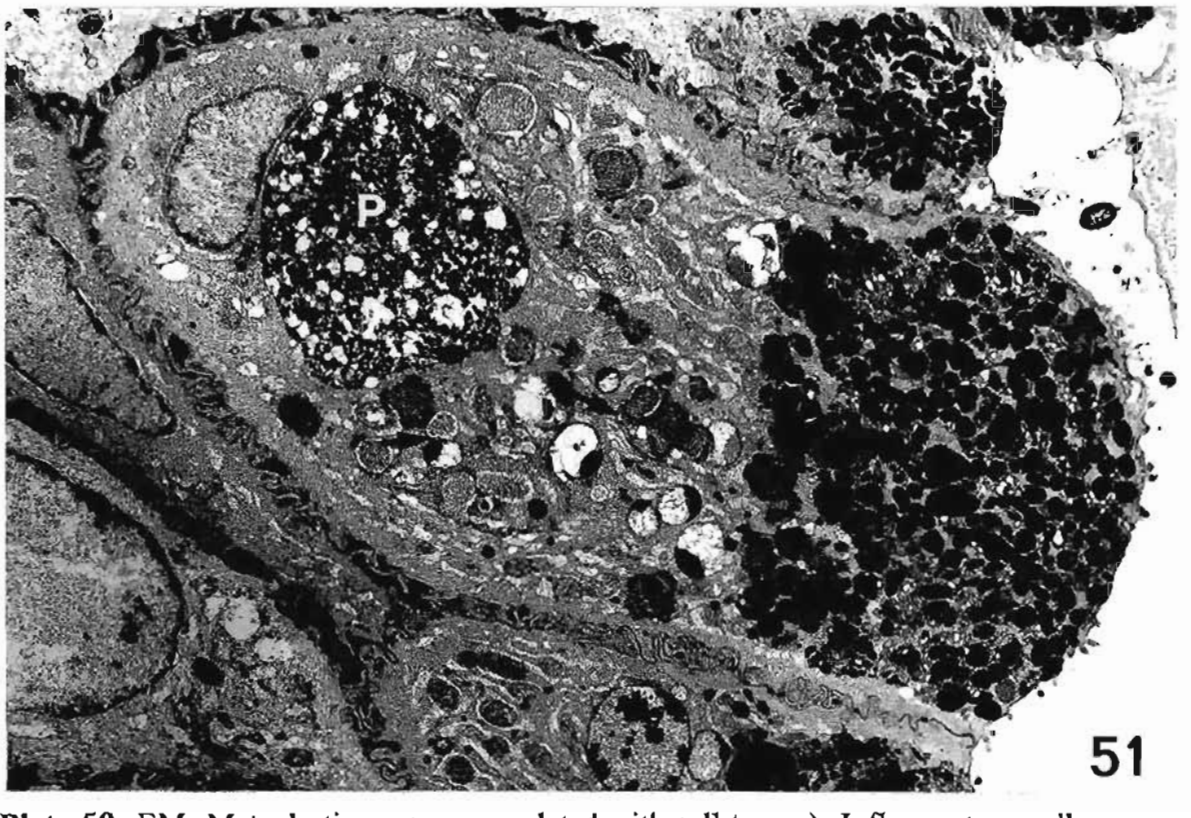
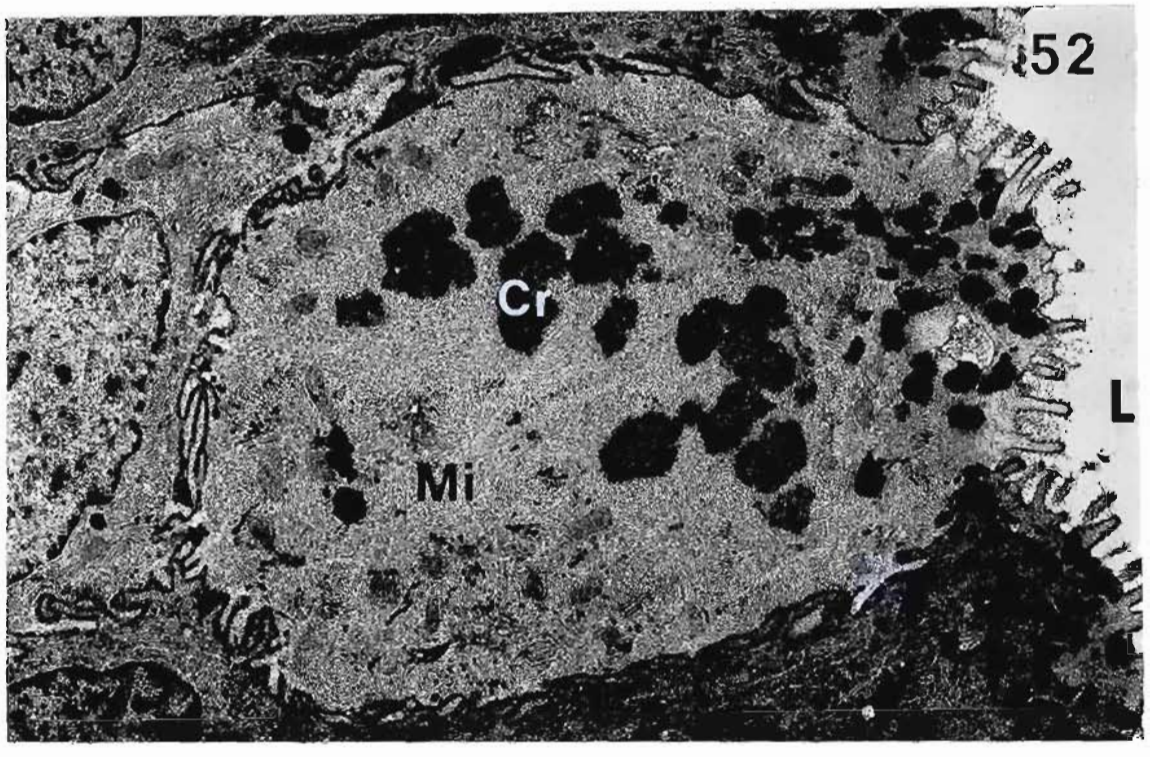


Plate 50: EM: Metaplastic mucosa populated with cell type a): Inflammatory cells are invading the mucosa. Magnification X 7500.

Plate 51: EM: Cell type a): Large phagosome in cytosol (P). The immature mucus granules in the golgi region (arrowed) suggests that this cell is actively synthesising mucosubstance. Magnification X 11000.



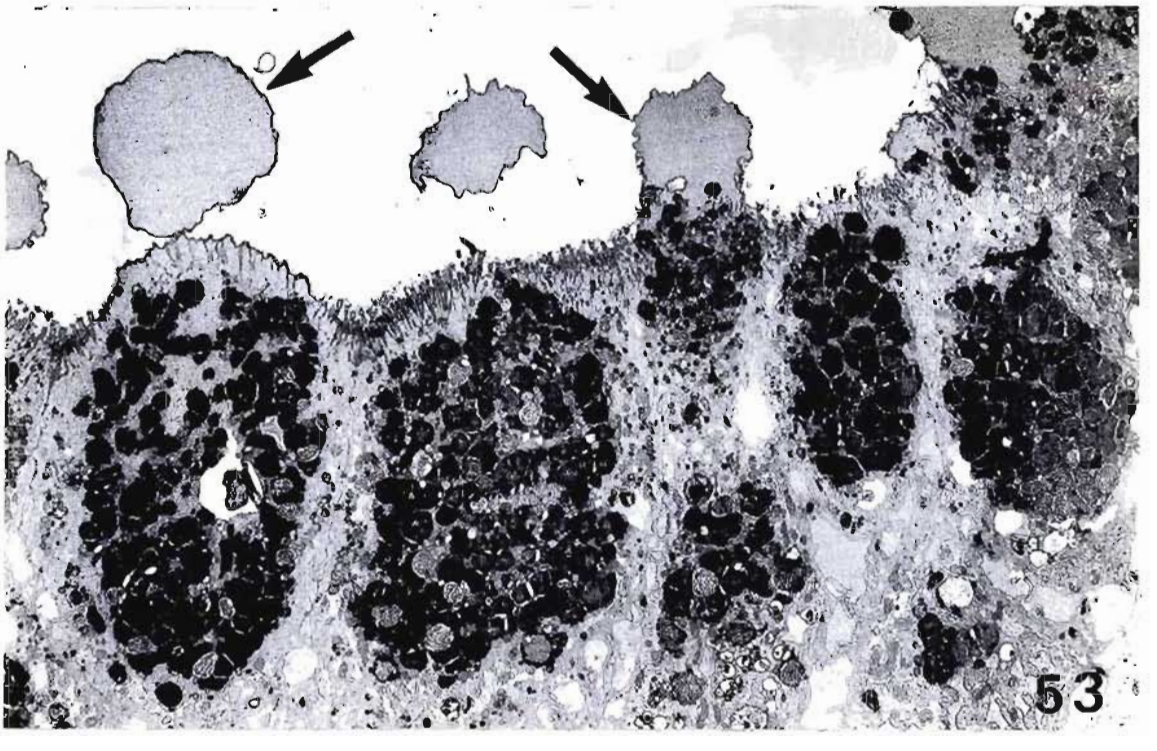


Plate 52: EM: Mitotic MSC in a crypt of Lieberkuhn populated with differentiating MSC. Note the secretory granules near the apex of the mitotic and adjacent cells. Chromatin (Cr). Magnification X 20000.

Plate 53: EM: Cells populating a crypt of Lieberkuhn in a specimen whose mucosa is exclusively populated with well differentiated MSC (cell type a). Note spheres of cytoplasm being extruded from the apices of cells (arrowed). Magnification X 7500.

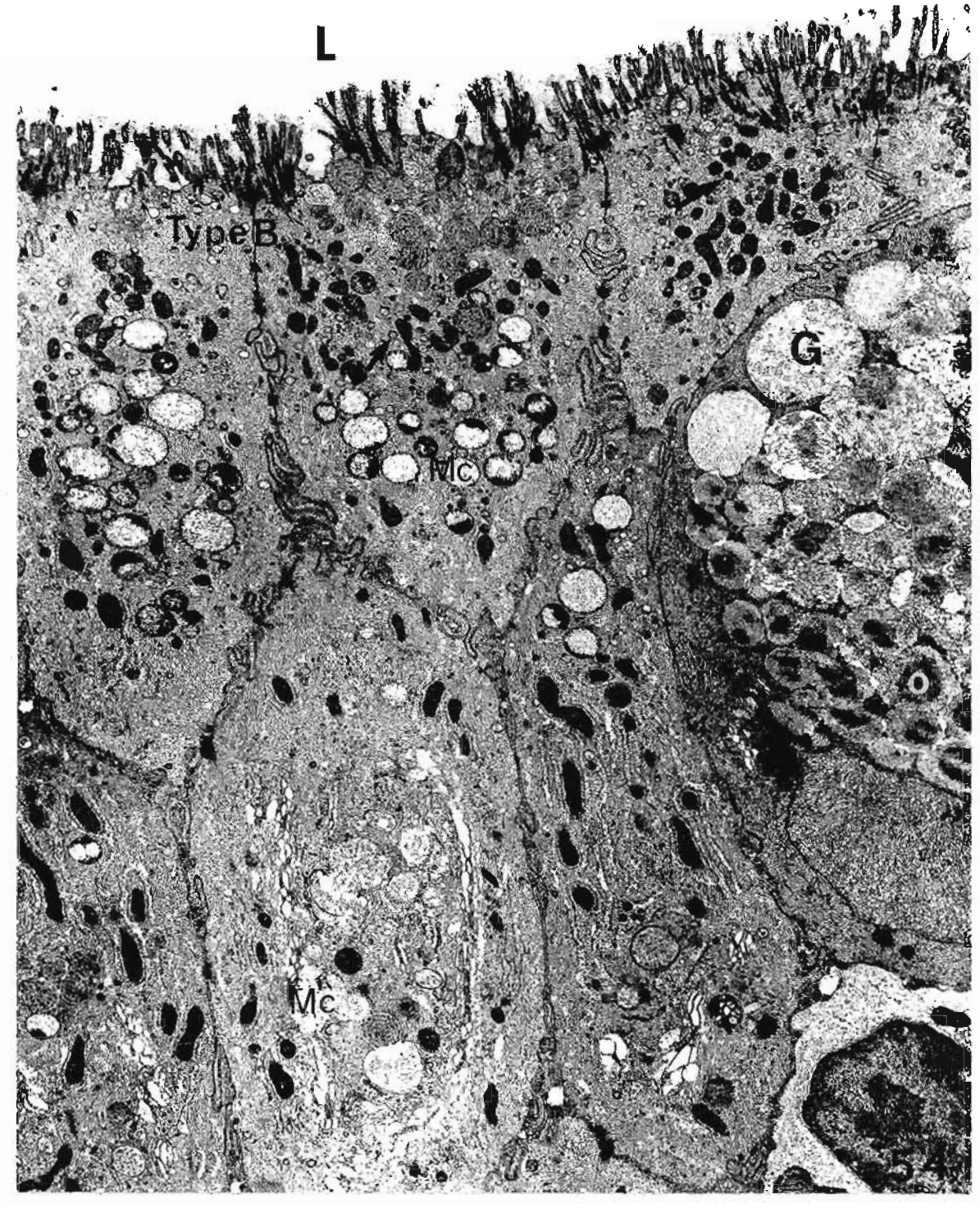
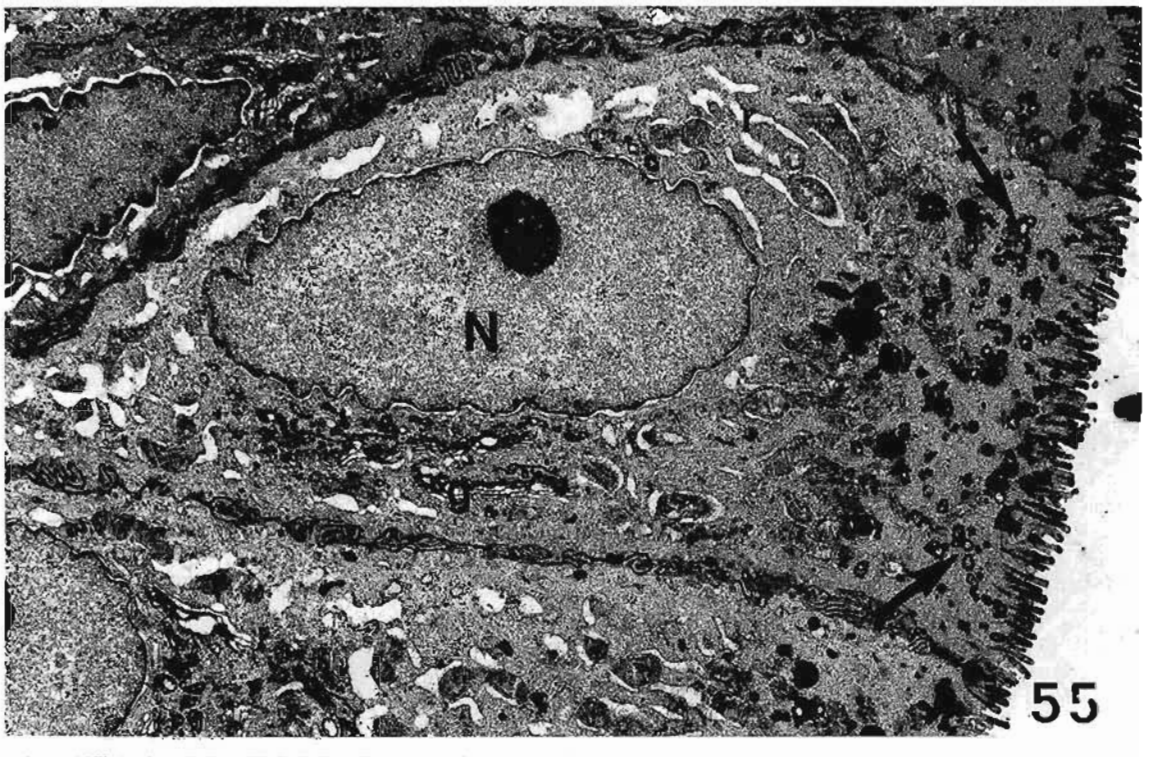


Plate 54: EM: Cell type b): MSC with smaller quantities of mucosubstance at their apices. The cells are actively secreting mucosubstance with immature mucin (Mc) being synthesised by the golgi and migrating towards the apex. There are numerous small osmiophilic vesicles present in the apical cytoplasm (arrowed). The Mv are longer and more numerous than in type a) cells. There is often a thin glycocalyx projecting from individual Mv. Note the abnormal goblet cell with mucus granules containing osmiophilic inclusions (o). Magnification X 10000.



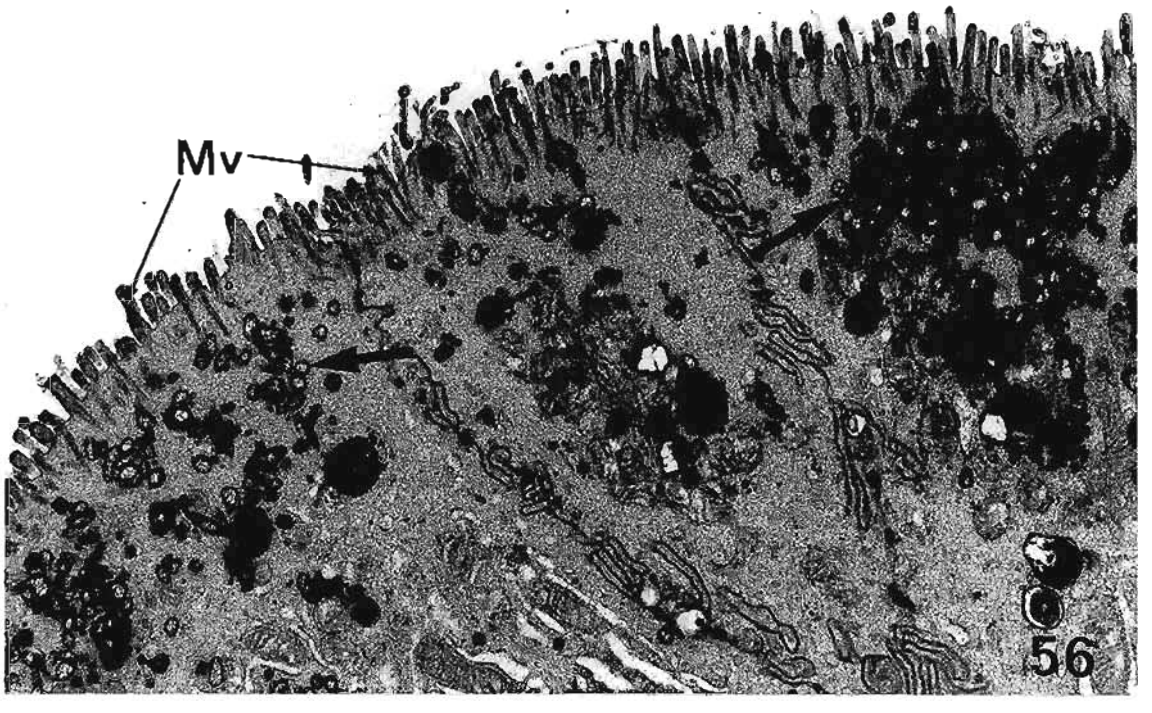


Plate 55: EM: Cell type b): A cell with numerous electron dense vesicles in the apical cytoplasm (arrowed). The RER is dilated and the golgi apparatus well defined. Note the absence of mucus droplets. The Mv are more numerous and shorter than those projecting from type a) cells. No glycocalyx is in evidence.
Magnification X 11000

Plate 56: EM: Cell type b): Detail of apical portion of cell showing aggregates of EDV (arrowed). Note numerous short Mv and absence of glycocalyx.
Magnification X 20000

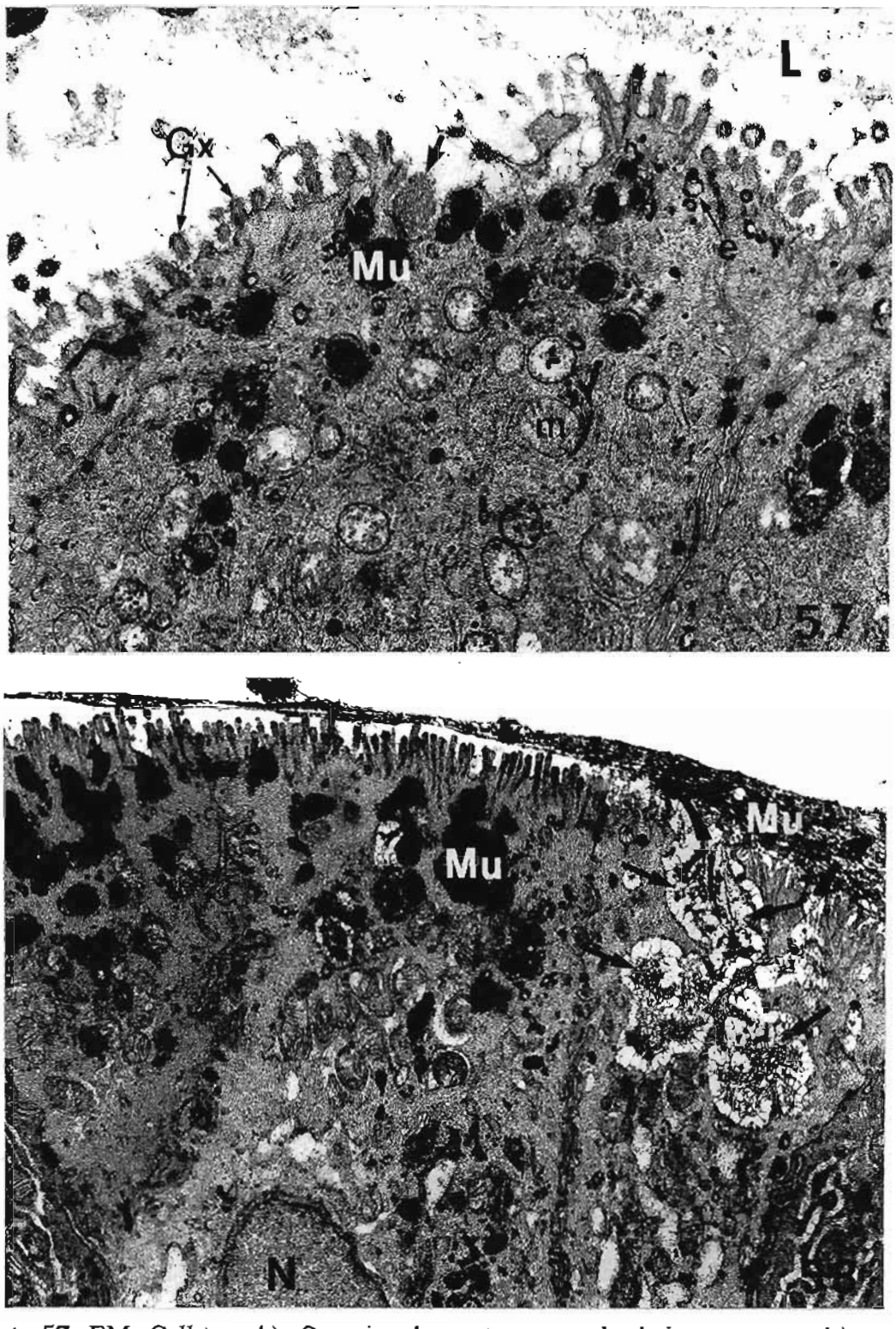
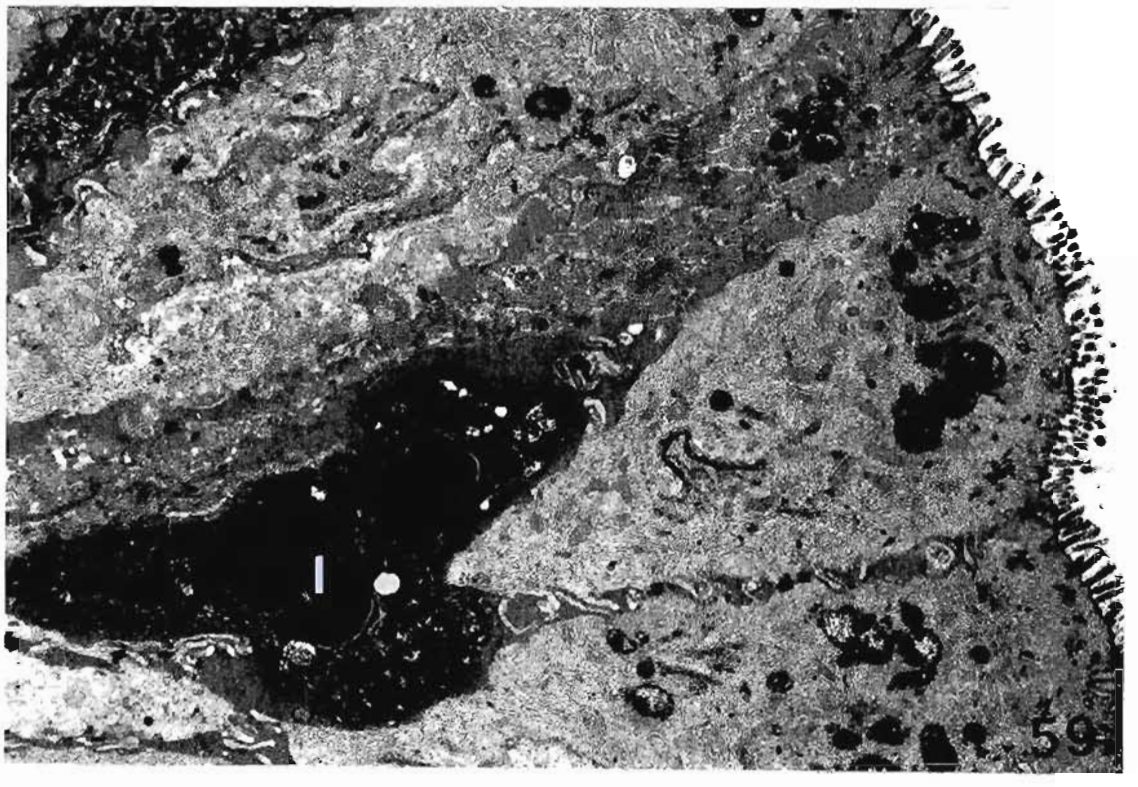


Plate 57: EM: Cell type b): Occasional secretory granules being exocytosed into the lumen (arrowed). Note short Mv from which projects a thin glycocalyx. A small number of electron dense vesicles (e) are present near the apical plasmalemma. Magnification X 22000.

Figure 58: EM: Cell type b): Small aggregates of apical mucus droplets fuse prior to exocytosis (arrowed). Note layer of mucosubstance spreading over the mucosal surface. Magnification X 11000.



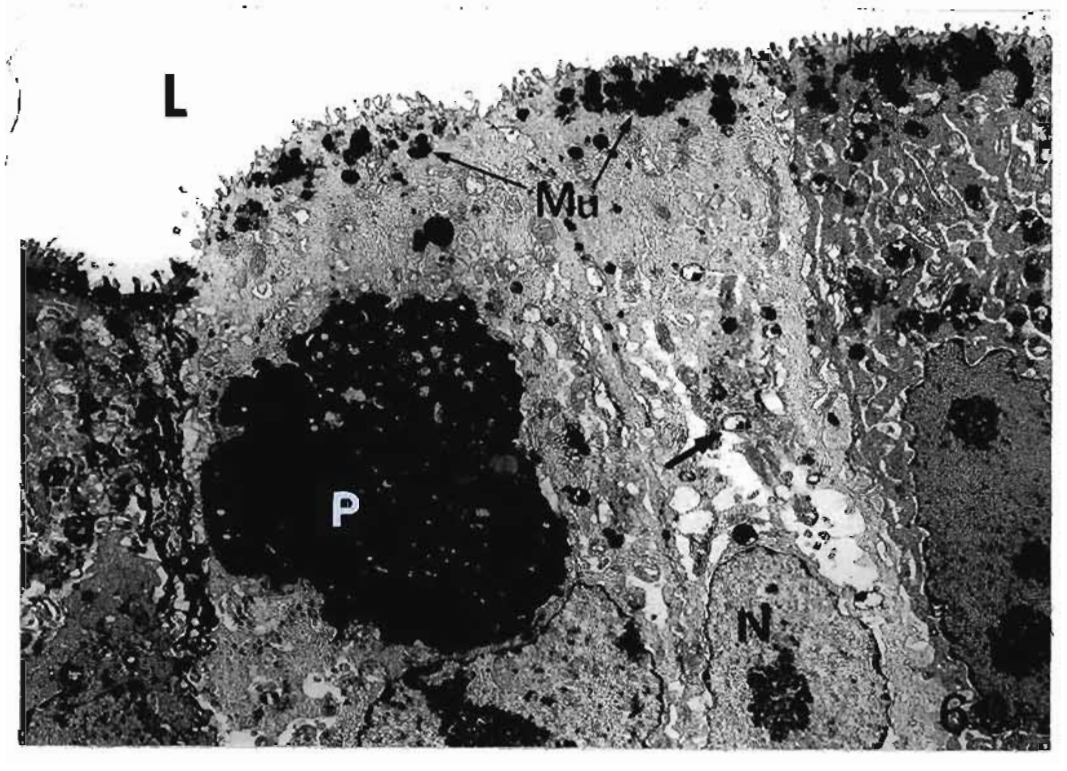


Plate 59: EM: Inflammatory cells invading a mucosa populated with type b) cells. Magnification X 8000.

Plate 60: EM: Phagosome (P) within a type b) cell. Aggregates of osmiophilic vesicles and small secretory droplets are present at the apex of each cell. Note the newly synthesised secretory droplets with osmiophilic inclusions near the golgi apparatus (arrowed). Magnification X 8000.

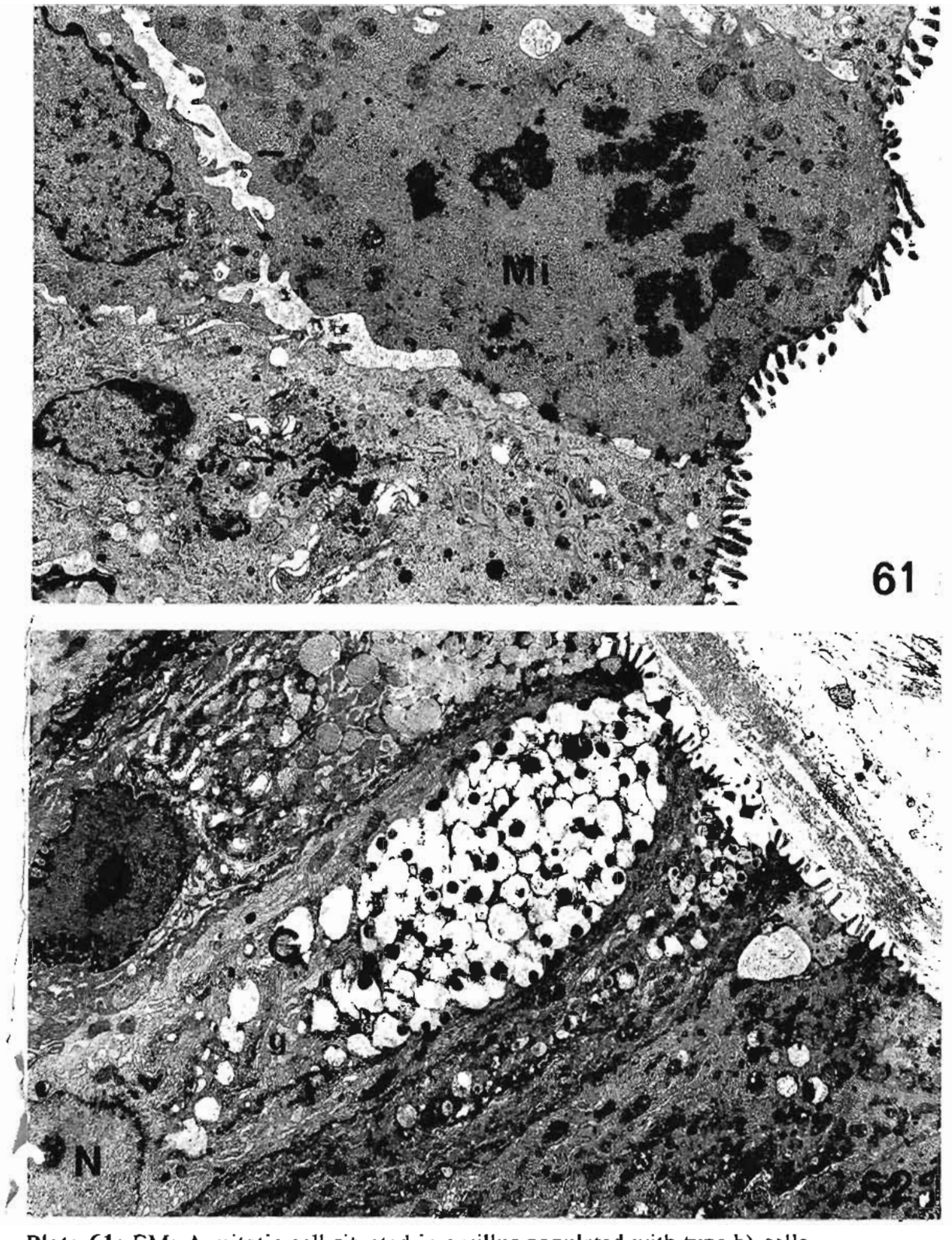


Plate 61: EM: A mitotic cell situated in a villus populated with type b) cells. Magnification X 11000.

Plate 62: EM: Cell type c): Abnormal mucus droplets in goblet cell. Note the osmiophilic inclusions are present in all mucodroplets from the newly synthesised droplets near the golgi apparatus to the more "mature" droplets at the apex of the cell. Magnification X 6500.

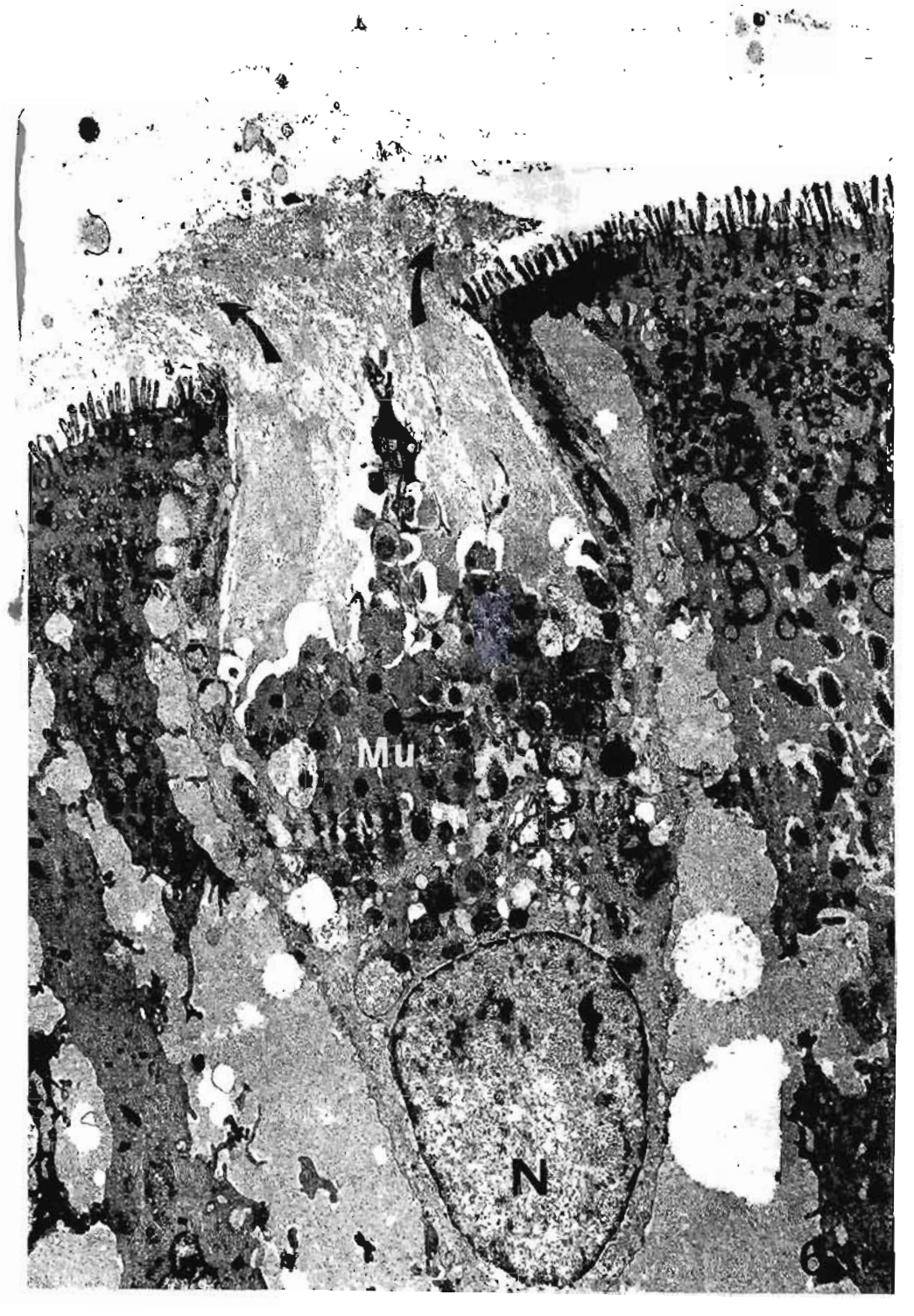


Plate 63: EM: Cell type c): Goblet cell containing morphologically "abnormal" mucus droplets within which are osmiophilic inclusions. These cells are usually found in association with type b) cells (B). Note the fusion of mucus droplets in the apex of the cell during exocytosis. Magnification X 7500.

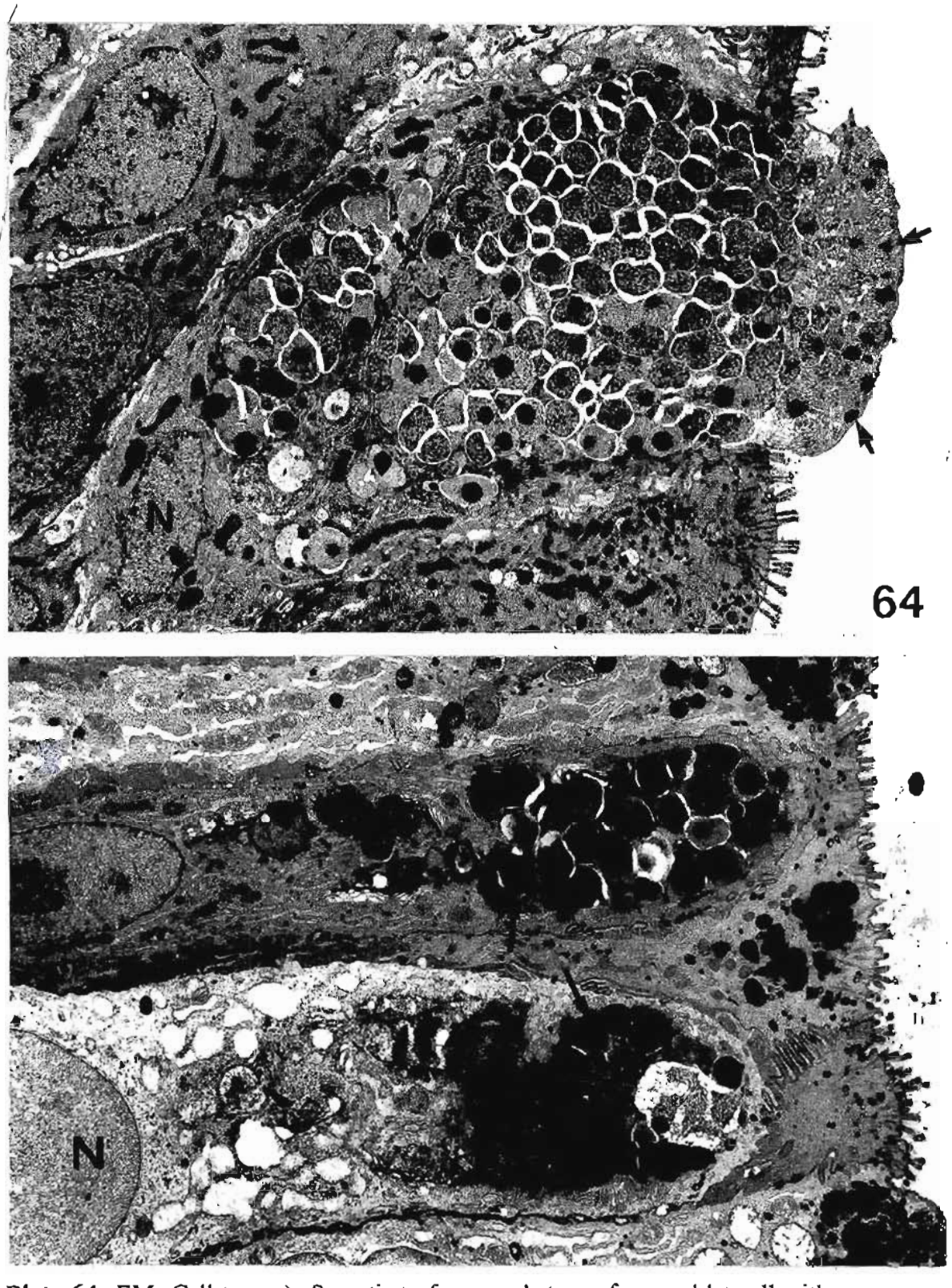


Plate 64: EM: Cell type c): Secretion of mucosubstance from goblet cell with morphologically abnormal mucus droplets. Note the osmiophilic inclusions are still present in the exocytosed mucus. Magnification X 6500.

Plate 65: Elongated immature goblet cells within the crypts of Lieberkuhn in specimens whose villi were populated with MSC in various stages of differentiation. Note the osmiophilic inclusions within the secretory granules (arrowed). Magnification X 8000

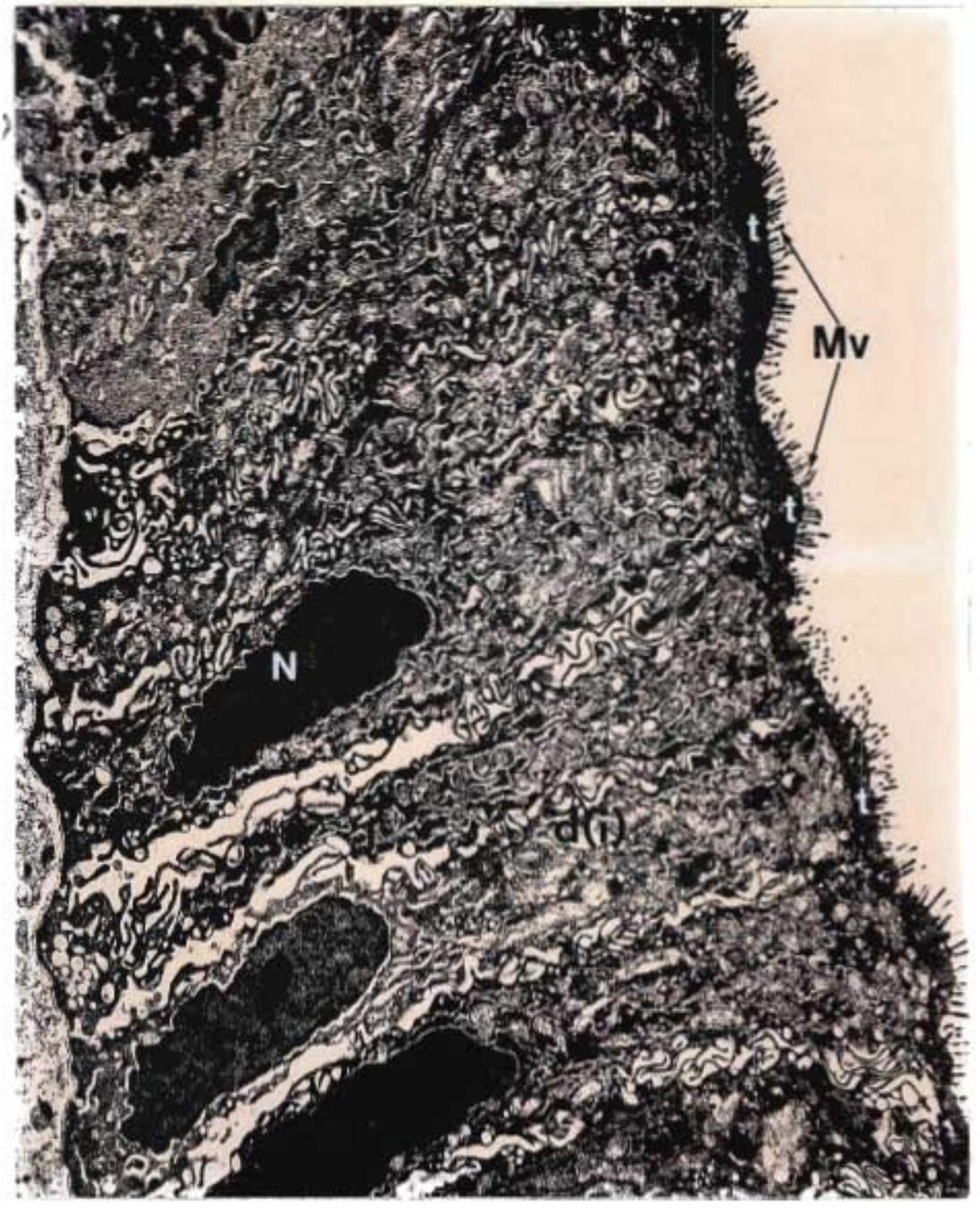
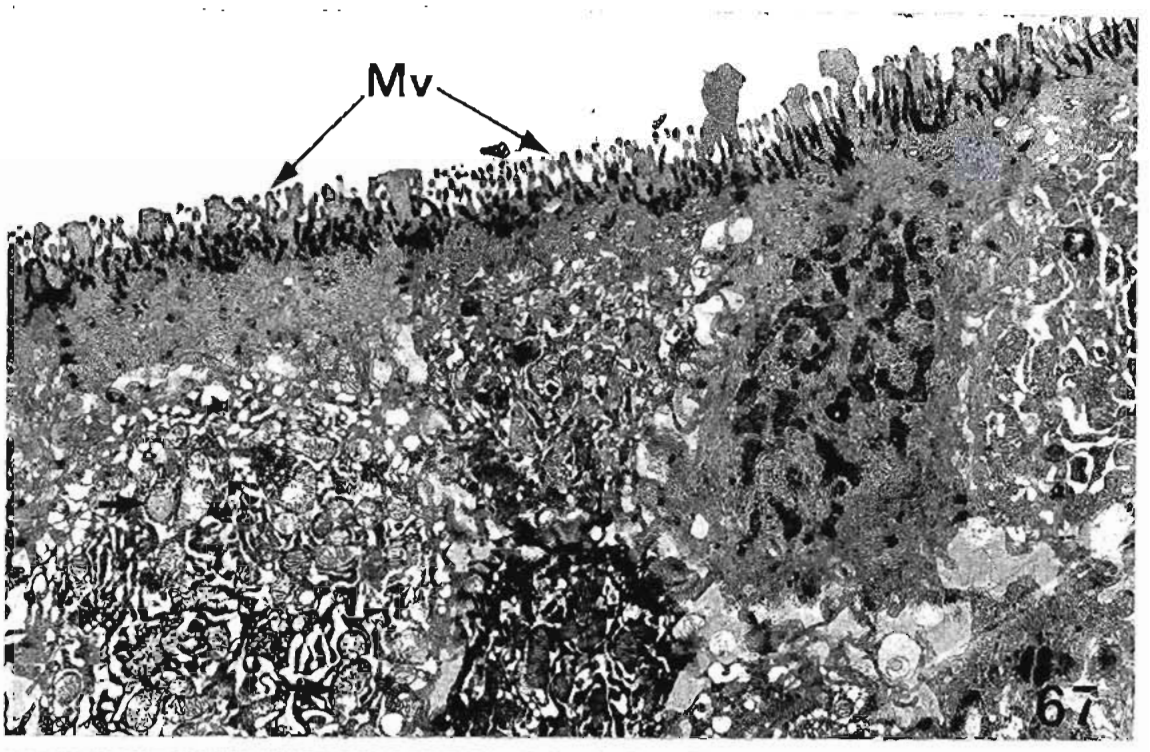


Plate 66: EM: Cell type d(i): The nuclei are crenated, the Mv short and quite sparse and they do not have a glycocalyx. The cytoplasm has condensed and is particularly electron dense in the position of the terminal web (t). The rough endoplasmic reticulum is dilated. Numerous interdigitating pseudopodia project from the lateral plasmalemma into the intercellular spaces. Magnification X 6500.



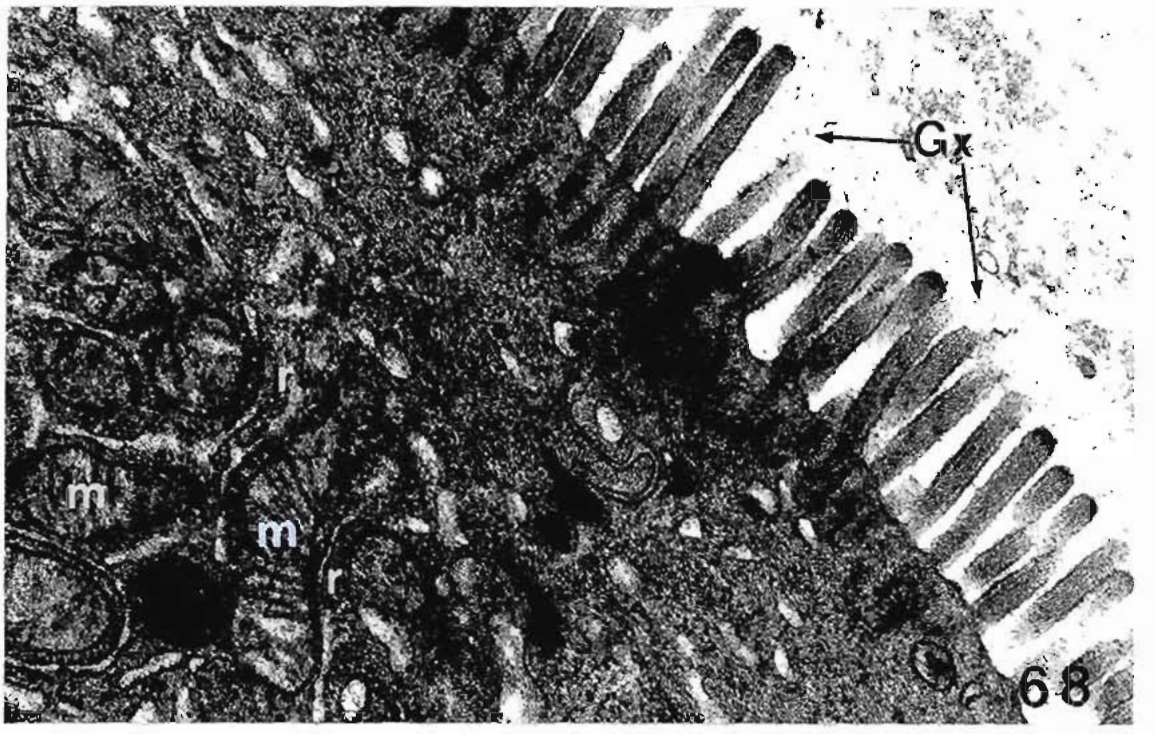
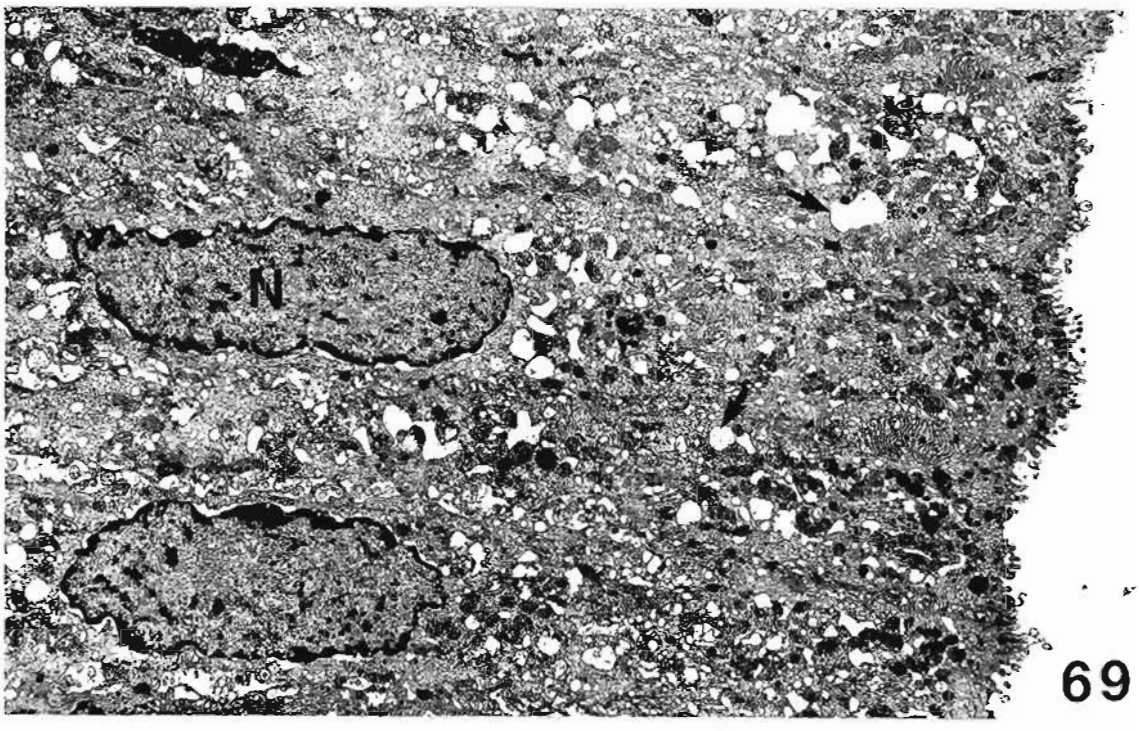


Plate 67: EM: Cell type d(i): Microvilli have fused to form cytoplasmic protrusions that project up to 3um into the lumen. The glycocalyx is absent. Note the electron-pale, swollen mitochondria in cells with dilated RER (arrowed) and the more normal mitochondria in less "vacuolated" cells.
Magnification X 8000.

Plate 68: EM: Cell type d(i): Detail of Mv projecting from a type d(i) cell with swollen RER. Note the presence of a "whispy", fragmented glycocalyx projecting from the Mv. Also note the whorls of RER surrounding mitochondria.
Magnification X 42000.



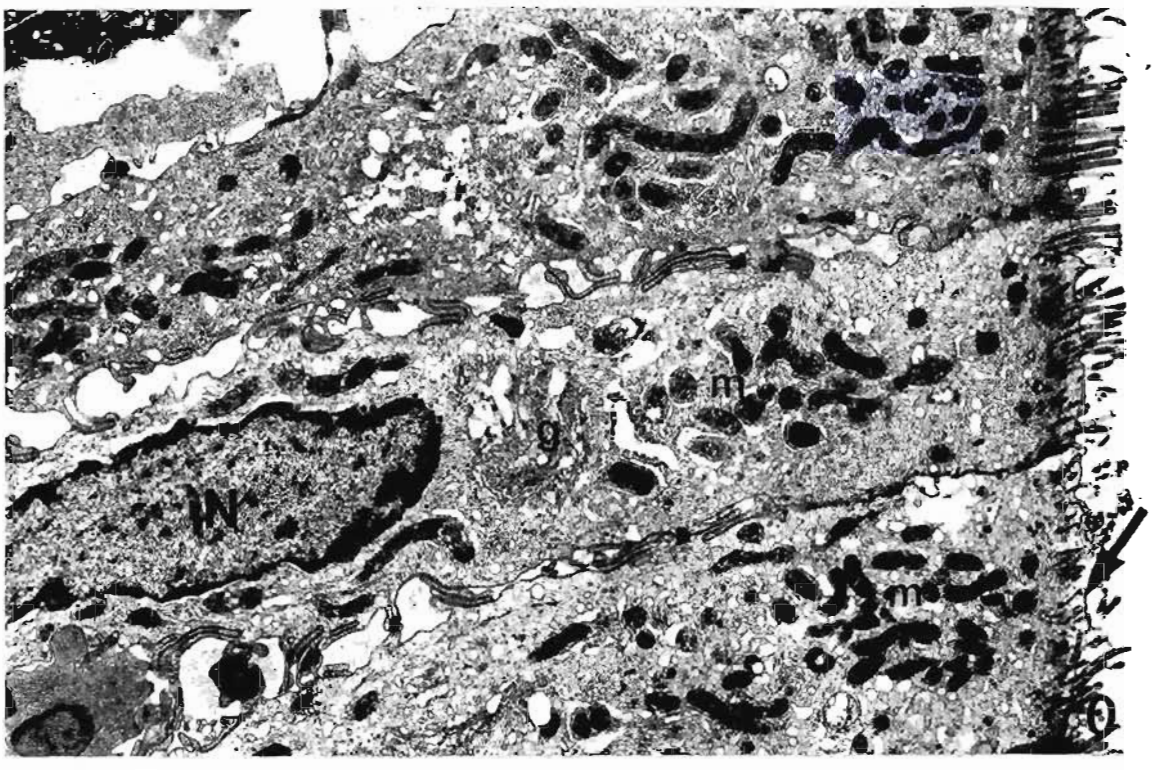
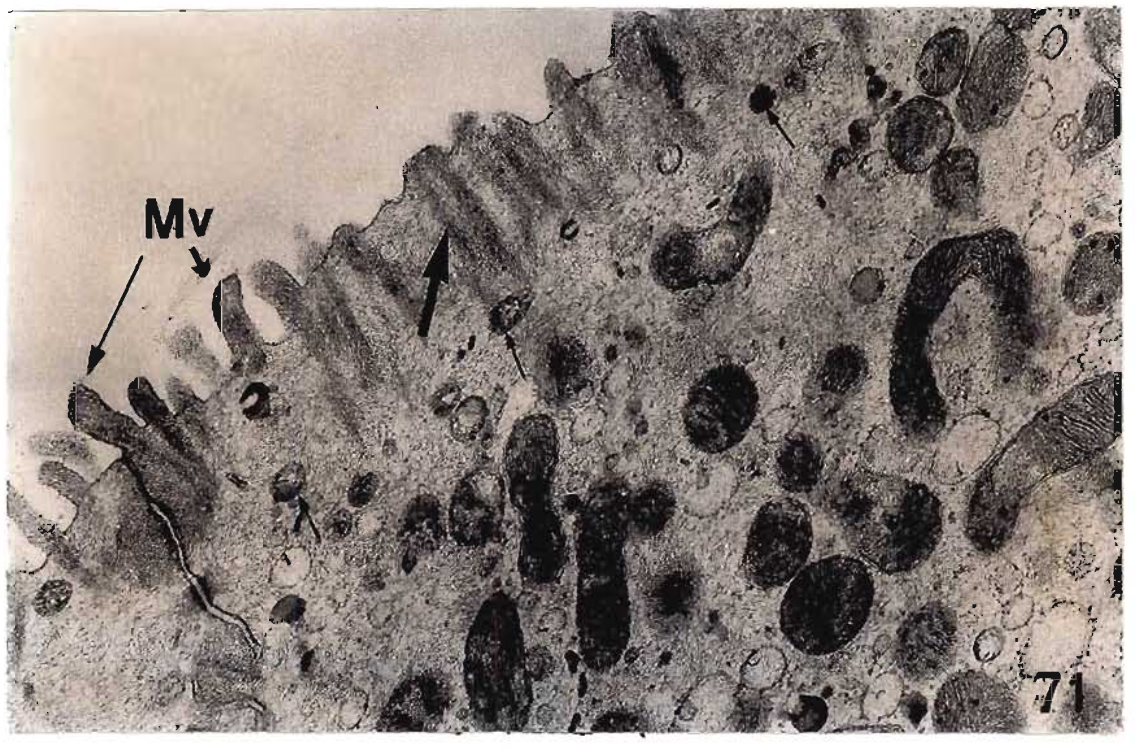


Plate 69: EM: Cell type d(i): An example of a type d(i) cell with less condensation of the cytoplasm. Elements of the RER and SER and golgi apparatus are extensively dilated (arrowed). The microvilli are short and in places, absent from the cell surface. There is no glycocalyx projecting from Mv. Magnification X 7000.

Plate 70: EM: Cell type d(i): An example of a type d(i) cell with minimal dilation of RER cisternae. The mitochondria appear normal. The Mv are generally longer than in Fig.69 but there are still areas where Mv are absent from the cell surface (arrowed). No glycocalyx is visible. Magnification X 8500.



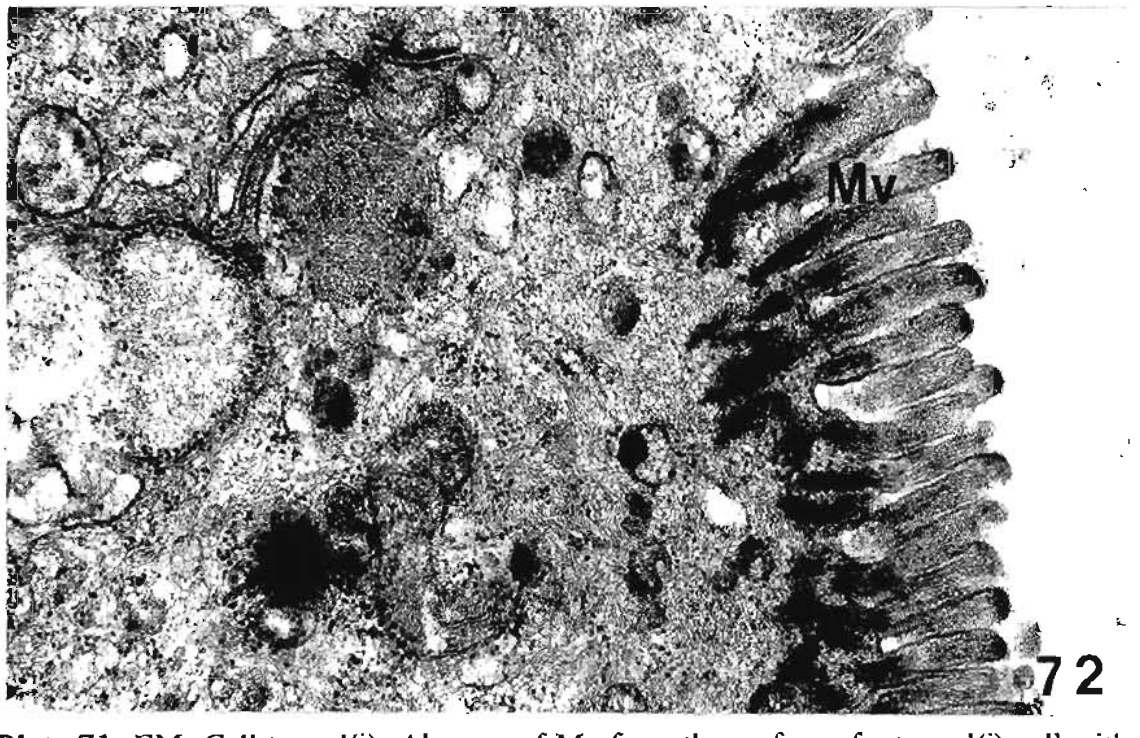


Plate 71: EM: Cell type d(i): Absence of Mv from the surface of a type d(i) cell with minimal RER dilation. Note the rootlets of absent Mv in the terminal web (arrowed). Also occasional EDV in the sub-terminal web cytoplasm (small arrows). Magnification X 40000.

Plate 72: EM: Cell type d(i): Densely packed Mv projecting from the surface of a cell with moderate RER dilation. Note absence of glycocalyx. Magnification X 50000.

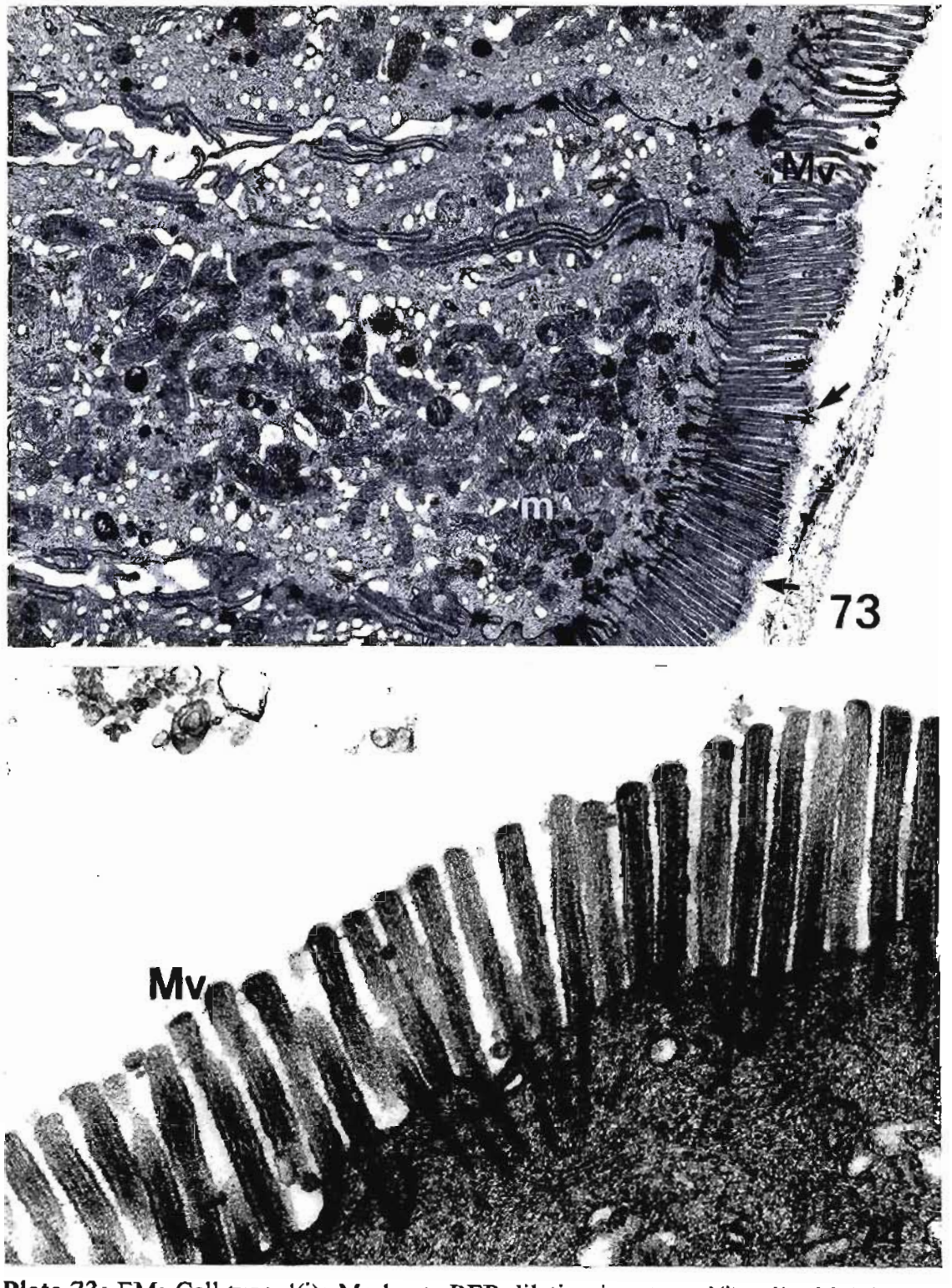
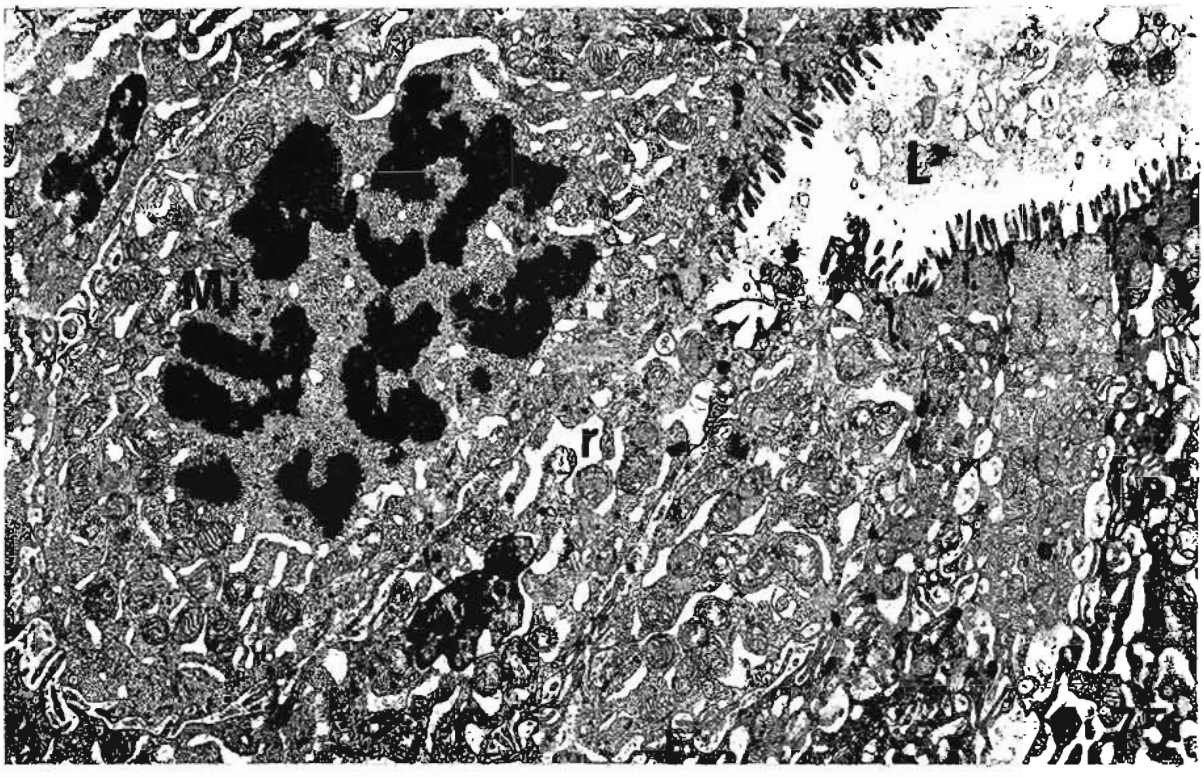


Plate 73: EM: Cell type d(i): Moderate RER dilation in a type d(i) cell with Mv of normal length. The glycocalyx is present over some areas (arrowed) and absent over others. Magnification X 12500.

Plate 74: EM: Cell type d(i): Detail of Mv from a type d(i) cell with moderate RER dilation. Note the glycocalyx is absent. Magnification X 46000.



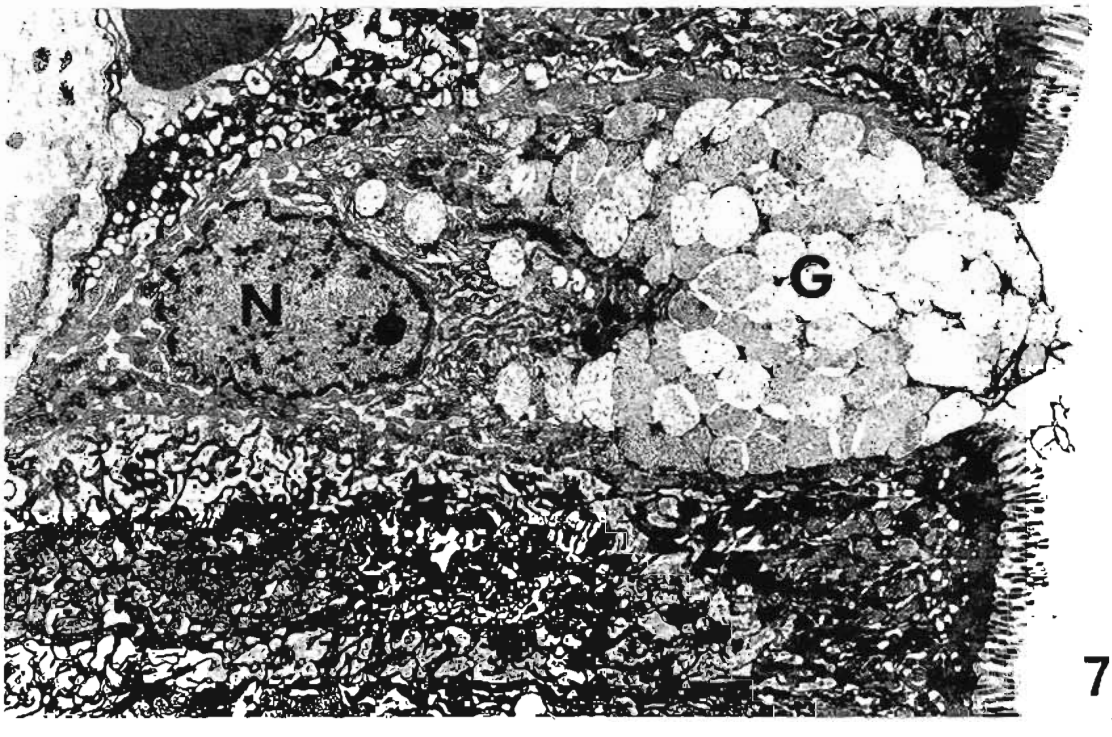


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Plate 75: EM: Mitotic cell in the germinal region of a crypt of Lieberkuhn at the base of a villus populated with type d(i) cells. Note that all crypt cells have dilated RER. Magnification X 10000.

Plate 76: EM: A goblet cell with normal mucus in a villus mucosa populated with type d(i) cells. Magnification X 7500.

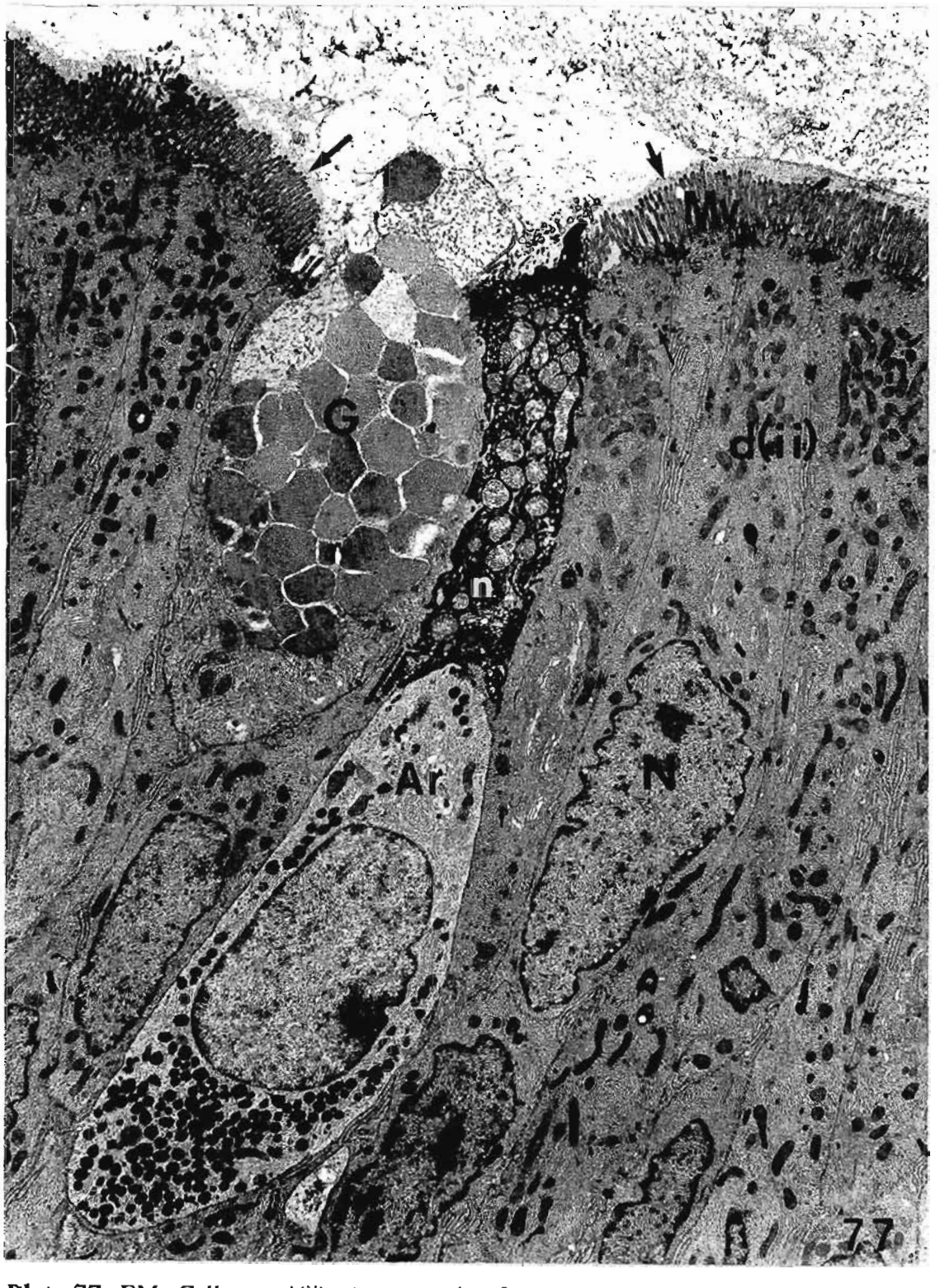
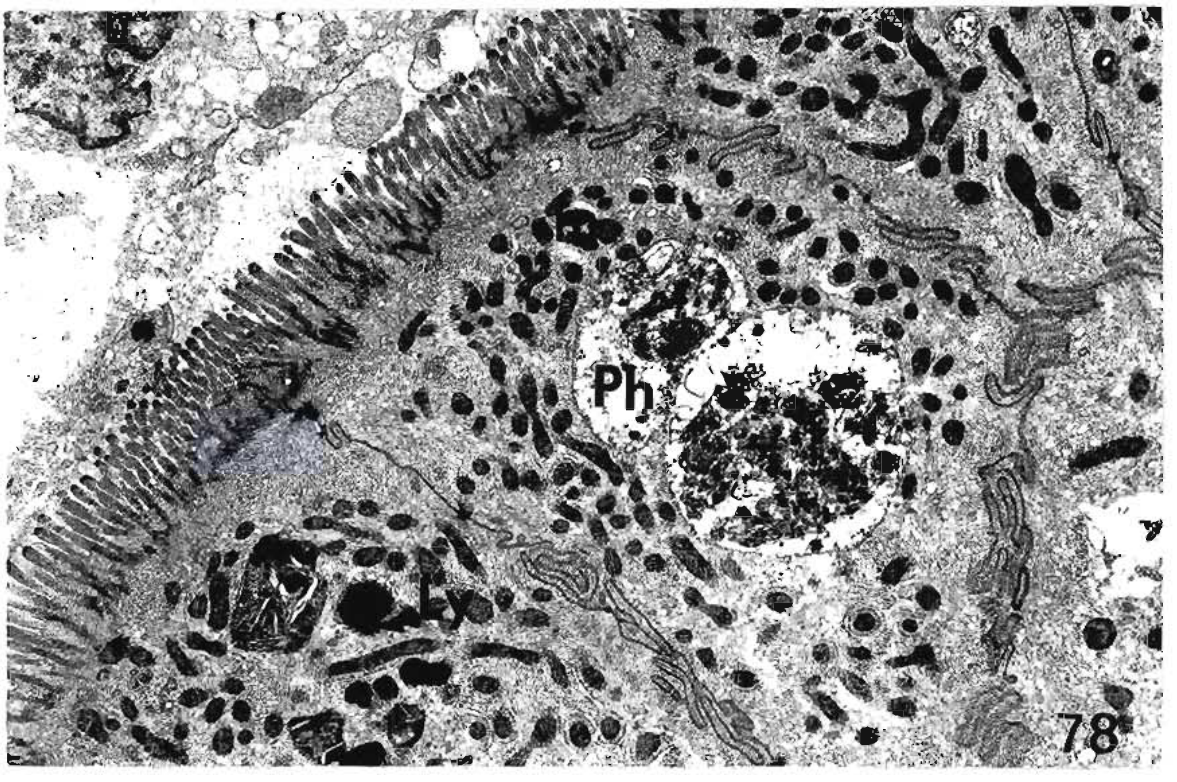


Plate 77: EM: Cell type d(ii): An example of near normal absorptive cells in an ulcerative mucosa. There is a necrotic cell (n) sandwiched between an absorptive and goblet cell. Also in this photograph is a rare example of an enteroendocrine cell (Ar) in the villous mucosa. Note the long, densely packed Mv from which emanates a thick glycocalyx (arrowed), also occasional EDV (small arrows). Magnification X 6250.



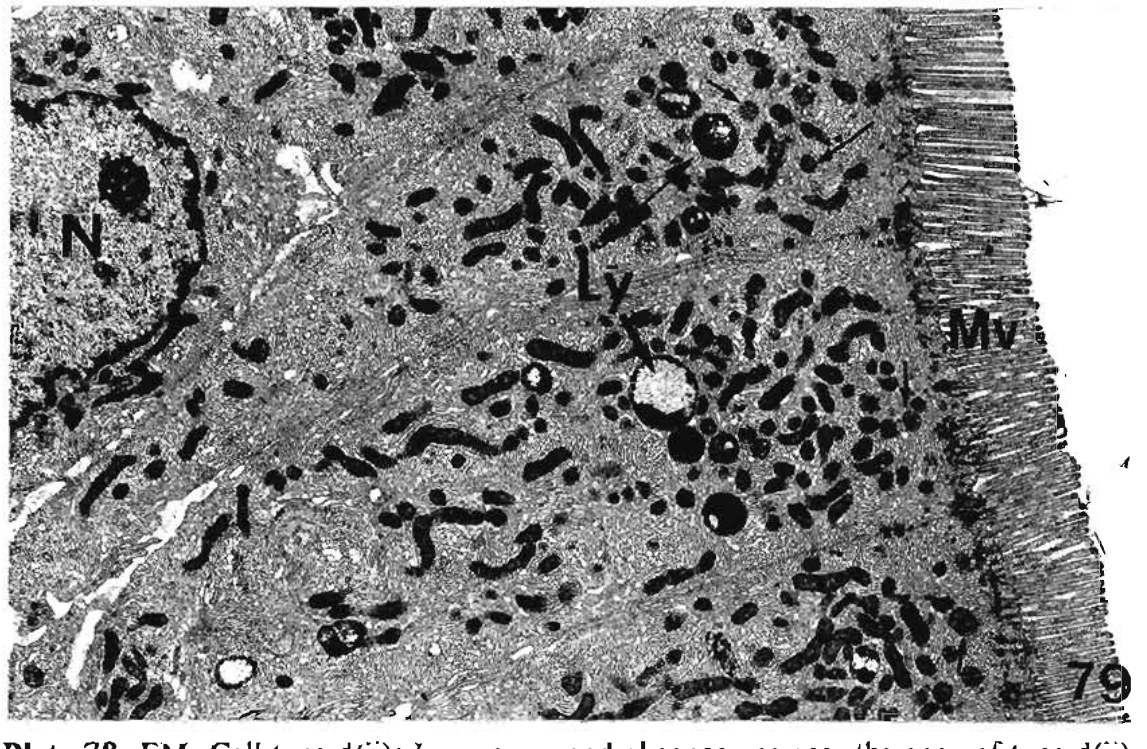
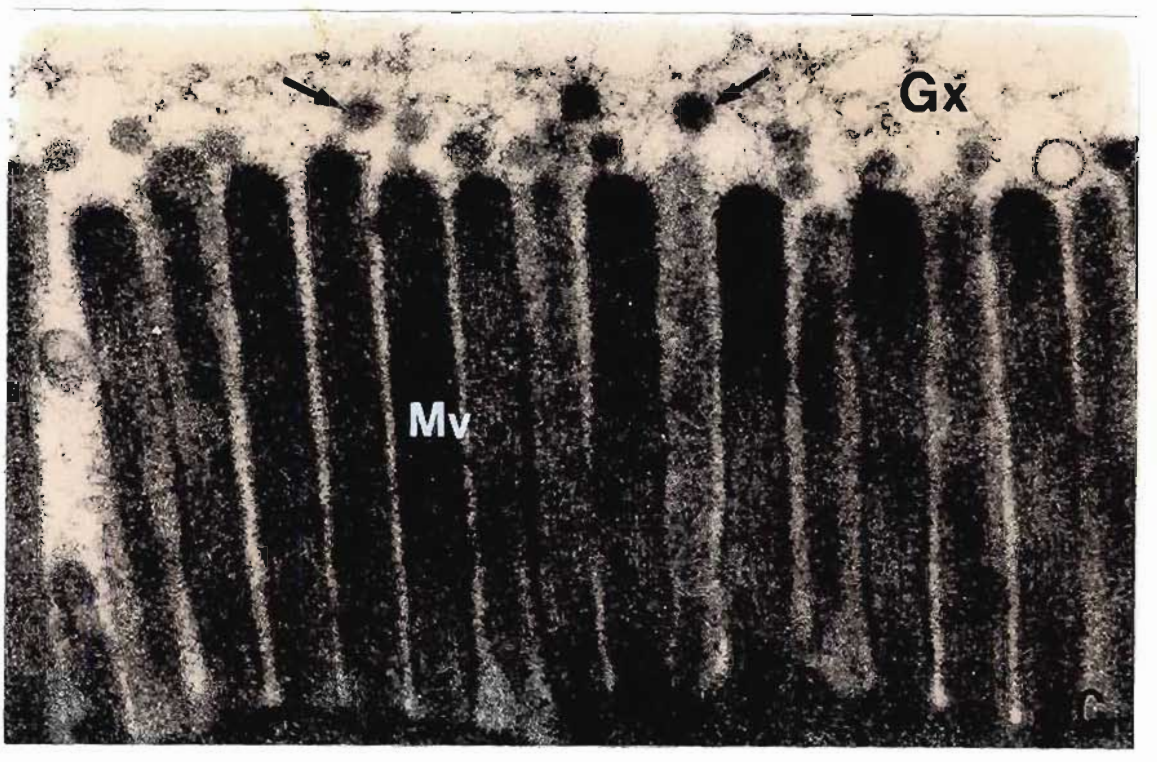


Plate 78: EM: Cell type d(ii): Lysosomes and phagosomes near the apex of type d(ii) cells. The mitochondria are normal in these cells. Note the absence of a well defined glycocalyx. Magnification X 15000.

Plate 79: EM: Cell type d(ii): Long, densely packed Mv from which there is no discernible glycocalyx. Note lysosomes and multivesicular bodies (small arrows) near the apices of cells. Magnification X 9000.



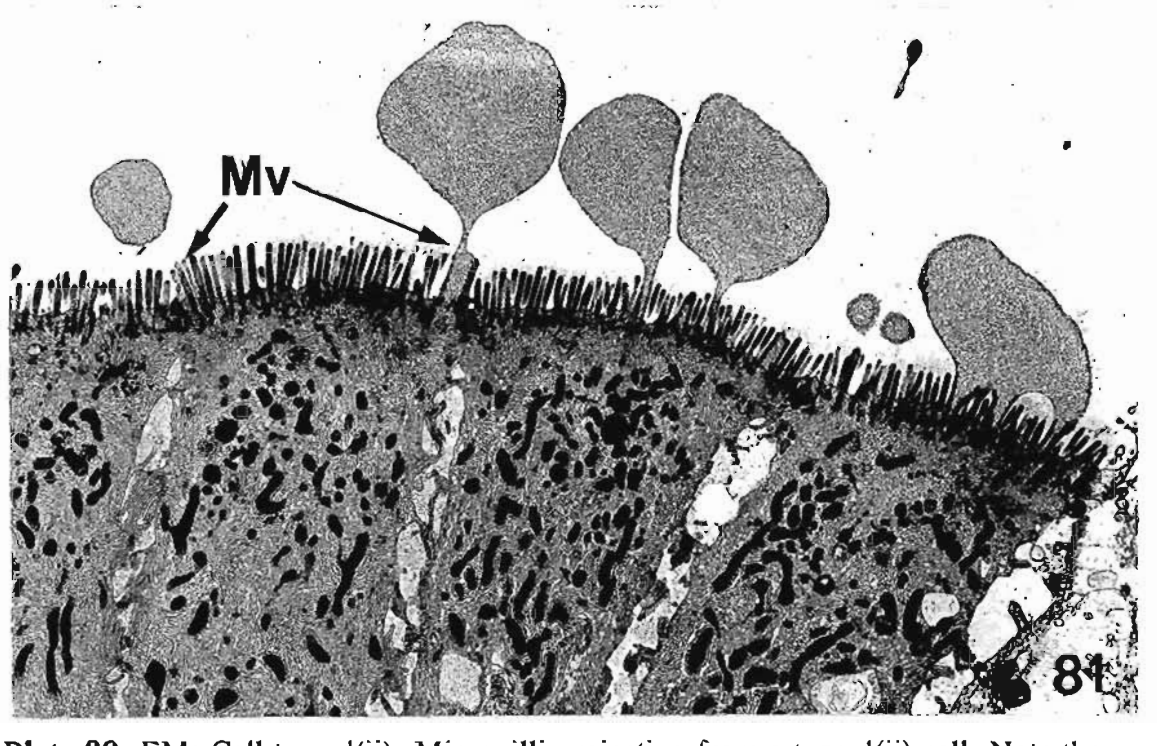


Plate 80: EM: Cell type d(ii): Microvilli projecting from a type d(ii) cell. Note the "whispy" glycocalyx within which are numerous glycocalyceal bodies (arrowed). Magnification X 90000.

Figure 81: EM: Cell type d(ii): Microvilli have fused and balloons of cytoplasm project up to 5um in to the lumen. Note absence of glycocalyx and presence of occasional EDV (small arrows). Magnification X 8000.

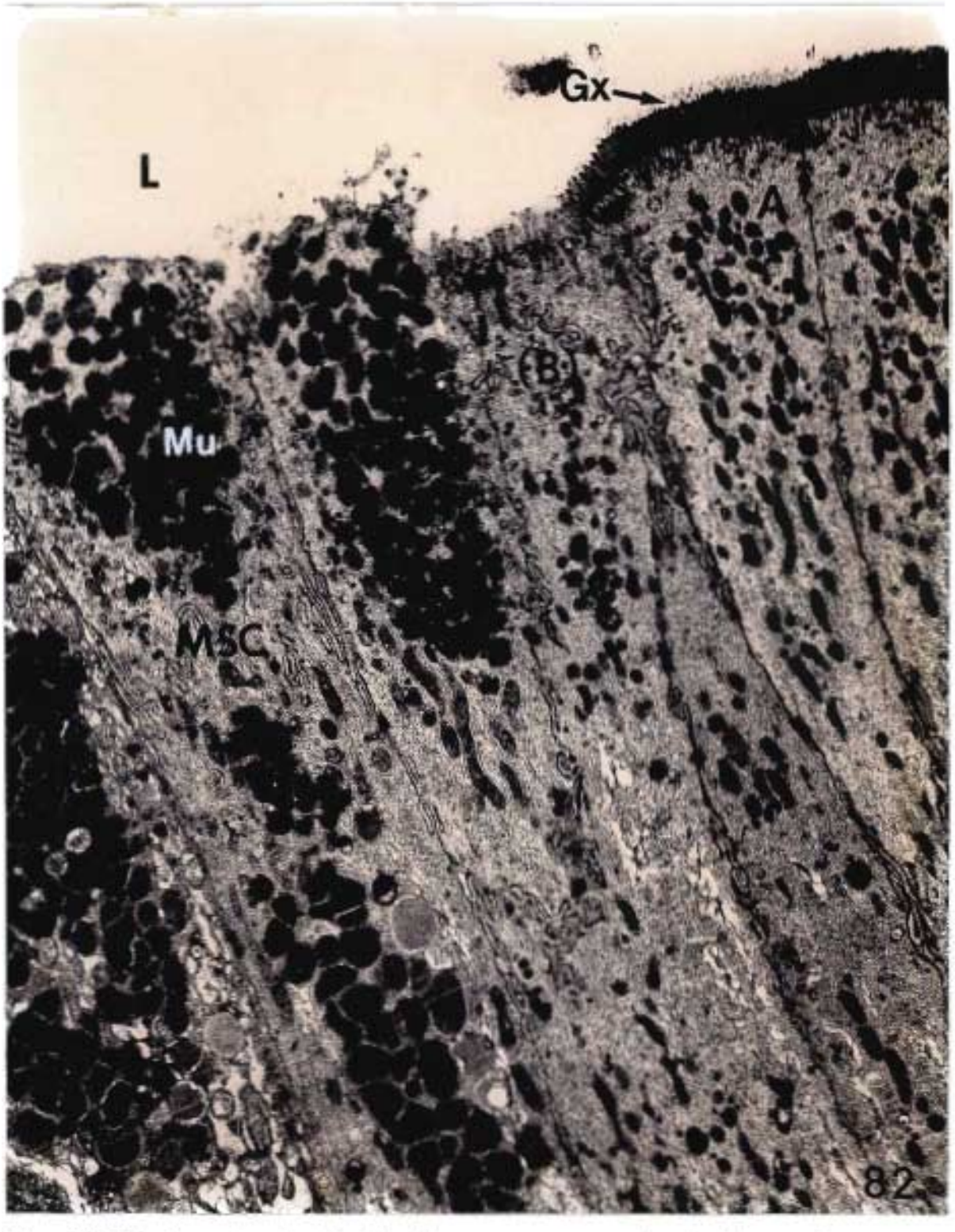
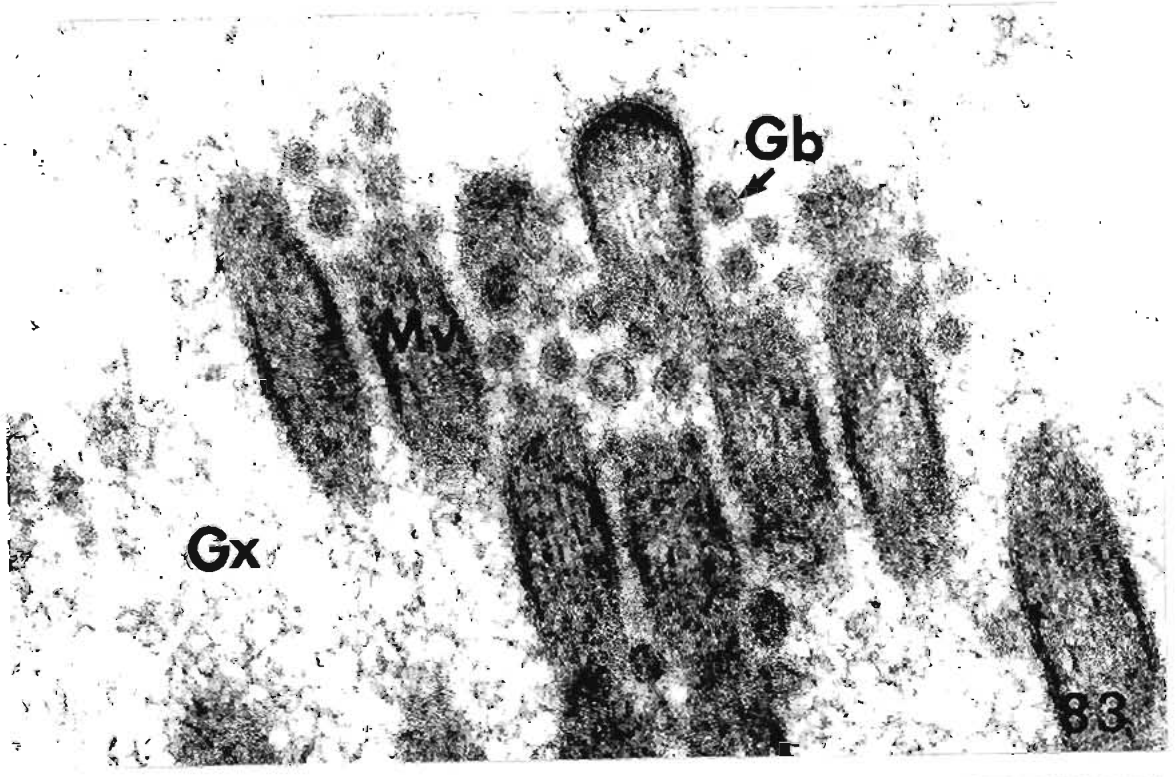


Plate 82: EM: Example of an interface between an area populated with normal absorptive cells (A) and one populated with well differentiated MSC. Note the type b) cell (B) between the absorptive and MSC. Magnification X 7000.

5.3.3.2. Glycocalyceal Bodies

Glycocalyceal bodies were seen as spherical/ovoid hollow vesicles and enclosd by a tri-laminar unit membrane. Ranging in diameter from 30nm to 70nm (Plate 83), these vesicles were similar in both size and structure to those within multivesicular bodies (Plate 84). Glycocalyceal bodies occurred in rows or clusters and lay in the glycocalyx investing the microvilli of partially differentiated MSC (type b cells - Plate 85) and some abnormal absorptive cells (type d(ii) - Plate 86). Multivesicular bodies were present in varying numbers in many normal absorptive cells (Plates 13 and 19) and some types di) (Plate 73), dii) (Plates 77, 79 and 81) and b) cells (Plate 57). In some instances, the limiting membrane of the multivesicular body appeared to have fused with the luminal plasmalemma of the enterocyte thereby releasing their vesicles into the extracellular space (Plate 87). In others, ruptured, exocytosed multivesicular bodies were present in the mucus adhering to the surface of microvilli (Plate 88).



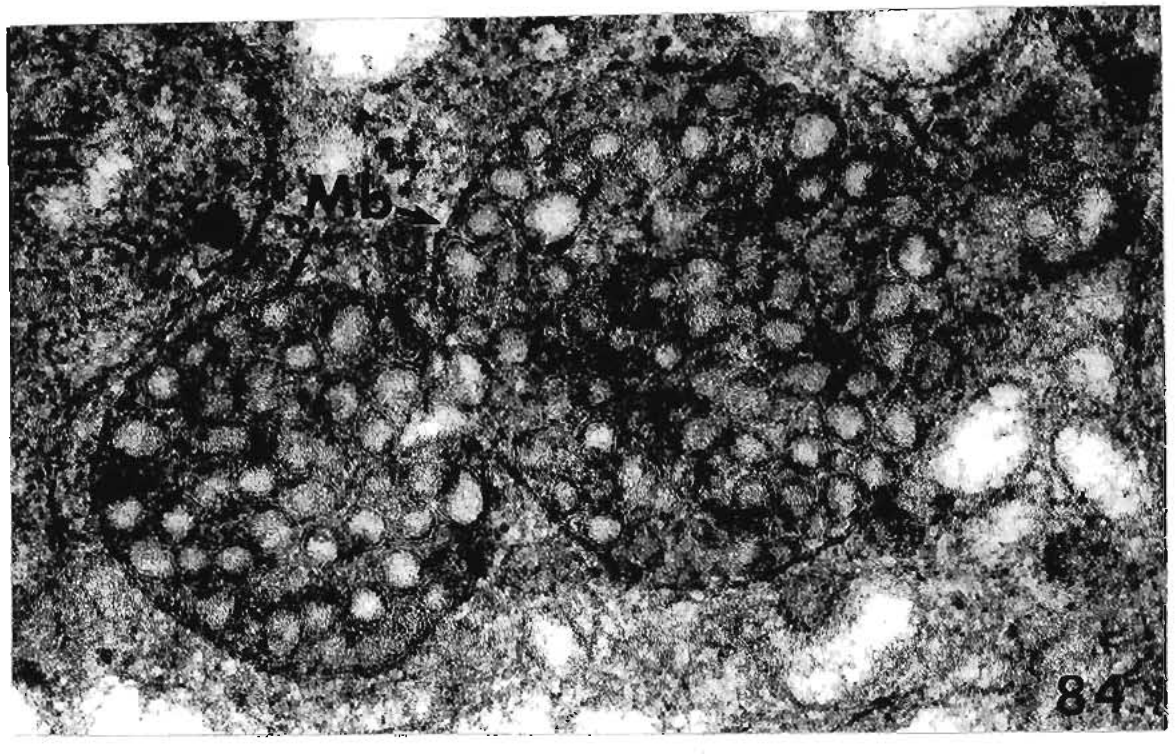
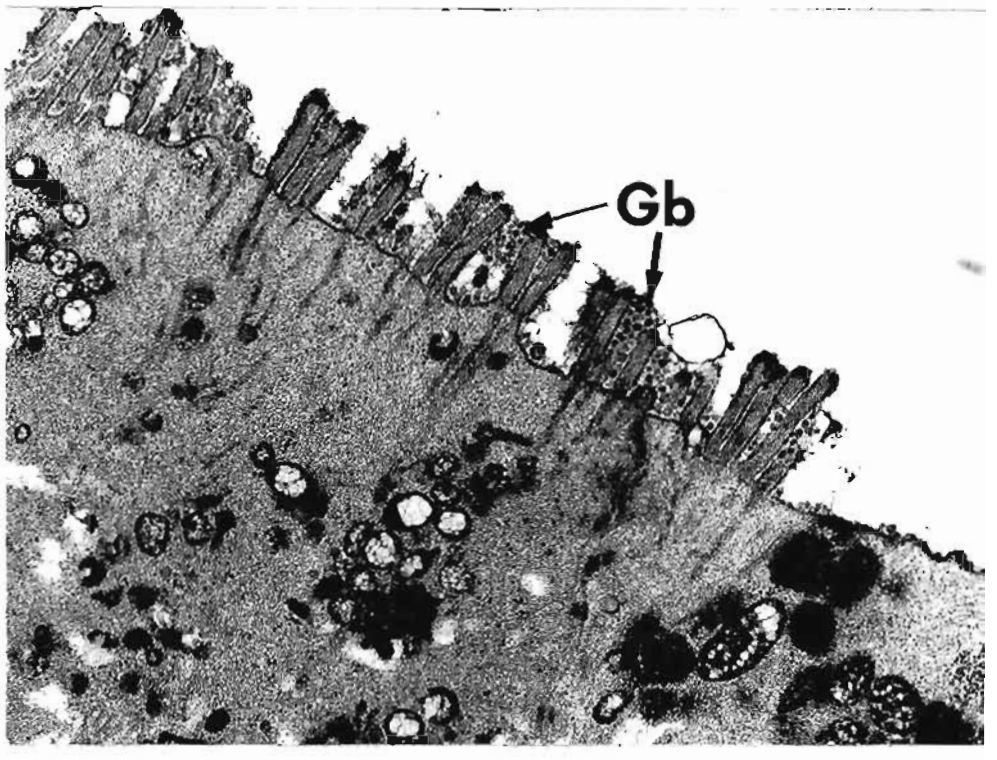


Plate 83: EM: Detail of glycocalyceal bodies in the glycocalyx associated with a type d(ii) cell. Magnification X 140000.

Plate 84: EM: Multivesicular bodies (Mb) near the apex of a type d(ii) cell. Note the vesicles within the the Mb are of similar sizes to the glycocalyceal bodies shown in Figure 83. Magnification X 140000.



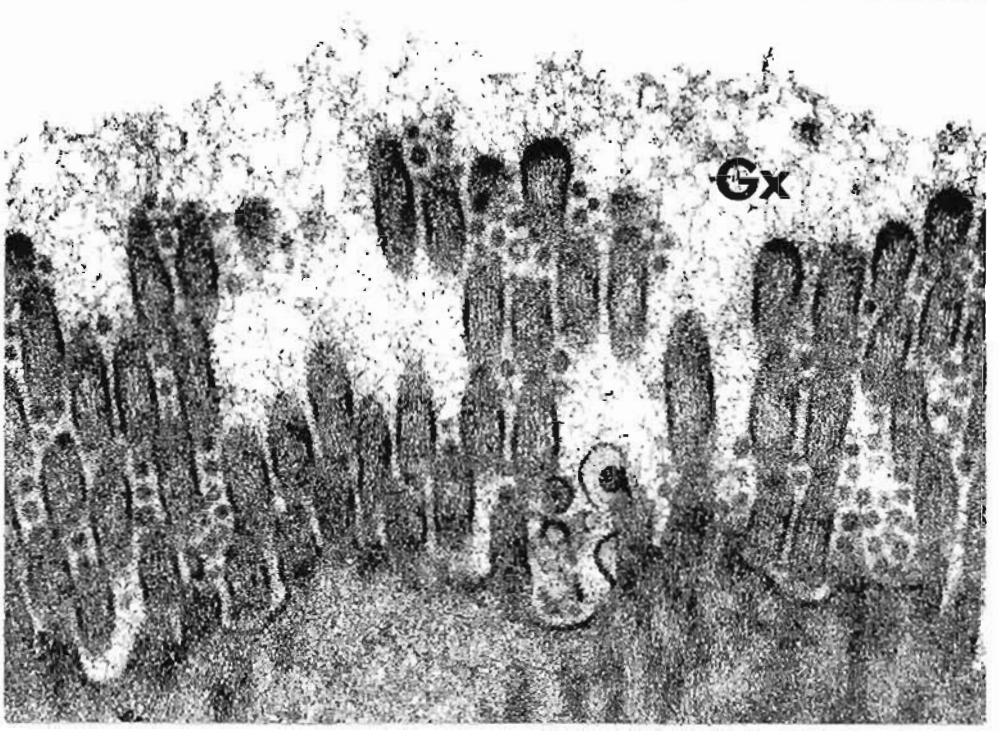
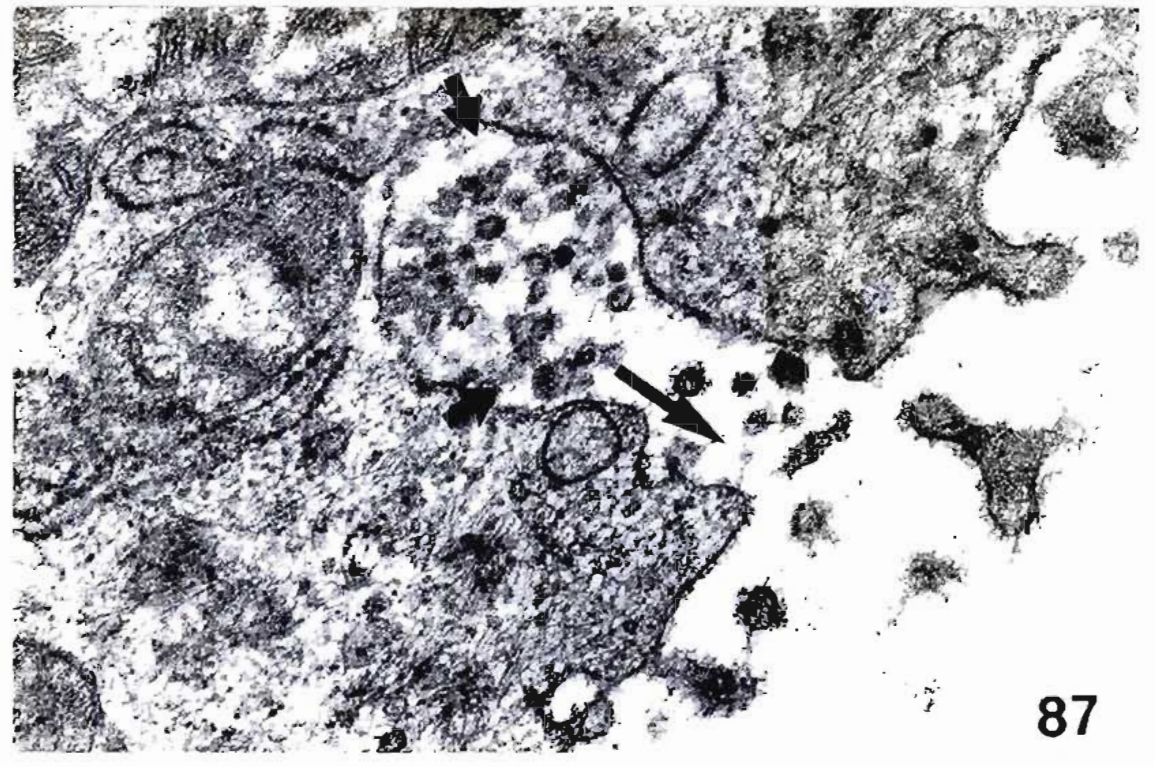


Plate 85: EM: Glycocalyceal bodies investing the fragmented glycocalyx of a type b) cell. Magnification X 25000.

Plate 86: EM: Glycocalyceal bodies investing the glycocalyx of a type d(ii) cell. Magnification X 62000.



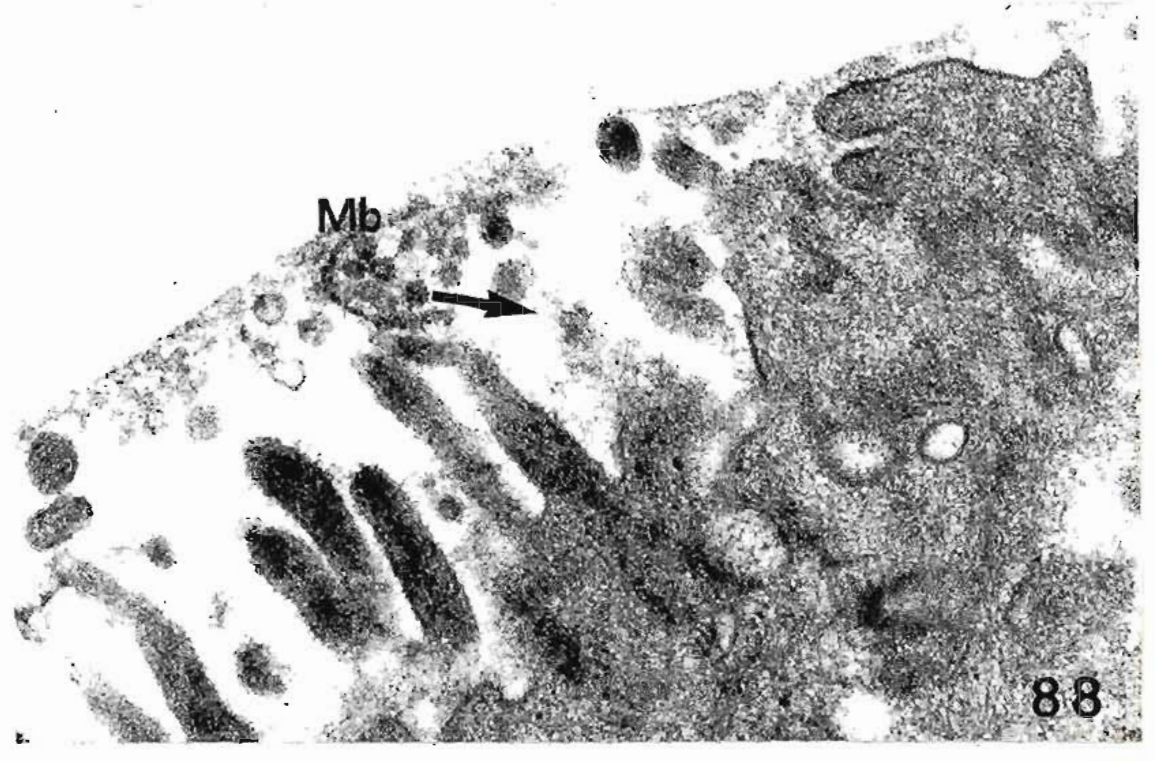


Plate 87: EM: Vesicles within an invagination of the plasmalemma of a type d(ii) cell. It is possible that the membrane limiting the multivesicular body has fused with the plasmalemma thereby releasing vesicles into the lumen (arrowed).
Magnification X 80000.

Plate 88: EM: A ruptured multivesicular body releasing vesicles into the glycocalyx of a type d(i) cell. Magnification X 70000.

5.3.3.3. Helicobacter Pylori.

Most personal observations on the bacilli which in retrospect were found to be Helicobacter pylori were made prior to the discovery and classification of the organism. H. pylori, either singly or in groups were found in approximately 50% of cases. The bacilli were sometimes densely packed over the mucosal surface (Plate 89). They were, however, only found in association with well differentiated MSC in specimens with a metaplastic mucosa. H.pyolri were from 1.5μm - 2.5μm in length and approximately 0,5μm in diameter and were characterised by a single "twist" over their length (Plate 90). While sometimes in intimate contact with the outer lamella of the enterocyte plasmalemma (Plate 90), they were usually found evenly distributed through the full thickness of the surface mucus coat (Plate 91). While generally remaining outside the epithelium, in some instances H.pylori had infiltrated the upper intercellular spaces between enterocytes (Plate 92). Here they appeared to have disrupted the lateral cell junctions. Very occasionally, H.pylori had penetrated further between cells (Plate 93). They were never, however, observed near the basal lamina.

In patients with a well differentiated metaplastic mucosa that extended into the crypts of Lieberkuhn, H.pylori was often found in great numbers near the surface and sometimes between enterocytes (Plate 94). There was no evidence to suggest that H.pylori had infected a cell. Nor was there any evidence to suggest that metaplastic cells had ingested H.pylori.

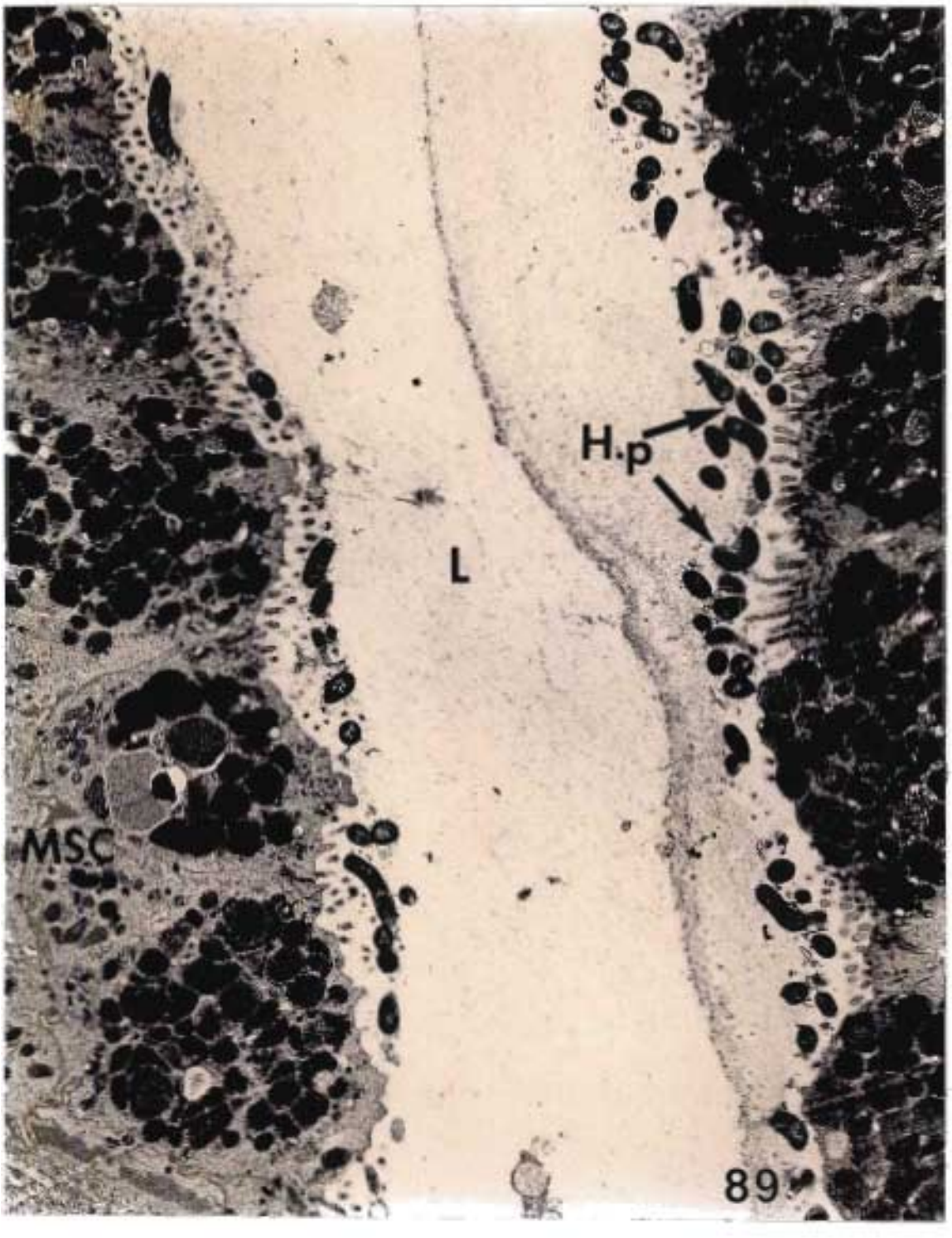


Plate 89: EM: Helicobacter pylori within the layer of mucus at the surface of well differentiated MSC. Magnification X 6000.

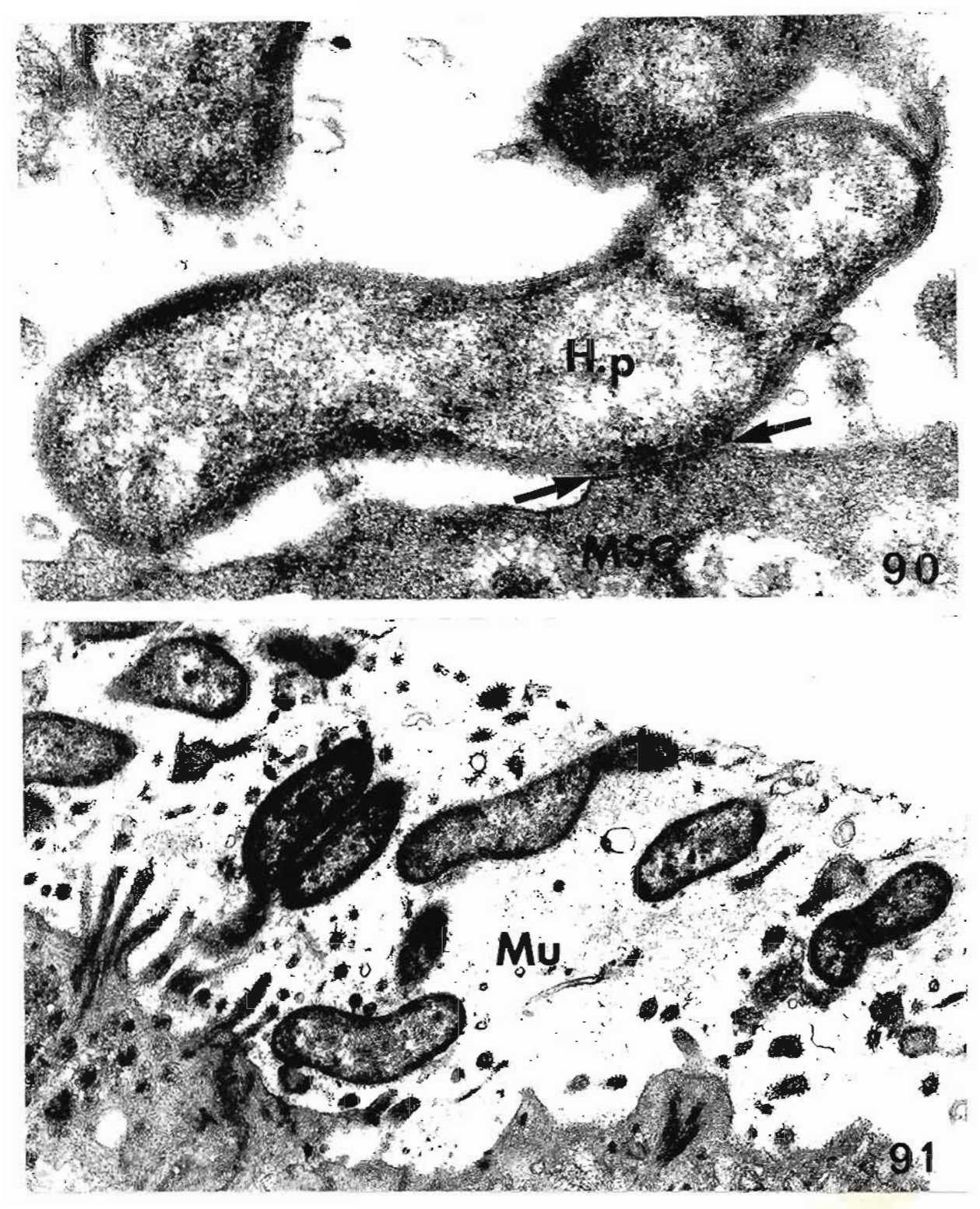


Plate 90: EM: Detail of H.pylori. This particular organism is 2.3um in length and 0.5um in diameter and is characterised by a single twist over its length. Note the close apposition between the outer membrane of the organism and the plasmalemma of the enterocyte (arrowed). Magnification X 70000.

Plate 91: EM: H.pylori distributed through the full thickness of the mucus coating the surface of a type b) enterocyte. Magnification X 22000.

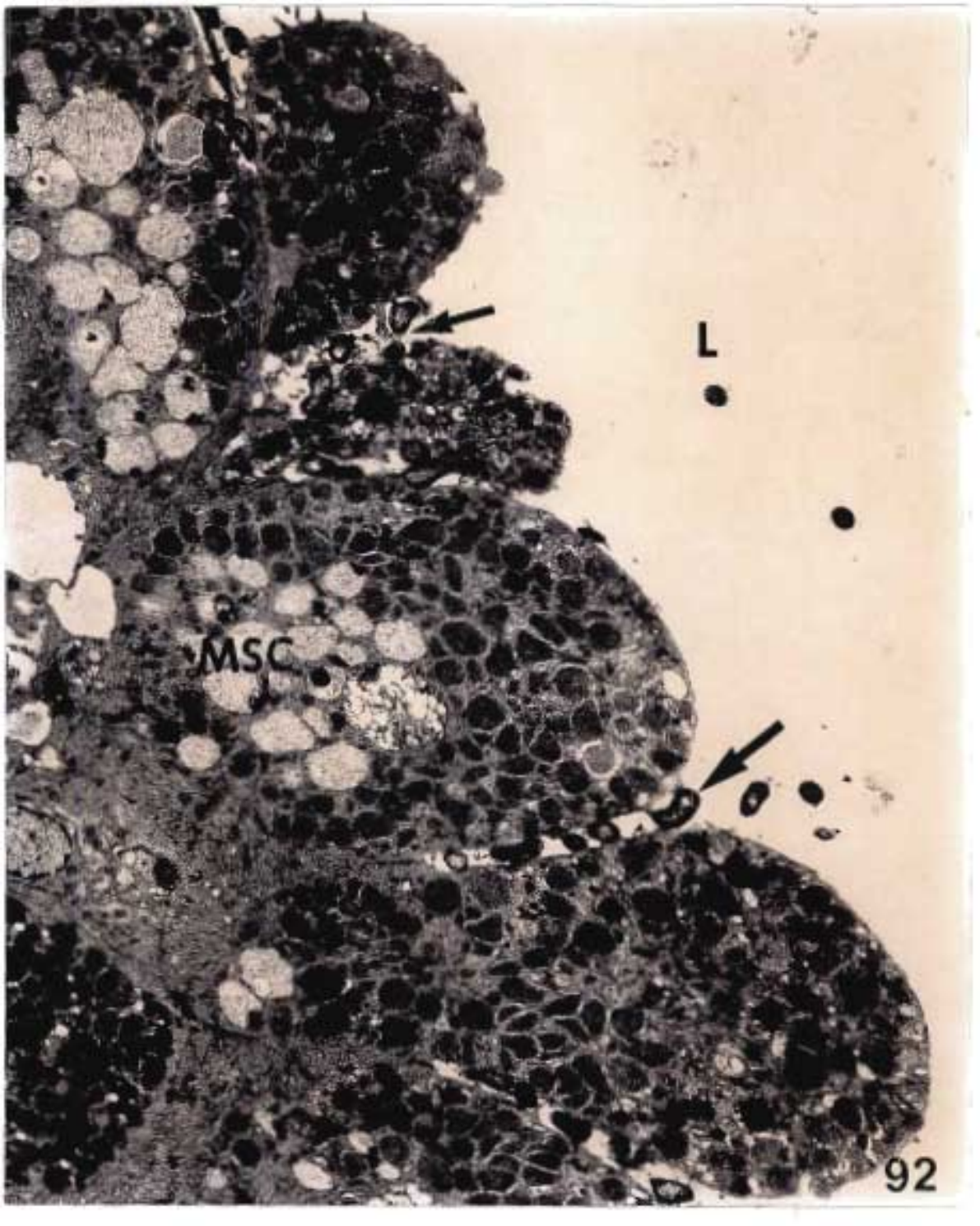


Plate 92: EM: H.pylori invading the upper intercellular spaces between well differentiated MSC (arrowed). Magnification X 7200.

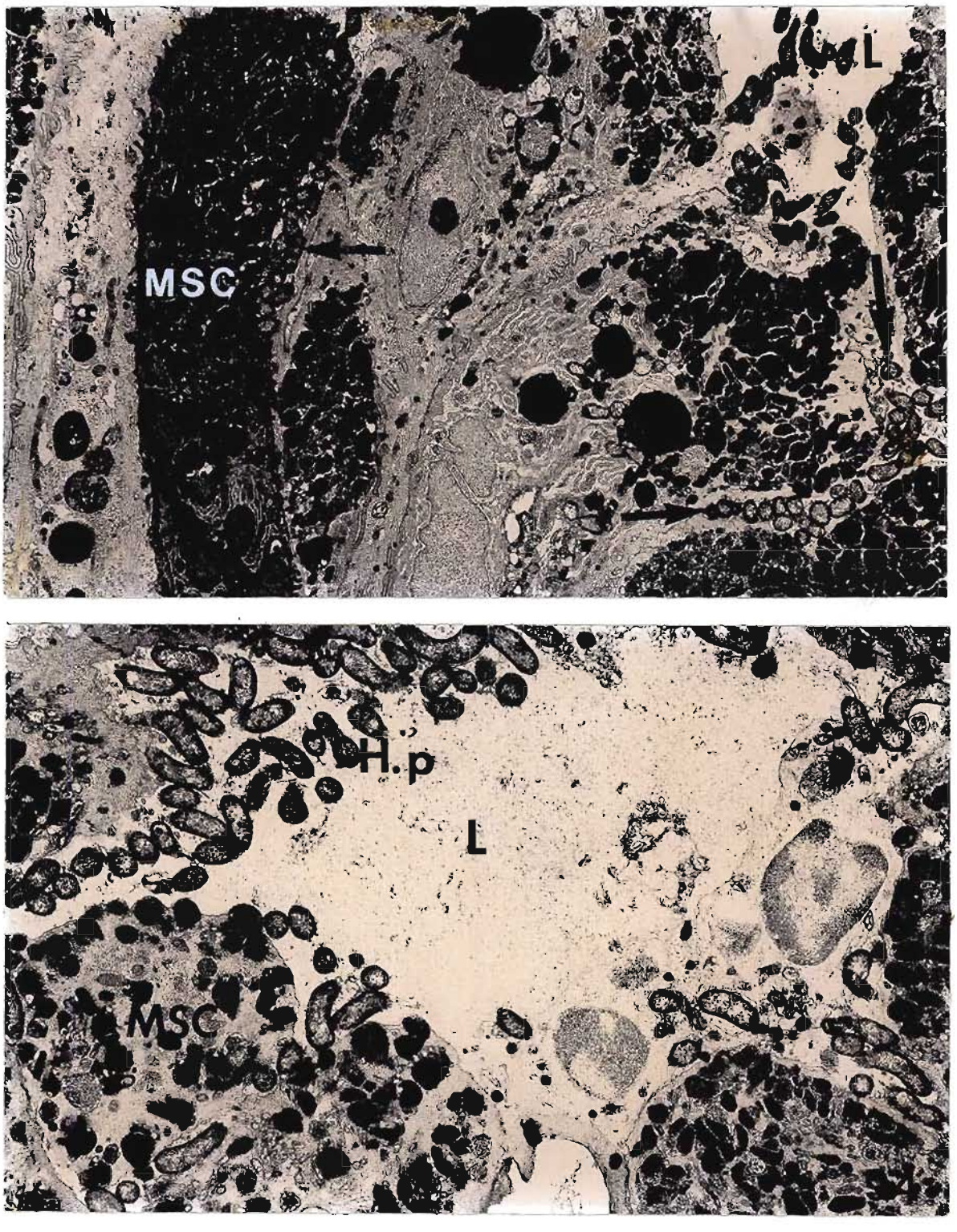


Plate 93: EM: H.pylori in the lumen and invading the deeper reaches of the intercellular spaces between well differentiated MSC (arrowed). Magnification X 6000.

Plate 94: EM: H.pylori in the lumen of a crypt of Lieberkuhn populated with MSC. Magnification X 8000.

5.4. Morphological Classification of the Juxta-DU Mucosa

Electron microscopy revealed the juxta-DU mucosa to be populated by one or more of 7 morphologically distinct cell "types" (see 5.3.3. p.90). Of particular interest was the observation that certain cell types were regularly associated with one another. Based on the presence and fine structure of individual cell types and their association with each other, the specimens that had been divided into two histological groups: metaplastic and non-metaplastic, were further subdivided into the following three morphological classes.

Metaplastic Specimens (Histological group 1)

MA: Entirely metaplastic: These specimens were populated entirely with metaplastic cells. Well differentiated, actively secreting type a) cells predominated. They were interspersed with occasional type b) cells.

MB: Partially metaplastic: These specimens contained some type a), many type b) and some type c) cells. Type c) cells consistently featured in these specimens. The epithelium also contained varying numbers of type d(i), type d(ii), e) and f) cells depending on the extent of metaplasia.

Non-metaplastic Specimens (Histological group 2)

NM: Non-metaplastic (degenerative): These specimens were primarily populated with type d(i) and f) cells interspersed with occasional type d(ii) and some type e) cells.

The presence and proportion of cell types in each morphological class is described in Table III.

Class	Non-Metaplastic		Metaplastic	
	Normal	NM	MA	MB
Cell type				
a	-	-	XXX	X
b	-	-	X	XXX
c	-	-	-	XX
d(i)	X	XXX	-	X
d(ii)	X	X	-	X
e	XXX	X	-	X
f	XX	XX	-	X

Table III: Morphological classification of the juxta-DU mucosa. The distribution of cells populating normal control biopsies is included for comparison with those populating non-metaplastic (NM) specimens.

Normal = distribution of cells in normal control specimens (5.1.2. p. 60).
NM = " " " in degenerative non-metaplastic specimens.
MA = " " " in entirely metaplastic specimens
MB = " " " in partially metaplastic specimens
X - XXX= presence and relative number of cells of a particular type in specimens of a particular morphological class.
- = cell type not present in this morphological class of mucosa.

5.5. Morphological Classification of Scar Mucosa

After 6 weeks curative therapy with either sucralfate or cimetidine(1) or 4 weeks treatment with high or low dose misoprostol or cimetidine(2) the juxta-scar epithelium remained abnormal. The 7 cell types present in the villous mucosa prior to treatment were still present in the scar tissue, albeit in different proportions. Two specimens (P.No.s 53 & 54), however, appeared histologically normal. Electron microscopy revealed them to contain type d(ii), type e) and type f) cells interspersed with occasional type b) and d(i) cells. To accommodate the morphology extant in these specimens, and to encompass the full spectrum of morphological variation observed in all specimens, the ulcerative non-metaplastic mucosa was further subdivided

into degenerative (NM) and non-degenerative (ND) classes making four classes in all (see Table IV).

Class	Non-Metaplastic			Metaplastic	
	Normal	NM	ND	MA	MB
Cell Type					
a	-	-	-	XXX	X
b	-	-	X	X	XXX
c	-	-	-	-	XX
d(i)	X	XXX	X	-	X
d(ii)	X	X	XX	-	X
e	XXX	X	XX	-	X
f	XX	XX	XX	-	X

Table IV: Classification of the juxta-DU and scar mucosa. The presence and relative proportion of cells that characterise each of the four morphological classes (NM; ND; MA; and MB). The distribution of cell types in normal control biopsies is included for comparison.

ND = distribution of cells in near normal, non-degenerative specimens from scars
NM = " " " in degenerative non-metaplastic specimens.
MA = " " " in entirely metaplastic specimens
MB = " " " in partially metaplastic specimens
\- = cell type not present in this class of mucosa.

Table V shows the morphological classification of each specimen before and up to 1 year after therapy. Prior to treatment, 29 (35%) specimens were classified as MA, 33 (39%) MB and 22 (26%) NM. Of the 41 patients whose ulcers were healed after curative therapy, 1 (3%) had a scar mucosa classified as MA, 32 (80%) were MB, 5 (12%) were NM and 2 (5%) were ND.

5.6 Morphological Changes Associated With DU Healing And Remission After Treatment With Cimetidine, Sucralfate or Misoprostol

Differences in juxta-DU and scar morphology were observed during and after healing. No obvious drug-specific changes

P	D	H±	SL	S	DH	PT	TT	3m	6m	9m	12m	Rem ±6m
1	S	+	3	S	-	MA	MB	ns	MB	MB	MB	+
2	S	+	0	M	-	MB	MB	MB	MB	MB	MB	+
3	S	+	4	S	-	MB	MB	MB	#	-	-	+
4	S	+	4	S	-	MA	MB	MB	MB	R	-	+
5	S	+	0	M	-	MB	MB	ns	R	-	-	-
6	S	+	4	S	-	MA	MB	ns	#	-	-	+
7	S	+	0	M	-	**NM**	MB	R	-	-	-	-
8	S	+	0	M	-	MB	**NM**	R	-	-	-	-
9	S	+	4	S	-	**NM**	**NM**	R	-	-	-	-
10	S	+	0	M	-	**NM**	MB	R	-	-	-	-
11	C1	+	0	M	-	MA	MB	MB	MB	ns	MB	+
12	C1	+	4	S	-	MA	MB	MB	MB	MB	MB	+
13	C1	+	4	S	-	MB	MB	MB	MB	MB	MB	+
14	C1	+	1	M	-	MA	MB	MB	R	-	-	-
15	C1	+	1	M	-	**NM**	MB	R	-	-	-	-
16	C1	+	0	M	-	MB	MB	R	-	-	-	-
17	C1	+	2	S	-	MB	MB	R	-	-	-	-
18	C1	+	3	S	-	MB	MB	R	-	-	-	-
19	C1	+	0	M	-	MB	MB	R	-	-	-	-
20	C1	+	3	S	-	MB	$	-	-	-	-	-
21	C3	+	1	M	-	**NM**						
22	C3	+	2	S	-	MB						
23	C3	-	2	S	1	MB						
24	C3	-	1	M	0	MA						
25	C3	-	4	S	3	MB						
26	C3	-	0	M	0	**NM**						
27	C3	+	0	M	-	MA						
28	C3	+	2	S	-	MB						
29	C3	+	2	S	-	**NM**						
30	P	-	4	S	1	**NM**						
31	P	-	3	S	1	**NM**						
32	P	-	3	S	3	MA						
33	P	+	3	S	-	MA						
34	P	+	0	M	-	**NM**						
35	P	-	4	S	3	**NM**						
36	P	-	1	M	1	MA						
37	P	-	2	S	1	**NM**						
38	P	-	2	S	1	MA						
39	P	+	3	S	-	MA						
40	HM	+	0	M	-	MB	MB	-	R	-	-	-
41	HM	+	1	M	-	MB	MB	-	MB	-	-	+
42	HM	+	1	M	-	MB	MB	-	**ND**	-	-	+
43	HM	+	0	M	-	MB	MB	-	**ND**	-	-	+
44	HM	+	1	M	-	MA	MB	-	**ND**	-	-	+
45	HM	+	2	S	-	MB	MB	-	R			
46	HM	+	0	M	-	MA	MB	-	R			
47	HM	-	2	S	0	**NM**						
48	HM	-	2	S	1	MA						
49	HM	-	4	S	0	**NM**						
50	HM	-	4	S	0	**NM**						
51	LM	+	2	S	-	MB	MA	-	R	-	-	-
52	LM	+	0	M	-	MB	MB	-	**ND**	-	-	+

Continued/

P	D	H±	SL	S	DH	PT	TT	3m	6m	9m	12m	Rem ±6m
53	LM	+	4	S	-	**MA**	**ND**	-	MB	-	-	+
54	LM	+	0	M	-	MB	**ND**	-	R	-	-	-
55	LM	+	1	M	-	MB	MB	-	**NM**	-	-	+
56	LM	+	0	M	-	MB	**NM**	-	**NM**	-	-	+
57	LM	+	1	M	-	**NM**	**NM**	-	MB	-	-	+
58	LM	+	3	S	-	MB	MB	-	R			
59	LM	-	2	S	2	MA						
60	LM	-	0	M	0	**NM**						
61	LM	-	2	S	0	**NM**						
62	C2	+	0	M	-	MA	MB	-	MB	-	-	+
63	C2	+	1	M	-	MB	MB	-	R	-	-	-
64	C2	+	1	M	-	**NM**	MB	-	R	-	-	-
65	C2	+	0	M	-	MB	**NM**	-	R	-	-	-
66	C2	+	1	M	-	MB	MB	-	R	-	-	-
67	C2	+	1	M	-	MA	MB	-	MB	-	-	+
68	C1	-	4	S	0	MA						
69	C1	-	4	S	1	MA						
70	C2	-	4	S	1	MA						
71	C2	-	0	M	2	**NM**						
72	C2	-	4	S	1	MA						
73	C1	-	4	S	1	**NM**						
74	C1	-	1	M	1	**NM**						
75	S	-	4	S	0	MA						
76	S	-	4	S	1	MA						
77	S	-	4	S	0	MB						
78	S	-	1	M	2	MB						
79	S	-	3	S	0	MA						
80	S	-	4	S	1	MA						
81	C1	-	4	S	1	MA						
82	MH	-	4	S	2	MA						
83	C2	-	1	M	2	MB						
84	C2	+	1	M	-	MB						

Table V: Summary of all endoscopic and morphologic data. The incidence of healing after treatment and duration of remission (more or less than 6 months) is shown.

KEY

Morphological Class: MA = Very metaplastic (Histology Class 1).
MB = Partially metaplastic (Histology Class 1).
NM = Non-metaplastic (Histology Class 2 with degenerative cells)
ND = Non-metaplastic (Histology Class 2 - near normal/non-degenerative)

PT = Morphological appearance of mucosa before therapy.

TT = Morphological appearance of mucosa after curative therapy.

3m - 12m = Morphological appearance of the mucosa at 3, 6, 9 and 12 months after curative therapy.

Rem ±6m = Patient remained in remission for more (+) or less (-) than 6 months after the termination of treatment.

R = Patient relapsed with new DU.

ns = EM Unit did not receive specimen from GI Unit.

= Patient did not arrive for endoscopic examination.

$ = Biopsy not received by EM Unit

were noted in scar mucosa after treatment with any one of the drugs used. Table V outlines the general mucosal changes associated with healing by sucralfate, cimetidine or misoprostol. A more detailed appraisal of scar morphology with special reference to possible drug mediated alterations is reported in Chapters 6 & 7.

5.6.1. Morphological Changes During The Course Of Healing After Treatement With De-Nol

Five patients were studied in detail during the healing phase with biopsies taken before treatment and at 1 and 6 weeks during treatment with De-Nol. The results were compared with the data recorded in 5.6. above. Prior to therapy, each of the 5 DU was surrounded by a metaplastic mucosa. The degree of metaplasia was reduced after 1 week and further reduced after 6 weeks of therapy. At the termination of treatment, the mucosa surrounding scars was of an ND type in 3 and an MB type in 2 cases (Figure 4).

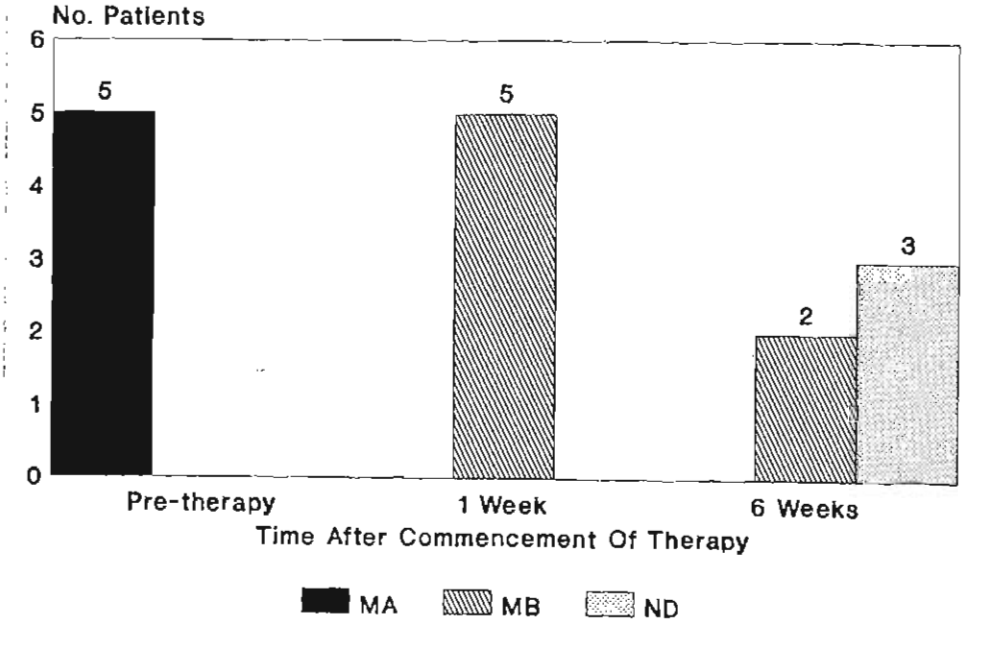


Figure 4: The morphological appearance of five DU before treatment with De-Nol and after healing. MA = Entirely metaplastic, MB = partially metaplastic, ND = near normal (non-degenerative).

5.6.2. Details Of Changes In Scar Morphology After Therapy

The overall morphological appearance of the juxta-DU and scar mucosa in 40 patients prior to and after curative therapy with either cimetidine, sucralfate or misoprostol (see Table V) is shown in Figure 5.

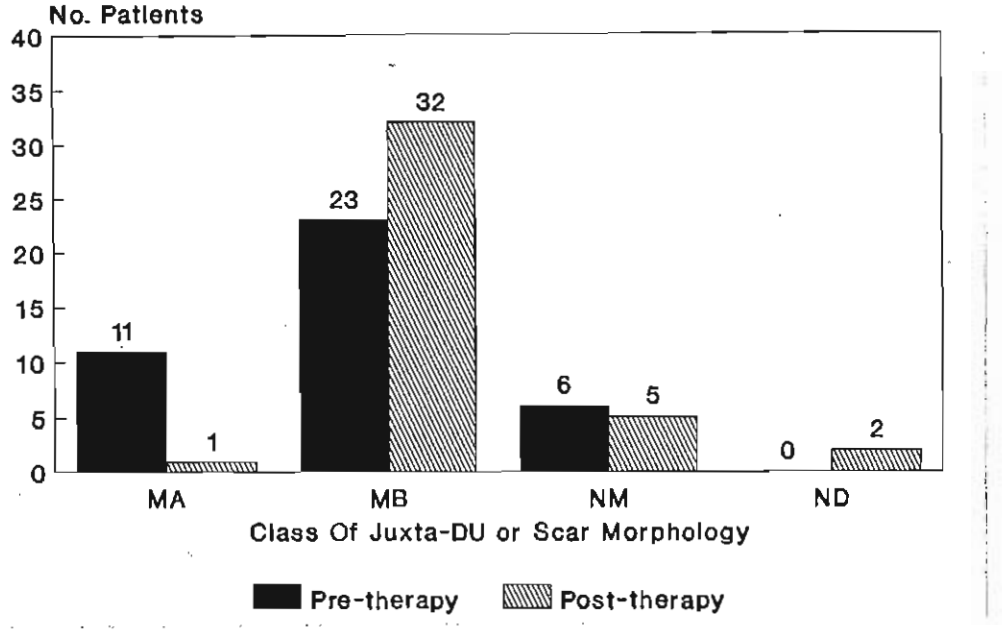


Figure 5: The appearance of the juxta-DU and scar mucosa in 40 patients who healed after therapy with either cimetidine, sucralfate or misoprostol.

Table VI summarises the distribution of juxta-DU and scar mucosal morphology before and after the above treatment.

	PT	AFTER THERAPY (TT)			
		MA	MB	NM	ND
MA	11	0	10	0	1
MB	23	1	18	3	1
NM	6	0	4	2	0
ND	0	0	0	0	0
Tot.	40*	1	32	5	2

Table VI: Classification of juxta-DU and scar mucosal morphology before and after therapy. The morphological appearance of juxta-DU biopsies (PT) was correlated with the appearance of scars from the same patients after curative therapy (TT). Forty one patients healed after therapy with cimetidine, sucralfate or misoprostol. The scar biopsy from patient No.20* had no mucosal surface and was excluded from the analysis.

Table VI shows that lesions surrounded by well differentiated MSC (MA) healed with a moderately metaplastic scar (MB) while most lesions circumscribed by a class MB mucosa healed leaving a class MB scar. In the case of DU surrounded by non-metaplastic tissue (NM), lesions either healed with a class MB scar or retained their original degenerative non-metaplastic appearance.

5.6.3. Morphological Appearance Of Scars During Period Of Remission After Treatment With Cimetidine, Sucralfate Or Misoprostol

The overall range of juxta-scar morphology of patients still in remission after 3,6,9, and 12 months (see Table V) was similar to that seen before and after therapy. No cells with different morphology were found and the proportions of cells of a particular type in remission specimens generally mimicked those at the termination of treatment. Figure 6 shows that of 33 biopsies taken during periods of remission, 27(82%) had scars with an MB mucosa, 4(12%) had an ND and 2(6%) an NM mucosa. No scars had well differentiated MSC.

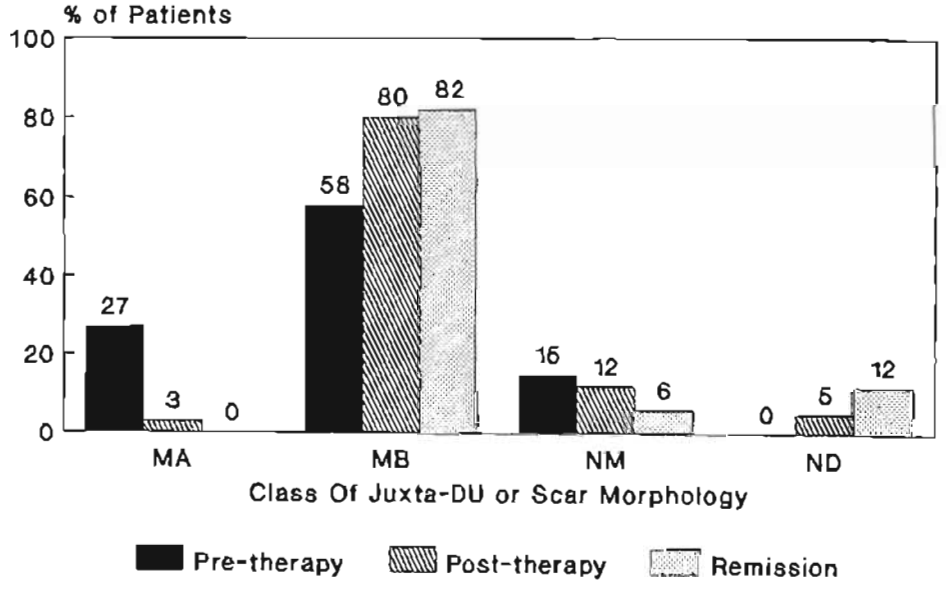


Figure 6: The morphological class of mucosa in specimens obtained from the juxta-DU mucosa and from scars after treatment with sucralfate cimetidine or misoprostol and during remission.

5.7. Summary of Morphological Results.

1. The optimal position for biopsy is from 4mm - 8mm from the edge of DU or scars.
2. The juxta-DU villous mucosa may be categorised as being either gastric metaplastic or non-metaplastic. These categories can be further subdivided into 4 morphological classes; entirely metaplastic (MA), partially metaplastic (MB), degenerative non-metaplastic (NM) and non-degenerative non-metaplastic (ND)
3. When metaplastic, the degree of metaplasia decreases with distance from the ulcer crater and the mucosa is often normal 12mm from the ulcer edge.
4. Irrespective of:
   a) the endoscopic severity of the lesion, or
   b) the class of the juxta-DU villous mucosa, and
   c) whether the biopsy was taken from near the DU prior to therapy or from near the scar at various periods of time after healing:

   the villous mucosa was populated with one or more of 7 morphologically identifiable cell "types", namely metaplastic types a), b), dii) and some di), normal (type f) and abnormal goblet (type c) and normal absorptive cells (type e).
5. After curative therapy, most scars have a moderately metaplastic mucosa. This type of mucosa persists during the period of remission.
6. Glycocalyceal bodies were present in the glycocalyx of some type b) and e) and many type d(i) and d(ii) cells.
7. Helicobacter pylori were found in association with type a) cells in approximately 50% of cases prior to therapy. They were rarely seen in post-therapy specimens.

5.8. Discussion

The range of morphology extant in cells populating crypts of Lieberkuhn and villi in normal tissue was similar to that reported by other authors (Ham 1979 p.677, Shiner 1983 p.5, Wheater et al. 1987 p.214, Leeson et al. 1988 p.434, Junqueira et al. 1992 p.297). Positional variations in crypt and villus epithelial cell morphology occurred as enterocytes migrated from crypt base to villus tip. In the case of absorptive cells, the length of microvilli and the thickness of the glycocalyx differed from villous base to tip. This concurs with reports by other authors (Shiner 1983 p.8, Leeson et al. p.445) and is an important observation for it suggests that apparently mature cells continue to differentiate and/or undergo change during their migration from the villous/crypt interface to the villous tip.

The morphological appearance of the juxta-DU and scar mucosa was particularly confusing, each biopsy probably being influenced by the aetiology of the parent DU (1.2.3.; 1.2.4. pp9-20), phase of ulcerogensis or healing(Chapter 2 p.20), level of luminal acidity(1.2.4.1. p.14) and/or degree of mucosal ischaemia(1.2.4.2. p.19). Only by taking cognisance of, and summarising particularfeatures of villous cells within individual biopsies and thereafter dividing these enterocytes into 7 morphological "types" (5.3.3. pp.90-97), could some sense be gleaned from the data.

5.8.1. Morphological Appearance of Metaplastic Cells

Throughout this study, the columnar gastric metaplastic secretory cells (MSC) on villi were identified by the presence of mucus droplets and/or varying numbers of electron dense vesicles (EDV) within their cytosol. There

appeared to be a correlation between numbers of secretory droplets, number and size of EDV, length and number of microvilli and thickness of glycocalyx. Fully differentiated MSC (type a) had numerous secretory droplets, few EDV and fewer and moderately long microvilli with a more pronounced glycocalyx. Cells with numerous EDV (type b) often had few secretory droplets and generally had shorter more numerous microvilli and a thinner, often discontinuous glycocalyx. Where only occasional EDV were present and cytoplasmic organelles were similar to those in normal absorptive cells (type dii), microvilli were both profuse and long. The glycocalyx associated with such cells, however, was often fragmented or missing. Occasional, minimally vacuolated type di) cells occurred either singly or in small clusters in partially metaplastic tissue, especially in the healed mucosa after treatment. Although often characterised by dilated RER and/or SER, most organelles appeared healthy. These and type dii) cells appeared to form a morphological link between metaplastic and normal villous absorptive cells. In summary, as evidence for secretory activity diminished so the structures associated with absorption became more prominent.

5.8.2. Postulated Differentiative Pathway Of MSC

It is important to note that specimens were obtained at one unknown moment in time from a continuum of ulcerogenesis and/or natural healing of each DU. Furthermore, the precise aetiology of each lesion and pH of the luminal environment was not known. The possible cell differentiation, degeneration and maturation scenarios outlined below are, therefore, speculative being created from the appearance of villus cells from one (pre-treatment) to five (up to 1 year after treatment) single frames in 84 individual DU developmental continua.

In some specimens from untreated patients whose villi and crypts of Lieberkuhn were populated exclusively with MSC, well differentiated MSC (type a) lay adjacent to stem cells. This suggested differentiation directly from stem to MSC without any obvious intermediary stages. In other partially metaplastic specimens from untreated, healing and healed DU, the villous mucosa was primarily populated with varying numbers of type a), b), dii) and e) cells. Very occasional di) cells exhibiting minimal organelle swelling and containing a few EDV were present in some instances. The change in proportions of mucus droplets and EDV, length and number of microvilli and presence, thickness or absence of the glycocalyx of type a), b) dii) occasional di) and normal absorptive cells suggested a differentiative continuum from absorptive to fully differentiated MSC and vice-versa.

The presence of many of these morphological cell "types" in some mucosa suggested either that individual MSC each reached particular and perhaps, predetermined levels of metaplastic differentiation at random positions on the villus or that normal or metaplastic villous cells had the capacity to change during their passage to the villus tip. This latter premise is in keeping with the observations on normal villous cell alteration during migration in 5.7. above. It is not known how long cells take to migrate from crypt base to villus tip in ulcerative tissue. Nor is it known how long these abnormal cells spend on villi prior to exfoliation. The time spent may be too short for cells to undergo complete transition from absorptive to well differentiated MSC. It does, however, appear possible that cells in a particular stage of development may change from one to another nearby stage in the differentiative continuum.

At a certain stage of ulceration, MSC may develop directly from stem cells. At other phases of ulcerogenesis and healing, cells in various stages of metaplastic transformation may differentiate towards normal absorptive or fully differentiated MSC and vice-versa. Irrespective of the mechanisms of change, alteration from one to the other morphological "type" of cell probably occurs as a consequence of local variations in the lumenal environmental (eg: variations in pH) or perhaps focal mucosal differences in vascular perfusion. These concepts will be examined in more detail in Chapter 8.9 (pp.230-237).

5.8.3. Development Of Non-metaplastic Cells In The Ulcerative Mucosa

While there appeared to be a differentiative pathway between normal absorptive and gastric metaplastic cells, most absorptive type di) cells did not fit into this continuum. Whereas cells in the metaplastic continuum showed some evidence of secretory activity or crinophagy (EDV), most type di) cells were void of these features being characterised by various degrees of organelle pathology and/or degeneration.

Severe pathomorphology was most common in specimens obtained prior to therapy and was characterised by cytoplasmic vacuolation, organelle swelling and dilatation and crenation of nuclei, all features synonymous with cellular ischaemia in other tissues (Goldstein 1979, Gregory and Mars 1992). In addition to the above, microvilli were sometimes fused and disrupted and the glycocalyx was thin or absent. Degenerate di) cells were interspersed with goblet cells, some containing swollen cytoplasmic organelles. Severe cellular pathology often extended to the epithelium in the crypts.

Most di) cells showed no evidence of metaplastic differentiation but were characterised by severe cellular pathomorphology. The lack of morphological evidence to link these cells to the metaplastic differentiative scenario suggested that the DU from which they were obtained were caused by factors other than luminal hyperacidity. It is postulated, therefore, that these cells together with local goblet and crypt cells, had responded to an ischaemic event that had caused general mucosal degeneration. In these specimens, less abnormal cells may indicate earlier stages in a process of degeneration. These were particularly important observations for they suggested that DU surrounded by degenerate cells may indicate an aetiology of focal/general mucosal ischaemia.

In summary, the morphological evidence suggested that biopsies were taken from DU with differing aetiologies. Where hyperacidity had stimulated a metaplastic response (2.3. p.35), cells exhibited features ranging from type e) to type a). Where ischaemia might be the primary cause of ulceration, degenerative type di) cells predominated. These and other possible scenaria explaining the variations in juxta-DU and scar morphology are discussed further in Chapter 8.

5.8.4. Abnormal Goblet Cells

Moderately metaplastic villi were characterised by the presence of goblet cells whose mucus droplets contained a well defined osmiophilic inclusion. These were similar in appearance to the mucus droplets in immature goblet cells in the crypts of Lieberkuhn and the newly synthesised droplets near the forming face of the Golgi apparatus of normal villous goblet cells. These data suggest that the secretory droplets within type c) cells were immature structures

within otherwise normal cells. This premise was considered more probable than that type c) cells were of a unique type. It may be that the droplets do not mature through an anomaly in goblet cell differention. Whether this has negative connotations in that immature mucus is less resistant to acidic chyme or whether the mucus has additional acid resistant properties and therefore affords additional protection to ulcerative tissue was not determined.

It should be noted that while goblet cells in a mucosa that contained many fully differentiated MSC were exclusively abnormal (in that they contained abnormal mucus droplets), those in a mucosa primarily populated with partially differentiated MSC occurred together with normal goblet cells. The relative numbers of abnormal to normal goblet cells in metaplastic epithelia was used later in the key for the morphological index as one means of gauging the degree of metaplastic differentiation of the tissue as a whole.

5.8.5. Goblet Cell Numbers

Goblet cells occurred in a ratio of approximately 1:5 in normal tissue (2.0:GC/100μm). In non-metaplastic tissue prior to therapy, goblet cell numbers increased significantly to 3.7:GC/100μm. Gastric metaplasia, *per-se*, is probably a protective response by the mucosa to luminal hyperacidity. An increase in the ammount of mucus secreted from more numerous goblet cells may be an alternative mechanism of protecting the mucosa from low pH fluids in the lumen. Alternatively, goblet cell hyperplasia may be a protective response by the mucosa to ulcerogenic factors that occur in a normal luminal environment. These possibilities together with the premise that goblet cell hyperplasia may indicate an early phase in ulcerogenesis is discussed in more detail in Chapter 8.

CHAPTER 6

MORPHOLOGICAL ANALYSIS OF DUODENAL MUCOSA

A primary aspect of this study was to determine whether ulcerative duodenal mucosal morphology influenced and/or indicated the potential for DU healing and/or remission. To address these questions, mucosal morphology prior to and after therapy was correlated with the incidence of DU healing and duration of remission.

In collecting adequate data to identify morphological criteria that may indicate prognosis, the following premises were adopted:

a) The dose and/or type of therapy did not significantly alter the proportion of patients healed at a given time (Marks 1980, Bardhan et al 1986, Lipsey et al 1990, Marks et al 1991).
b) The dose and/or type of therapy did not significantly alter scar morphology (Tovey et al. 1989a,b).

This enabled the pre- and post-therapy information from the drug studies to be pooled. These premises will be critically examined in Chapter 7.

6.1. Identification of Prognostic Criteria

Potential prognostic criteria include the severity of individual DU as determined by endoscopy, the morphological appearance of the mucosa surrounding lesions prior to and the scars after treatment and the type of curative regimen.

The first 3 criteria are examined in this Chapter. The possible influence that drugs may have on the duodenal mucosa and DU prognosis is examined in Chapter 7.

6.1.1. The Severity Of DU As Determined By endoscopy Correlated With The Incidence Of Healing

At the termination of the various therapeutic regimens, 50 patients were healed. Figure 7 shows the incidence of healing correlated with endoscopic severity.

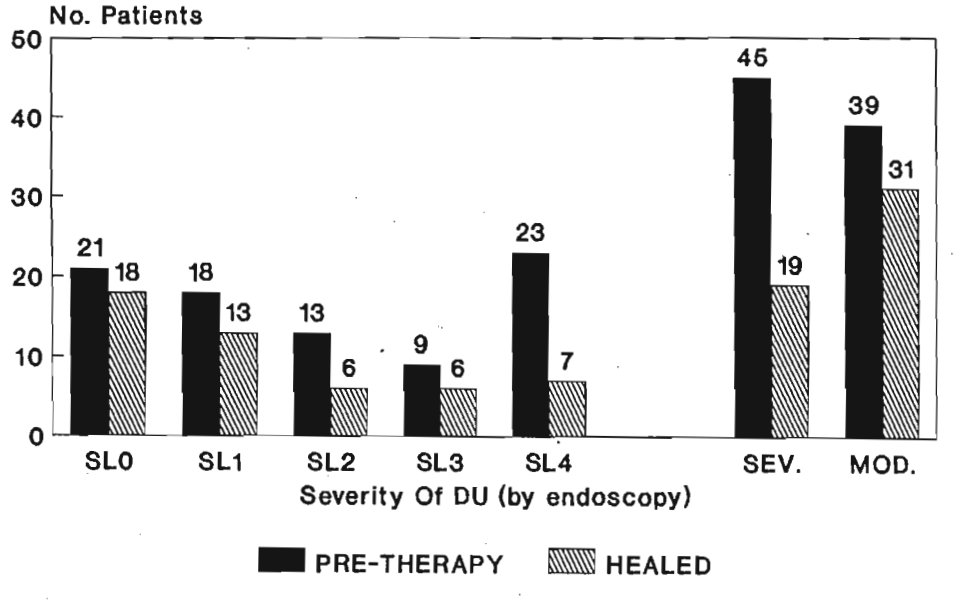


Figure 7: The incidence of healing correlated with the severity of DU as estimated by endoscopy. Note that most patients with small (SL:0) **MOD**erate lesions healed whereas most of those with **SEV**ere (SL:4) lesions did not.

Only 19 (42%) of the 45 DU from the group graded endoscopically as severe (SL-4;3;2) healed whereas 31 (82%) of 39 DU graded as moderate (SL-1;0) healed after therapy. There was a significant difference in the incidence of healing between endoscopically severe and moderate lesions (P-test: $p = 0{,}00052$). These data showed that the endoscopic severity of a lesion prior to therapy influenced the probability of healing after a fixed period of treatment.

In the case of the 34 DU that did not heal, 26 were classified by endoscopy as severe and 8 as moderate. Of the severe and moderate DU, 5 (19%) and 3 (38%) respectively showed improvement at the termination of therapy (Figure 8). The apparent disproportionate improvement exhibited by moderate lesions was not significant ($p < 0.05$).

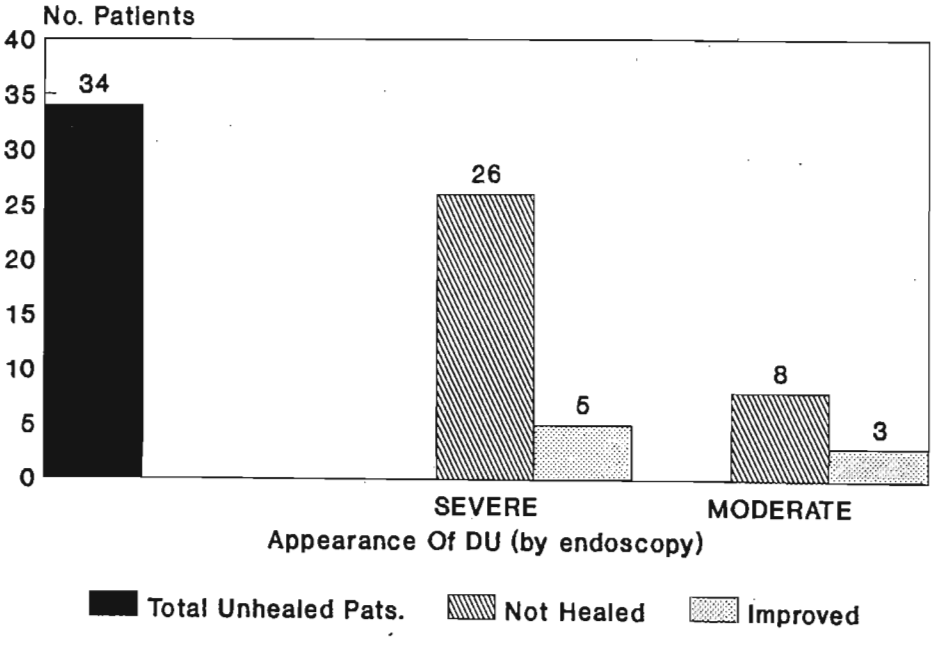


Figure 8: The endoscopic appearance of unhealed mucosa after therapy.

6.1.2. Pre-therapy mucosal morphology correlated with DU healing.

Figure 9 shows the morphologic class of juxta-DU mucosa correlated with the incidence of healing. Of the 84 paired biopsies evaluated prior to therapy, 62 (74%) were metaplastic (MA & MB) and 22 (26%) non-metaplastic (NM). Of the former group, 42 (67%) healed while only 9 (40%) non-metaplastic DU healed. There was a significant difference in the incidence of healing between lesions surrounded by metaplastic and non-metaplastic mucosa (P- test: p = 0,0483).

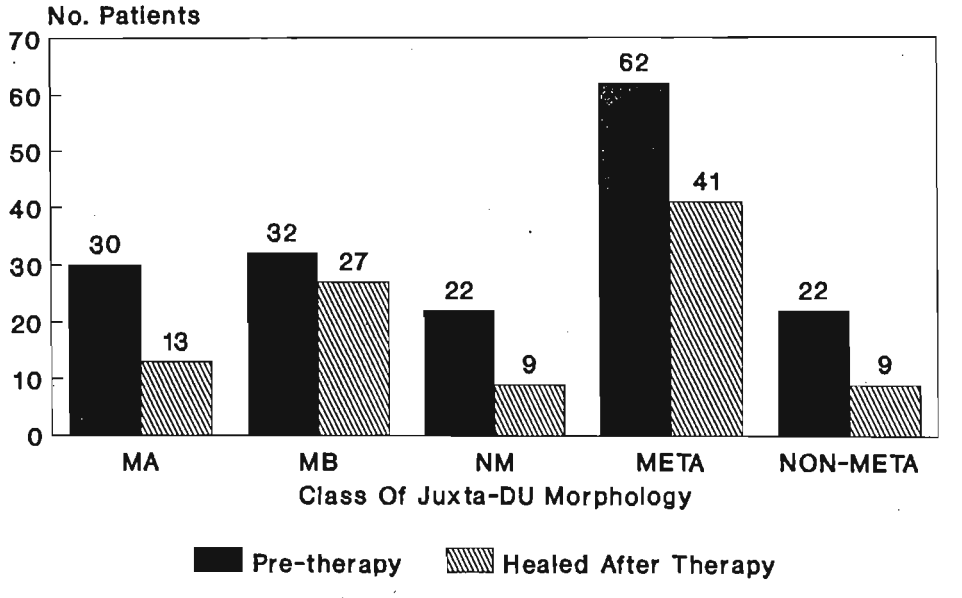


Figure 9: The morphological class of the mucosa in juxta-DU biopsies correlated with the incidence of healing. The 84 juxta-DU biopsies divided into 3 groups: **META**plastic (MA and MB) and **NON-META**plastic (NM).

Figure 10 correlates the incidence of healing and non-healing with the type of juxta-DU mucosa.

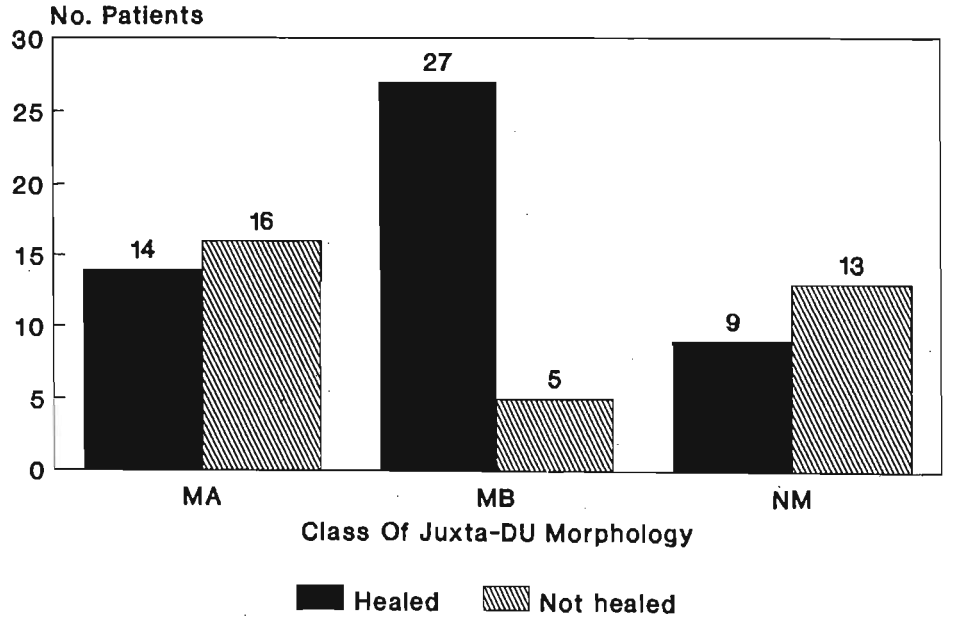


Figure 10: The morphologic class of juxta-DU mucosa correlated with the incidence of healing and non-healing.

There was a significant difference in the overall pattern of healing/non-healing between morphological classes of lesion ($p = 0,0043$). There was no difference in the incidence of healing/non-healing of patients whose lesions were surrounded by type MA (14/16 patients) or NM (9/13 patients) mucosa, but a very significant difference in those with an MB mucosa (27/5 patients.: $p = 0.0002$).

These data revealed a difference in the incidence of healing of metaplastic and non-metaplastic lesions. Metaplastic DU, particularly those surrounded by a moderately metaplastic (MB) mucosa had a high probability of healing while DU circumscribed by non-metaplastic tissue were less likely to heal.

6.1.3. Changes in the type of juxta-scar mucosa at the termination of curative therapy.

Figure 5 (page 135) shows the number of patients with a particular morphological class of juxta-DU and scar mucosa before and after therapy. There was a significant difference in mucosal morphology prior to and after healing. Scar mucosa was characterised by a reduction in the number of specimens exhibiting well differentiated metaplasia (MA: $p = <0,0001$).

6.1.4. Scar morphology correlated with the duration of remission.

Figure 11 shows the appearance of the scar mucosa at the termination of therapy in patients who remained in remission for more or less than 6 months. There was no difference ($p = 0,47$) in post-treatment mucosal morphology between patients who relapsed within 6 months or who remained in remission

for longer. These data suggested that the morphological quality of healing after curative therapy did not influence remission prognosis.

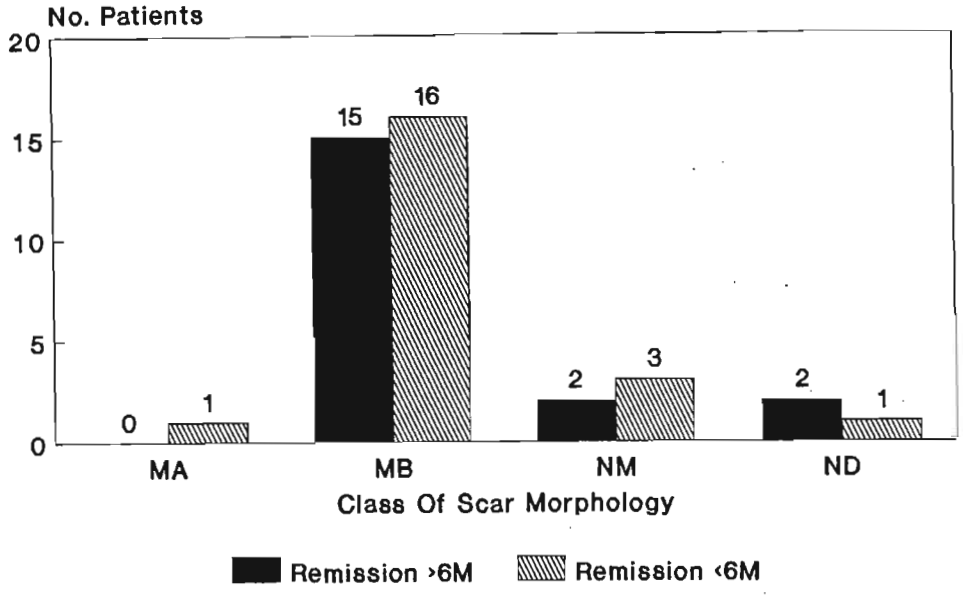


Figure 11: The morphological class of scar mucosa in patients that experienced remission for more or less than six months. There were no significant differences in the number of specimens with a particular type of morphology in either group ($p = 0.47$).

6.1.5. Summary Of Morphological Results

1. Endoscopically moderate DU were more likely to heal than severe lesions ($p = 0,00052$).
2. A metaplastic mucosa predisposed towards DU healing ($p = 0,04$).
3. A moderately metaplastic juxta-DU mucosa (MB) was a particularly favourable phenomenon associated with DU healing ($p = 0,0001$).
4. Degenerative non-metaplasia (NM) was associated with persistant DU ($p = 0.0483$).
5. There was a difference between juxta-DU and scar mucosal morphology ($p = 0,022$), the most obvious change being a general reduction in the "degree" of gastric metaplasia from type MA to MB ($p = 0,0001$).

These results show that endoscopically small or medium sized DU were the lesions most likely to heal and that juxta-DU metaplasia was a positive and degenerative non-metaplasia a negative prognostic criterion for DU healing.

6.2. Quantitative Morphological Analysis

The preceding results showed that the endoscopic and morphologic appearance of the untreated juxta-DU mucosa indicated healing prognosis. These important observations were derived from analyses of groups of specimens that had been morphologically classified as either exhibiting gastric metaplastic or degenerative non-metaplastic features. Although able to confirm a relationship between morphology and prognosis, these categories were too vague to enable correlative analyses to determine the probable prognosis of individual DU. In order to investigate the possibility that the morphologic appearance of individual lesions may indicate specific short and/or long-term prognoses, a more sensitive method of assessing juxta-DU and scar mucosa had to be devised.

The present section outlines the rationale for the formulation of a numeric morphological index that more accurately described the morphological state of the mucosa

6.2.1. The Morphological Index

Morphological indices are formulated about the positive and negative criteria of the phenomena to be studied (Cooper et al, 1985, Tovey et al 1989). In this study, the phenomena to be studied were healing and remission prognoses and the positive and negative criteria determined in 6.1. above were mucosal gastric metaplasia and degenerative non-metaplasia.

In accord with these observations, the morphological appearance of the juxta-DU mucosa was prognostically graded as follows:

FAVOURABLE

i) Well differentiated gastric metaplasia (MA).
ii) Poorly differentiated gastric metaplasia (MB).
iii) Degenerative non-metaplasia (NM).

UNFAVOURABLE

6.2.2. Selection of Morphologic Parameters

Certain qualitative morphologic variables were interlinked to describe and qualify the presence and degree of gastric metaplasia and degenerative non-metaplasia in every specimen. The following variables were employed in the key to the morphological index.

Light Microscopy

a) **The size and shape of villi** together with the general distribution and approximate numbers of a particular cell type within the villous mucosa

Electron Microscopy

b) **Amount of mucosubstance in metaplastic cells** (much or little).
c) **Length of microvilli** (long or short).
d) **Number of microvilli** (numerous or few)
e) **Presence and thickness of the glycocalyx.**

Well differentiated gastric metaplastic cells had numerous secretory droplets, few and short microvilli and a thin, sometimes discontinuous glycocalyx. In less well differentiated cells there appeared to be a concommitant reduction in the numbers of secretory droplets with an

increase in the numbers apical electron dense vesicles, length of microvilli and thickness of the glycocalyx.

f) **Type and number of goblet cells.**

g) **Type of goblet cell mucodroplets.**

In well differentiated metaplastic specimens, goblet cells were sparse and generally contained abnormal mucodroplets with large osmiophilic inclusions. In less well differentiated specimens, goblet cell numbers increased and fewer cells contained abnormal mucus droplets.

h) **Degree of organelle pathology in degenerative cells.**

In severely degenerative, non-metaplastic cells, the cytoplasm was condensed, cytoplasmic organelles were swollen, nuclei crenated and microvilli were often eroded, sparse and shortened and the glycocalyx absent. In less denerative cells, the microvilli were longer and more numerous, the glycocalyx was fragmented or absent and there were often numerous glycocalyceal bodies associated with the disrupted glycocalyx. Cytoplasmic organelles were less swollen than above. In the least degenerative cells, microvilli were of normal length and number while the glycocalyx remained thin. Glycocalyceal bodies were often present. Cytoplasmic organelles were normal. Cells contained more lysosomes and sometimes contained phagocytosed inclusions.

6.2.3. The Morphological Key.

The light and electron microscopical features described above were incorporated into 4 parameters (**S1-S4**) that formed the basis of the morphological key (Appendix 3).

S1: General Appearance of the Mucosa. This parameter describes the general level of metaplasia and non-metaplasia in each pair of specimens. The level was determined by light and electron microscopy. Cognisance

was taken of villus shape and the proportion of metaplastic and non-metaplastic cells in each specimen.

S2: Goblet Cell Morphology. The presence and approximate numbers of goblet cells populating the villous epithelium was recorded by light microscopy. The type of mucosubstance (normal or abnormal) was determined by electron microscopy. This parameter helped qualify the metaplastic aspects of S1.

S3: General Ultrastructural Appearance of the Majority of Metaplastic Cells in the Mucosa. This parameter was designed to describe the degree of metaplastic differention in a specimen and helped to qualify the metaplastic aspects of S1.

S4: General Ultrastructural Appearance of the Majority of Non-Metaplastic Cells in the Mucosa. This parameter was designed to describe the degree of degenerative change in non-metaplastic cells in each specimen and helped to qualify the non-metaplastic aspects of S1.

Each parameter (S1-S4) was awarded a score 0-4 (S1 & S2) or 0-3 (S3 & S4) - maximum of 14 points - that described the degree of metaplasia or degenerative non-metaplasia in each specimen. All features associated with a well differentiated metaplastic mucosa had a high score which was reduced with decreasing metaplastic differentiation. Normal or near normal specimens had a moderate score. Pathological non-metaplastic mucosa had a low score which, depending on the "severity" of cyto-pathology, ranged from just below "normal" values to zero. The sum of scores (0-14) for individual cases was converted to a percentage and expressed as a (M)orphological (I)ndex (MI:0-100).
The combination of morphological features and scores awarded to such features are described in detail in the Morphological Key (Appendix 3).

6.3. Acquisition of Numeric Data from Endoscopic Biopsies

All endoscopic biopsies were re-evaluated by light and electron microscopy. Included in the re-appraisal were all light and electron micrographs obtained during the earlier morphological analysis of pre- and post-therapy specimens. All patient data and details of the light and electron microscopic appearance of each specimen were recorded on a form designed specifically for this purpose (See Appendix 4). The microscopist (MAG), by referring to the morphological key, scored each specimen directly while examining the tissue with the light and electron microscope. Light and electron-photomicrographs were made of important features for later reference.

6.4. Morphological "Ranges".

The morphological index was designed to numerically describe the morphological appearance of the ulcerative mucosa. In Chapter 5, the ulcerative mucosa was divided in to 4 classes (MA; MB; NM; ND). In order to afford the reader a better understanding of how the morphological scores describes morphology, the following explains how these classes of mucosa may be expressed as MI.

6.4.1. Degenerative Non-metaplasia (NM).

A degenerative, non-metaplastic mucosa has no MSC, a plethora of degenerative and occasional normal absorptive cells and perhaps a normal or increased complement of goblet cells. In numeric terms, the most degenerative mucosa has an MI of 0. The degree of pathology is primarily determined by the ratio of degenerative to normal absorptive cells in the mucosa together with an overall assessment of the severity of absorptive cell pathomorphology. The interface between a

mucosa displaying minimal evidence of non-metaplastic degeneration (NM) and a normal mucosa displaying no evidence of metaplasia is numerically described as follows:

S1:1.0; S2:1.0; S3:0,5; S4:2.0 MI:32

Degenerative non-metaplasia, therefore, ranges from MI:0 to approximately MI:32.

6.4.2. Normal Mucosa.

A "normal" mucosa is populated with a normal complement of healthy absorptive and goblet cells. There may be occasional single cells or small foci of MSC, some of which may be well differentiated. In numeric terms, the most metaplastic "normal" specimen is described thus:

S1:1,5; S2:1,5; S3:2.0; S4:2.0 MI:50

A normal mucosa, therefore, could range from approximately **MI:32-50.**

6.4.3. Metaplastic Mucosa (MA & MB).

A metaplastic mucosa is exclusively or predominantly populated with metaplastic cells in various phases of metaplastic differentiation. In the most extreme cases, the mucosa is exclusively populated with well differentiated MSC and goblet cells are absent. Such a mucosa is numerically described thus.

S1:4,0; S2:4,0; S3:3,0; S4:3,0 MI:100

The degree of mucosal metaplasia is assessed by the relative number of MSC, the overall degree of MSC differentiation and numbers of normal absorptive and goblet cells populating the epithelium. A mucosa displaying minimal metaplastic

characteristics could fit the numeric morphological description bordering on mucosal normality (**MI:50**). Depending on the degree of metaplasia, therefore, a metaplastic mucosa is deemed to range from **MI:100** to (**MI:50**). However, as explained in 6.4.2. above, some evidence of metaplasia may be present in specimens whose MI range down to MI:32.

The morphological index, therefore, numerically describes a morphological continuum from exclusively metaplastic (**MI:100**) via moderately metaplastic (**MI:50**) to minimally metaplastic/minimally degenerative (**MI:32**) and severely degenerative (**MI:0**).

6.5. Summary

The morphological index was designed to "quantify" variations in the ulcerative duodenal mucosa and to express as a percentage the general state of the mucosa with regards the prognostic severity of lesion pathomorphology. The method of morphological indexing identified stereo-specific morphological features and correlated these features to the degree of mucosal gastric metaplasia and degenerative non-metaplasia. Numeric values awarded to morphological parameters were biased in such a way as to maximally differentiate between favourable metaplastic features associated with a good prognosis for healing and the non-favourable, degenerative non-metaplastic features associated with non-healing.

CHAPTER 7

QUANTITATIVE RESULTS

The morphological results revealed a correlation between the appearance of untreated DU and the incidence of DU healing. In this chapter, by correlating morphological scores with healing and remission outcome, this relationship will be critically examined. In addition, the numeric data will be examined to determine other possible relationships between pre- and post-therapy mucosal morphology and incidence of DU healing and/or duration of remission. Chapter 7 is divided into 3 sections. In Section 1, all available data are evaluated to detect general relationships between morphology and healing and duration of remission. These relationships will be further examined in Section 2 with special reference to how they apply to particular curative regimens. In this way it was hoped to determine whether a particular type of therapy alters mucosal morphology in such a manner as to influence DU healing or remission. Section 3 will integrate the data collected in sections 1 & 2 and use the information to predict DU healing and/or duration of remission. In addition, the prognostic data will be employed to provide an accurate means of comparing the relative efficacy of different curative regimens.

Section 1: Morphological and Prognostic Correlates

It is important to note, that in this study, neither before nor at any time after therapy, did the mucosa from a single patient appear morphologically normal. Morphological scores within the normal range (**MI:32-50**) generally described

abnormal, mildly metaplastic specimens previously classified as ND. These specimens contained foci of normal epithelium in a mucosa populated with poorly differentiated MSC rather than foci of well differentiated metaplasia in a predominantly normal epithelium. In addition, there was no clear demarcation between minimally metaplastic and degenerative non-metaplastic specimens near the NM/ND interface (**MI:32**).

The Electron Microscope Unit (EM) and hospital (GI) data, the morphological scores for each parameter (S1-S4) and the composite morphological score (MI) for each pre-therapy specimen are summarised in Table VII.

7.1. The Prognostic Value Of Metaplasia As An Indicator For DU Healing.

In Chapter 6, a prognostic relationship between pre-therapy, juxta-DU metaplasia and the incidence of healing was described. This relationship was re-examined using the morphological index. In this section, specimens with a score **MI:>50** are categorised as metaplastic and those with a score **MI:<49**, non-metaplastic.

7.1.1. Metaplasia (MI:>50) and Non-Metaplasia (MI:<50) Correlated with Incidence of Healing.

From Table VII, 53 (63%) patients had pre-therapy morphological scores MI:>50. Of these 34 (64%) healed. Thirty-one patients (37%) had scores MI:<50. Of these, 15 (48%) healed. Statistical analysis revealed no significant difference in the incidence of healing between the metaplastic and non-metaplastic groups ($p = 0.14$).

Table VII

PATIENT DATA					MORPHOLOGICAL SCORES					
P	EM	GI	D	H±	SL	S1	S2	S3	S4	MI%
1	898	20079	S	+	3	3	3	2	3	78
2	928	20772	S	+	0	1.5	2	2.5	1.5	53
3	1061	22399	S	+	4	2.5	2	1.5	2	57
4	1068	22519	S	+	4	3.5	4	2.5	0.5	75
5	1097	22727	S	+	0	2.5	4	2.5	0	67
6	1146	23530	S	+	4	3.5	4	2.5	3	92
7	875	19543	S	+	0	1	0	2.5	1	32
8	894	20009	S	+	0	2	3	2	1	57
9	901	20171	S	+	4	0.5	1	0	1	18
10	1077	22667	S	+	0	1	2	2	0.5	39
11	897	20054	C1	+	0	3.5	3	2.5	3	85
12	906	20281	C1	+	4	3.5	4	2.5	0.5	75
13	967	21233	C1	+	4	2.5	2	2.5	1.5	60
14	1095	22724	C1	+	1	3.5	3	2.5	2	78
15	889	19840	C1	+	1	0.5	0.5	0	0	7
16	893	20007	C1	+	0	2	2	1.5	1	46
17	902	20195	C1	+	2	2.5	0.5	2.5	1	46
18	968	21234	C1	+	3	3	2	2.5	0	53
19	1123	23246	C1	+	0	2.5	4	2	0.5	64
20	1147	23531	C1	+	3	2.5	3	2.5	0	57
21	1447	27886	C3	+	1	3.5	4	1.5	1	71
22	1446	27882	C3	+	2	1.5	2	1.5	1	43
23	1448	27890	C3	-	2	3	3	2.5	1	68
24	1450	27903	C3	-	1	3	4	1.5	1	68
25	1488	29105	C3	-	4	1.5	2	2	1.5	50
26	1494	29294	C3	-	0	1	1.5	0	1.5	29
27	1495	29300	C3	+	0	3.5	4	2.5	1	79
28	1500	29396	C3	+	2	2.5	2	1.5	1	50
29	1502	29429	C3	+	2	1	1	0	1.5	25
30	1443	27757	P	-	4	0	0	0	0.5	4
31	1462	28071	P	-	3	1	1	0	1.5	25
32	1466	28419	P	-	3	3.5	3.5	3	1.5	82
33	1481	28762	P	+	3	3	3.5	1.5	3	79
34	1482	28835	P	+	0	0	3	0.5	0.5	29
35	1534	29042	P	-	4	0.5	2.5	0.5	1	32
36	1489	29174	P	-	1	3.5	3	2.5	1	71
37	1492	29248	P	-	2	1	2	0.5	1.5	36
38	1493	29250	P	-	2	3.5	4	2.5	3	93
39	1501	29428	P	+	3	4	4	3	3	100
40	1225	24657	HM	+	0	3	3	2.5	0.5	64
41	1318	25551	HM	+	1	3	2.5	2.5	3	79
42	1357	26013	HM	+	1	0.5	1	0	1.5	21
43	1391	26429	HM	+	0	2	3	2.5	0.5	57
44	1396	26510	HM	+	1	3	4	2.5	1	75
45	1384	26379	HM	+	2	2.5	0	1.5	1	36
46	1385	26329	HM	+	0	3	4	2	3	85
47	1389	26412	HM	-	2	0.5	1	0	1	18
48	1248	24769	HM	-	2	4	4	2.5	3	96
49	1241	24709	HM	-	4	0.5	0	0.5	0.5	11
50	1370	26130	HM	-	4	0.5	1.5	0	1.5	25
51	1244	24692	LM	+	2	3	3	2.5	1.5	67

Continued/

PATIENT DATA					MORPHOLOGICAL SCORES					
P	EM	GI	D	H±	SL	S1	S2	S3	S4	MI%
52	1280	25053	LM	+	0	3	2	2.5	1.5	64
53	1286	25080	LM	+	4	3.5	4	3	3	96
54	1336	25855	LM	+	0	2.5	0.5	2.5	0.5	43
55	1354	25995	LM	+	1	2.5	2.5	2.5	0.5	57
56	1363	26055	LM	+	0	3	2	2.5	0.5	57
57	1367	26107	LM	+	1	1	1	0	1.5	25
58	1390	26413	LM	+	3	1.5	2	2.5	1	50
59	1223	24189	LM	-	2	3	4	1.5	1	75
60	1359	26031	LM	-	0	0	0	0.5	0.5	14
61	1245	24722	LM	-	2	1	1.5	0	0.5	21
62	1224	24653	C2	+	0	2.5	3	3	1	68
63	1264	24916	C2	+	1	2	3	1.5	0.5	50
64	1298	25201	C2	+	1	0.5	2	0.5	0	21
65	1310	25391	C2	+	0	2.5	2	2.5	0.5	54
66	1320	25564	C2	+	1	2	2	2	0.5	46
67	1379	26232	C2	+	1	2	2.5	2.5	0.5	54
68	878	19684	C1	-	4	4	4	3	3	100
69	899	20115	C1	-	4	3	3	2.5	3	82
70	1271	24965	C2	-	4	3	4	2	1	92
71	1395	26508	C2	-	0	1	1	0	1	21
72	1358	26016	C2	-	4	4	4	3	3	100
73	1078	22693	C1	-	4	0.5	2	1.5	1.5	39
74	879	19685	C1	-	1	0	0	0	0	0
75	877	19538	S	-	4	4	4	3	3	100
76	1096	22726	S	-	4	3	3	1.5	1	53
77	869	19608	S	-	4	2.5	2	1.5	1.5	53
78	939	20957	S	-	1	2	3	1	0.5	46
79	890	19829	S	-	3	3.5	4	1.5	3	85
80	882	19749	S	-	4	3.5	4	2.5	3	93
81	880	19701	C1	-	4	4	4	3	3	100
82	1335	25813	MH	-	4	2.5	0	1.5	1	36
83	1350	25941	C2	-	1	1.5	0.5	2.5	1.5	43
84	1353	26018	C2	+	1	2	2.5	2.5	0.5	54

Table VII: Specimen numbers and summary of, type of drug, endoscopic, DU healing and numeric morphological data for all biopsies included for correlative analyses.

KEY

P = Thesis patient number
EM = Electron Microscope Unit specimen number.
GI = Gastrointestinal Unit patient number.
D = Type of drug therapy.
H± = States whether the patient healed or not.
SL = Severity of DU as determined by endoscopy (grades 0-4).
S1-S4 = Morphological parameters included in the morphological key - the scores are in accord with those determined using the morphological key.
MI = Morphological Index expressed as a %.

7.1.2. Metaplastic Score (MI) Correlated with the Incidence of Healing.

Table VII and Figures 12 and 13 detail the pre-therapy MI of each DU that healed (group 1) and did not heal (group 2).

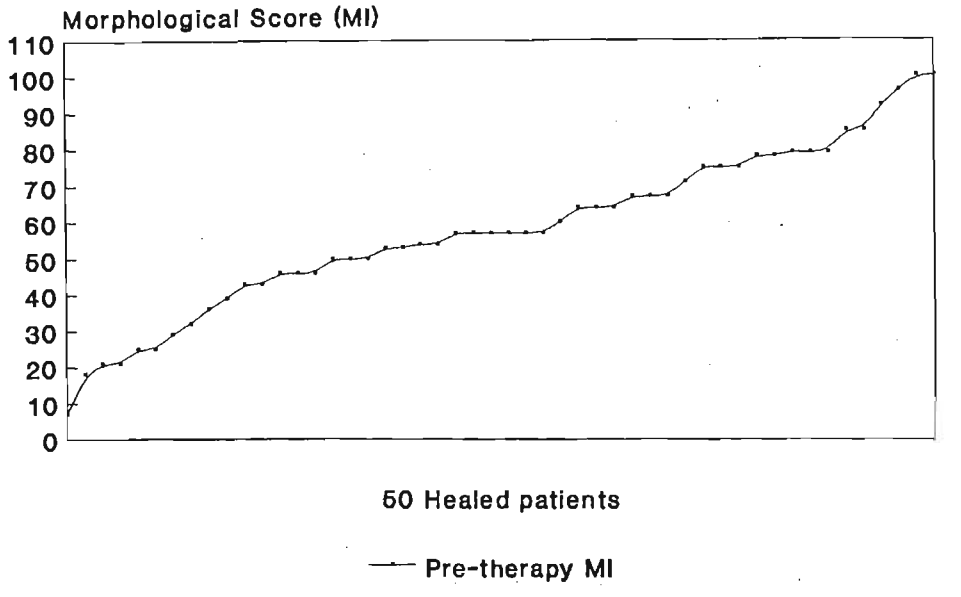


Figure 12: Morphological scores of juxta-DU mucosa from 50 patients whose DU healed after therapy. The scores were sorted and arranged in ascending order.

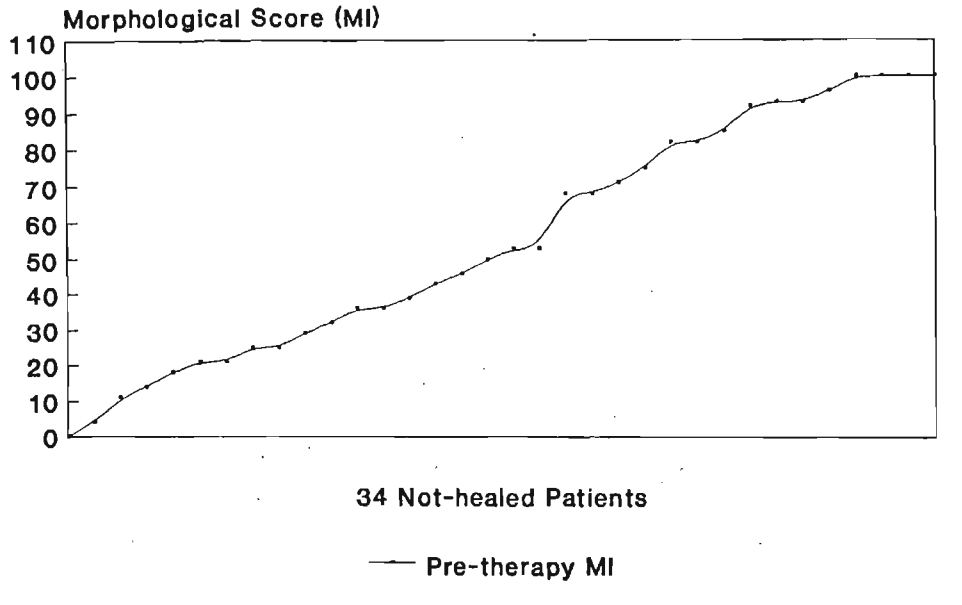


Figure 13: Morphological scores of juxta-DU mucosa from 34 patients who did not heal after therapy. The scores were sorted and arranged in ascending order.

The mean MI of group 1 was MI:58 and group 2 MI:55. The data is summarised in Table VIII.

	Group 1	Group 2
Mean MI	58	55
SD	22	32

Table VIII: Mean morphological scores (MI) of juxta-DU specimens in patients that healed (Group 1) and did not heal (Group 2). SD = Standard deviation.
Mann-Whitney p = 0,5176; F-distribution (Variance) p = >0.05

No significant difference was found between the two groups.

7.1.3. DU Healing Correlated With Juxta-DU MI

The pre-therapy morphological scores from lesions that healed and did not heal were collected and sorted in ascending order (MI:0-100). Based on their morphological score, each DU was asigned to one of 5 numeric "bins": **A:**0-20; **B:**21-40; **C:**41-60; **D:**61-80; **E:**81-100. The 5 MI data bins **A-E** were used as a format for all distribution analyses performed later in this Chapter. Table IX lists the data and Figure 14 shows the percentage of patients that healed in each morphological bin.

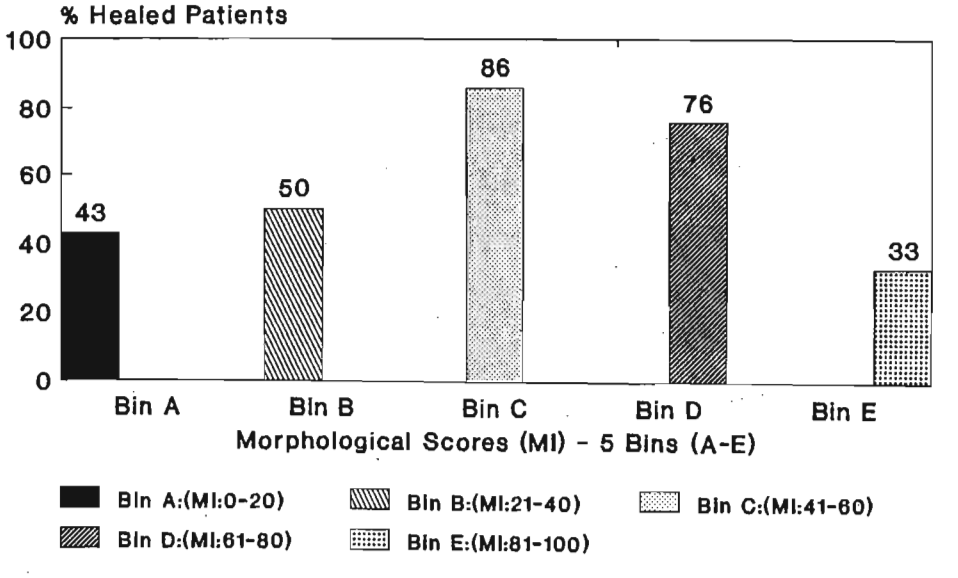


Figure 14: Morphology correlated with the incidence of healing. Note that most DU that healed had mucosa that was placed in bins C and D.

BIN 1 (MI:0-20)

P	9	47	60	49	15	30	74
M	18	18	14	11	7	4	0
H	+	-	+	-	+	-	-
S	4	2	0	4	0	4	1

Mean MI = MI:10
Mean SD = MI: 7
Percentage Healing = 43%
Mean SL = 2.1

BIN 2 (MI:21-40)

P	10	73	45	37	7	35	26	34	57	31	29	50	71	64	61	42
M	39	39	36	36	32	32	29	29	25	25	25	25	21	21	21	21
H	+	-	+	-	+	-	-	+	+	-	+	-	-	+	-	+
S	0	4	2	2	0	4	0	0	1	3	2	1	0	1	2	1

Mean MI = MI:29
SD = MI:6.2
Percentage Healing = 50%
Mean SL = 1.4

BIN 3 (MI:41-60)

P	56	20	55	43	3	8	84	18	65	2	77	58	28	63	25	17	66	16
M	57	57	57	57	57	57	53	53	53	53	53	50	50	50	50	46	46	46
H	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+
S	0	3	1	0	4	0	1	3	0	0	4	1	2	1	4	2	1	0

Mean MI = MI:51
SD = MI:5
Percentage Healing = 86%
Mean SL = 1.4

P	78	54	22	83
M	46	43	43	43
H	-	+	+	+
S	1	0	2	1

BIN 4 (MI:61-80)

P	14	27	41	33	1	12	4	44	59	36	21	5	23	51	62	24	40	52
M	79	79	79	79	79	75	75	75	75	72	72	67	67	67	67	67	64	64
H	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+
S	2	0	1	3	3	4	4	1	2	1	1	0	2	2	0	1	0	0

Mean MI = MI:71
SD = MI:6.5
Percentage Healing = 76%
Mean SL = 1.8

P	19	13	76
M	64	60	60
H	+	+	-
S	0	4	4

BIN 5 (MI:81-100)

P	81	67	72	75	39	68	48	53	80	82	38	6	70	69	11	46	79	32
M	100	100	100	100	100	100	96	96	92	92	92	92	92	82	85	85	85	82
H	-	+	-	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-
S	4	1	4	4	3	4	2	4	4	4	2	4	4	4	0	0	3	3

Mean MI = MI:93
SD = MI:6.5
Percentage Healing = 33%
Mean SL = 3.0

Table IX: Morphological scores correlated with incidence of healing (H) and endoscopic severity (S). Based on the morphological score (M) the data from each patient (P) was placed in one of 5 numeric bins. Shown are the mean morphological scores (Mean MI), the standard deviation (SD) of the mean and the percentage healing of patients in each bin. Also shown is the mean endoscopic severity of DU in each bin.

Eighty-six percent of patients with DU in bin C and 76% in bin D eventually healed.

The apparent prognostic importance of pre-therapy morphology is highlighted in Table X where the incidence of healing was compressed into 3 clearly defined morphological groups: non-metaplastic (MI:0-40 - Group 1); moderately metaplastic (MI:41-80 - Group 2); and very metaplastic (MI:81-100 - Group 3) DU.

Group	No.in Group	Healed DU	Compared	p =
1	23	11	1 & 3	**0,29**
2	43	35	2 & 1	**0,016**
3	18	6	3 & 2	**0,0006**

Table X: Comparison between the incidence of healing in non-metaplastic group 1 and non-metaplastic groups 2 and 3.
p = significance value from proportions test.

Thirty five patients (81%) healed in group 2, whereas only 11 (48%) and 6 (33%) patients healed in groups 1 & 3 respectively. There was a significant differences in the incidence of healing between groups 3 and 2 ($p = 0.0006$).

7.1.4. Distribution of Juxta-DU MI in Healed and Non-Healed Lesions.

The pre-therapy morphological scores from lesions that healed and did not heal were collected and sorted in ascending order (MI:0-100). Based on their morphological scores, the specimens were assigned to one of the 5 numeric bins. Figure 15 shows the number of patients whose DU healed and did not heal in each of the 5 MI bins. There was no significant difference in the range of MI between patients that healed or did not heal ($p = 0.05176$). There was, however, a difference between the numbers of patients that healed and did not heal in the 5 bins ($p = <0.047$). A very

significant difference existed between the number of patients who healed and did not heal whose morphological scores placed them in bins C ($p = 0.00003$) and D ($p = 0.00036$). There were no significant differences in the other 3 MI bins.

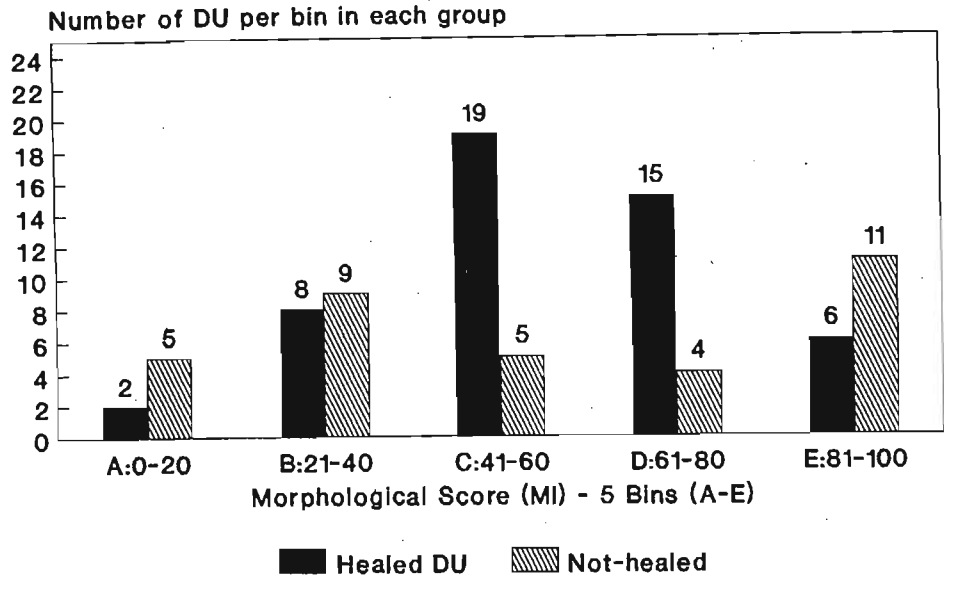


Figure 15: Incidence of DU healing and non-healing correlated with the morphological score.

7.1.5. The Endoscopic Severity of DU Correlated With Juxta-DU Morphology (MI)

In accord with previously described criteria, specimens were grouped according to the endoscopic severity of each lesion (SL:0-4). The morphological scores, incidence of healing and type of therapy was correlated with a particular severity level and listed in Table XI. For convenience and ease of comparison, DU were graded as endoscopically severe (SL:4;3;2:- 45pats.) or moderate (SL:1;0:- 39pats.). The morphological scores of each specimen from both groups were plotted in Figure 16.

Endoscopic Severity Level 4

P	**3**	**4**	**6**	**9**	**12**	**13**	**53**	**25**	**30**	**35**	**49**	**50**	**77**	**68**
MI	57	75	92	80	75	60	96	50	4	32	11	25	53	100
H±	+	+	+	+	+	+	+	-	-	-	-	-	-	-

P	**69**	**70**	**72**	**73**	**75**	**76**	**80**	**81**	**82**
MI	82	92	100	39	100	60	93	100	93
H±	-	-	-	-	-	-	-	-	-

Mean MI = MI:68.2
SD = MI:29.3
%age H = 30%

Endoscopic Severity level 3

P	**1**	**18**	**20**	**31**	**32**	**33**	**39**	**58**	**79**
MI	78	53	57	25	82	79	100	50	85
H±	+	+	+	-	-	+	+	+	-

Mean MI = MI:67.7
SD = MI:21.7
%age H = 66%

Endoscopic Severity level 2

P	**17**	**22**	**23**	**28**	**29**	**37**	**38**	**45**	**47**	**48**	**51**	**59**	**61**
MI	46	43	68	50	25	36	93	36	18	96	67	75	21
H±	+	+	-	+	+	-	-	+	-	-	+	-	-

Mean MI = MI:51.9
SD = MI:24.9
%age H = 46%

Endoscopic Severity Level 1

P	**15**	**21**	**24**	**36**	**41**	**42**	**44**	**55**	**57**	**63**	**64**	**66**	**74**
MI	57	71	68	71	79	21	75	57	25	50	21	46	0
H±	+	+	-	-	+	+	+	+	+	+	+	+	-

P	**78**	**14**	**67**	**83**	**84**
MI	46	78	100	43	54
H±	+	+	+	-	+

Mean MI = MI:53.4
SD = MI:24.4
%age H = 72%

Endoscopic Severity level 0

P	**2**	**5**	**7**	**8**	**10**	**11**	**16**	**19**	**26**	**27**	**34**	**40**	**43**	**46**
MI	53	67	32	57	39	85	46	64	29	79	29	64	57	85
H±	+	+	+	+	+	+	+	+	-	+	+	+	+	+

P	**52**	**54**	**56**	**60**	**62**	**65**	**71**
MI	64	43	57	14	67	54	21
H±	+	+	+	-	+	+	-

Mean MI = MI:52.7
SD = MI:19.5
%age H = 86%

	Severe (4:3:2)	**Moderate (1:0)**
Number of Patients	45	39
Mean MI	63.4	53
SD	27.7	21.9
%age Healing	42%	82%

Table XI: Endoscopic severity correlated with morphological score (MI) and incidence of healing (H±). Shown are the mean morphological scores (Mean MI) and standard deviation of each mean together with the percentage of patients healed at each level of endoscopic severity. Similar data is shown for DU graded as endoscopically severe (SL:4;3;2) and moderate (SL:0;1).

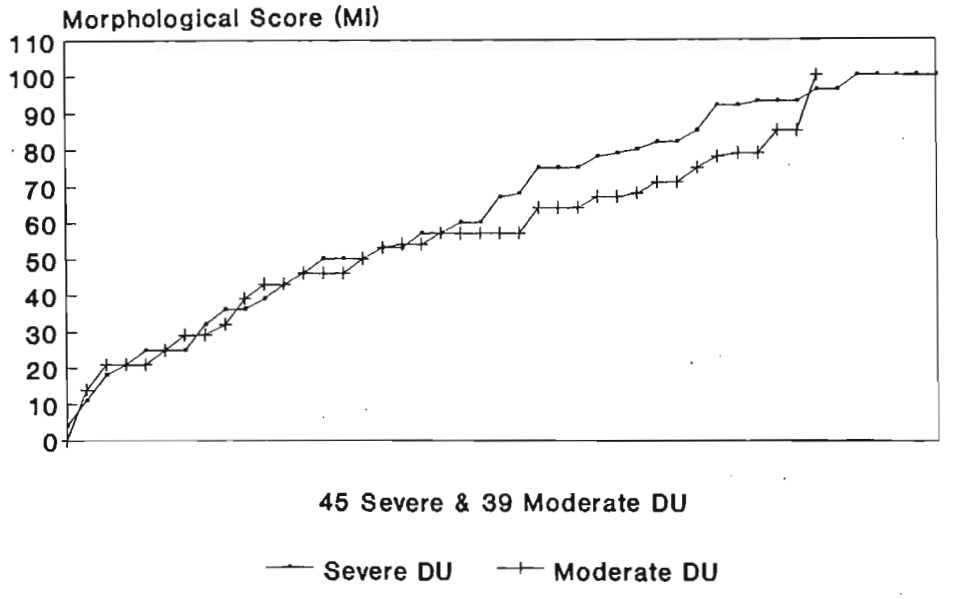


Figure 16: Morphological scores of endoscopically severe or moderate lesions. The MI of specimens from each group of lesions was sorted and plotted in ascending order.

The mean MI of endoscopically severe lesions was MI:63.4 and moderate DU MI:53 (Table XI). There was no significant difference in the range of MI data from severe and moderate lesions.

The scores of biopsies from lesions categorised as endoscopically severe or moderate were assigned to one of the 5 numeric bins. The data is summarised in Figure 17.

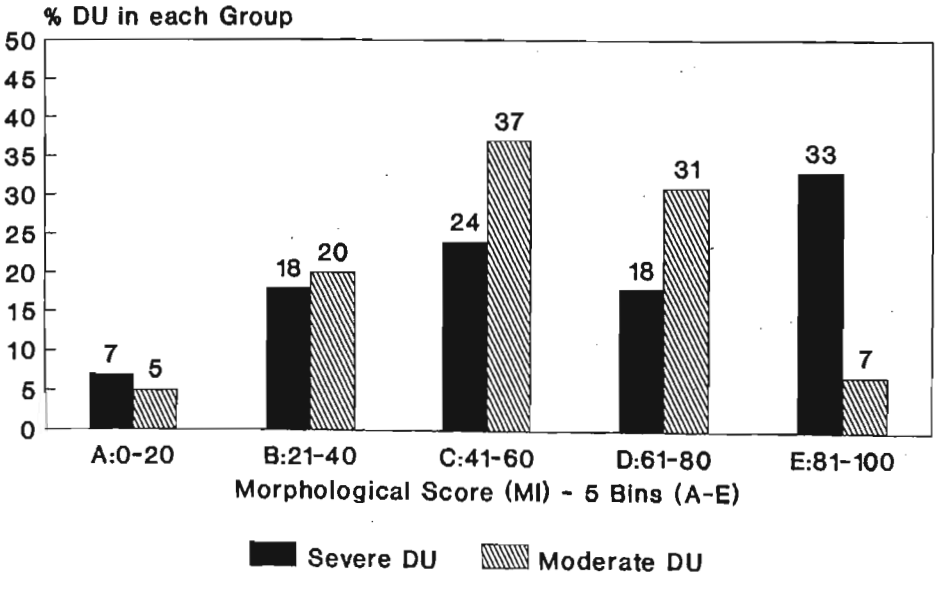


Figure 17: Percentage of patients in each numeric bin correlated with the severity of DU as assessed by endoscopy.

Bin E contained 28 patients (33%) with endoscopically severe DU and only 6 patients (7%) with moderate lesions. The difference in the numbers of DU from each endoscopic group in bin E was particularly significant ($p = 0.00006$).

To accentuate differences in morphology between endoscopically severe and moderate lesions, the MI of 23 patients with the most severe (SL:4) DU and 21 with the least severe (SL:0) DU were compared. The data from both groups of specimens was sorted in ascending order and plotted in Figure 18.

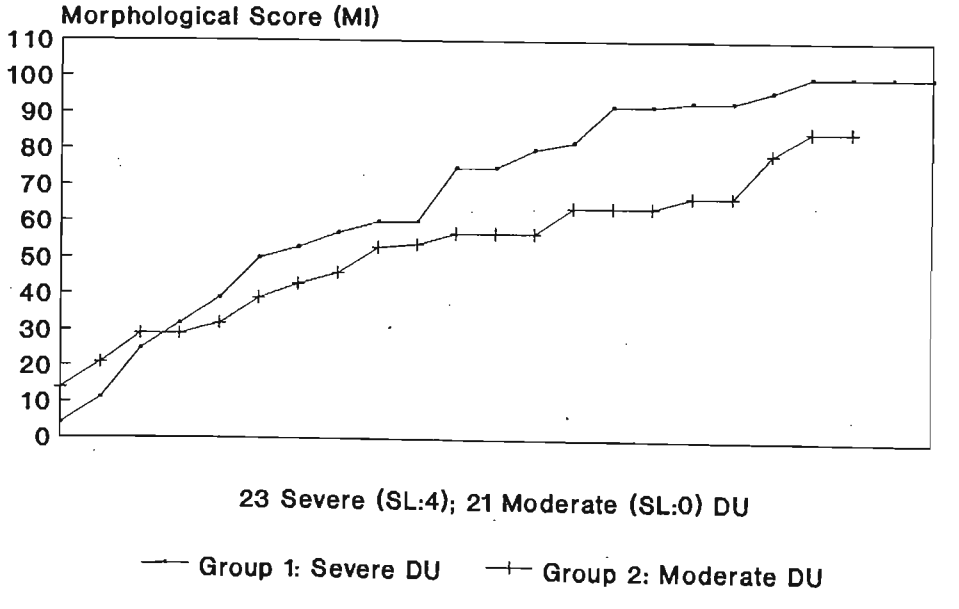


Figure 18: The morphological scores of the juxta-DU mucosa surrounding endoscopically the most (SL:4) and least (SL:0) severe lesions. The scores were sorted and plotted in ascending order.

There was a difference in MI between the groups of specimens ($p < 0.05$).

Based on their morphological scores (MI), specimens from DU categorised endoscopically as SL:4 or SL:0 were assigned to one of the 5 numeric bins. The data is summarised in Figure 19.

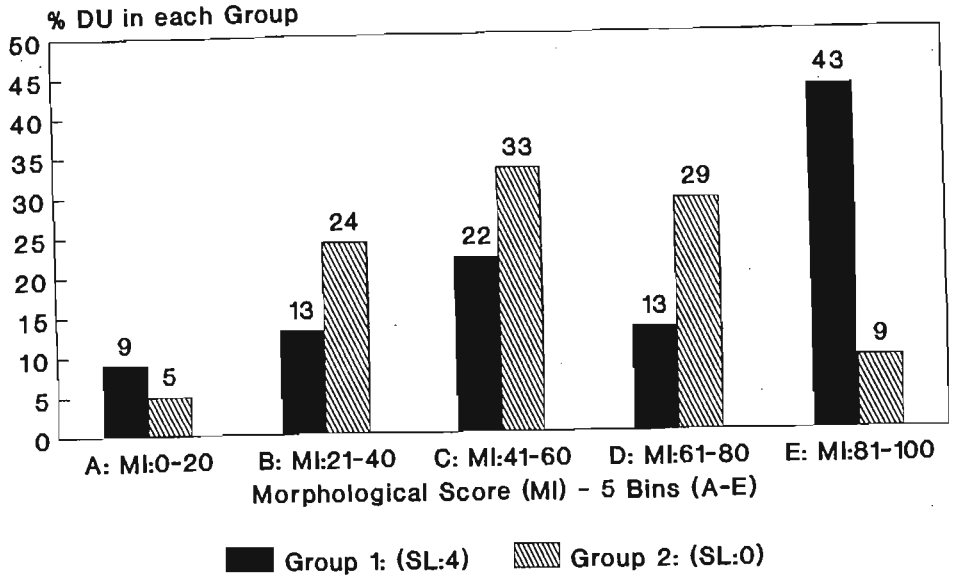


Figure 19: Comparison between the morphology of specimens from near the edge of endoscopically the most severe (SL:4) and least severe (SL:0) DU. The percentage of specimens in each numeric bin are recorded and compared.

There was a difference between the numbers of endoscopically severe and moderate DU in the 5 bins ($p = <0.0486$). Bin E contained 11 patients (43%) with SL:4 DU and only 2 (9%) with SL:0 lesions. The difference in the numbers of DU from each endoscopic group in bin E was significant ($p <0.001$).

7.1.6. Summary of Correlations Between Juxta-DU Morphology And Incidence of Healing

Gastric metaplasia in the juxta-DU mucosa is not necessarily a favourable prognostic criterion. However, moderate metaplasia (MI:40-80), especially when extant in the mucosa circumscribing endoscopically moderate lesions (SL:0-1), was invariably associated with DU healing. Well differentiated metaplasia (MI:81-100) and non-metaplasia (MI:0-40) were morphological phenomena that militated against DU healing. Many endoscopically severe, more difficult to heal DU, were

surrounded by a well differentiated metaplastic mucosa. This physico-morphological association rather than metaplasia, per-se, may explain why severe metaplasia was a poor prognostic criterion. In the case of DU surrounded by non-metaplastic mucosa, no physico-morphological reason for DU persistance could be proffered for there was no difference in the number of endoscopically severe or moderate lesions surrounded by this type of tissue.

7.2. Correlations Between Juxta-DU and Scar Morphology.

The EM Unit and hospital data, the morphological scores for each parameter (S1-S4) and the composite morphological score (MI) of biopsies obtained before and at the termination of 6 weeks curative therapy with either sucralfate or cimetidine(1) are listed in Table XII. Also shown are data obtained from patients in remission 13, 26, 39 and 52 weeks after the cessation of treatment. Table XIII summarises the numeric data obtained before, after 4 weeks treatment and 6 months after treatment was terminated with either low or high-dose misoprostol or cimetidine(2).

All juxta-DU and scar MI data from each of the 41 patients who had healed after drug therapy were collated and compared. Morphological differences in pre- and post-therapy mucosa were examined and the possibility that scar morphology may influence the duration of remission investigated.

SUCRALFATE - CIMETIDINE(1) STUDY

Pre-therapy Data

PATIENT DATA				MORPHOLOGICAL SCORES				
P	EM	GI	D	S1	S2	S3	S4	MI%
1	898	20079	S	3		3	2	3	79
2	928	20772	S	1.5		2	2.5	1.5	54
3	1061	22399	S	2.5		2	1.5	2	57
4	1068	22519	S	3.5		4	2.5	0.5	75
5	1097	22727	S	3		4	2.5	0	68
6	1146	23530	S	3.5		4	2.5	3	92
7	875	19543	S	1		0	2.5	1	32
8	894	20009	S	2		3	2	1	57
9	901	20171	S	0.5		1	0	1	7
10	1077	22667	S	1		2	2	0.5	39
11	897	20054	C1	3.5		3	2.5	3	86
12	906	20281	C1	3.5		4	2.5	0.5	76
13	967	21233	C1	2.5		2	2.5	1.5	61
14	1095	22724	C1	3.5		3	2.5	2	79
15	889	19840	C1	0.5		0.5	0	0	7
16	893	20007	C1	2		2	1.5	1	46
17	902	20175	C1	2.5		0.5	2.5	1	46
18	968	21234	C1	3		2	2.5	0	54
19	1123	23246	C1	2.5		4	2	0.5	64
20	1147	23531	C1	2.5		3	2.5	0	57

After 6 weeks Curative Therapy

1	919	20470	S	3	3	2	1	64
2	972	21275	S	1.5	1	1.5	1.5	39
3	1094	22273	S	1.5	1	0	1.5	29
4	1102	22760	S	3	3	2.5	1	68
5	1134	23384	S	2	2	2	2	57
6	1167	23888	S	1.5	2	0	1.5	36
7	900	20165	S	3	2.5	2	1	61
8	916	20367	S	2	0.5	2	0	32
9	924	20578	S	1	1	0	0.5	18
10	1120	23126	S	2	2	1.5	1.5	50
11	923	20054	C1	2	2	2	1	50
12	927	20698	C1	1.5	1.5	2.5	1.5	50
13	1002	21673	C1	2.5	2	2.5	1.5	61
14	1136	23428	C1	1.5	0.5	2.5	0.5	36
15	914	20343	C1	2	0	1.5	0	25
16	915	20359	C1	1.5	1	1.5	0.5	32
17	925	20628	C1	2.5	1	2.5	0.5	46
18	1001	21672	C1	2.5	0.5	2.5	0	39
19	1160	23620	C1	1.5	2.5	1.5	0.5	43
20	Specimen had no mucosal surface							

Continued/

13 Weeks After the Termination of Curative Therapy

PATIENT DATA			MORPHOLOGICAL SCORES					
P	EM	GI	D	S1	S2	S3	S4	MI%
1	Specimen had no mucosal surface - Patient in remission.							
2	1052	22209	S	2.5	2.5	2	1	57
3	1159	23619	S	3	1	1.5	3	61
4	1153	23564	S	3	2	2	2	64
5	Specimen had no mucosal surface - Patient in remission.							
6	No specimen received by EM Unit - Patient in remission.							
7-10	Each patient had relapsed - New DU							
11	995	21504	C1	1.5	1.5	1	1.5	39
12	996	21574	C1	1.5	1	2.5	1.5	39
13	1067	22449	C1	3	2.5	2.5	1.5	68
14	1183	24233	C1	0.5	2	2.5	1	57
15-20	Each patient had relapsed - New DU							

26 Weeks After the Termination of Curative Therapy

1	1053	22264	S	2.5	1.5	2.5	1	54
2	1111	22904	S	2	2	2	1	50
3	Did not arrive for examination							
4	1190	24291	S	3	1	2.5	1	54
5	Patient relapsed							
6	Did not arrive for examination							
11	1054	22307	C1	3.5	2.5	2.5	0	61
12	1065	22428	C1	2.5	2	2.5	0.5	54
13	1124	23269	C1	1.5	1	2	3	54
14	Patient relapsed							

39 Weeks After the Termination of Curative Therapy

1	1113	22981	S	2.5	2	2	0.5	50
2	Did not arrive for examination							
3	Patient relapsed - 33 weeks							
4	Patient relapsed							
6	Patient relapsed - 33 weeks							
11	Did not arrive for examination							
12	1121	23160	C1	2	2	2.5	0.5	50
13	1166	23889	C1	3	1.5	2	1	54

1 Year after the Termination of Curative Therapy

1	1161	23673	S	3	2	1	1	50
2	1175	24034	S	2.5	2	2	0.5	50
11	1152	23550	C1	2.5	2	2.5	0	50
12	1163	23864	C1	2	2	2.5	0	46
13	1237	24681	C1	2.5	3	2	0.5	61

Table XII: Summarises the thesis and hospital patient numbers (P;EM;GI), numeric morphological data from biopsies obtained before, after 6 weeks therapy and 13, 26, 39,and 52 weeks after treatment with sucralfate or cimetidine(1).

CIMETIDINE: HIGH AND LOW DOSE MISOPROSTOL STUDY

Pre-therapy Data

PATIENT DATA			MORPHOLOGICAL SCORE					
P	EM	GI	D	S1	S2	S3	S4	MI%
40	1225	24657	HM	3	3	2.5	0.5	64
41	1318	25551	HM	3	2.5	2.5	3	79
42	1357	26013	HM	0.5	1	0	1.5	21
43	1391	26429	HM	2	3	2.5	0.5	57
44	1396	26510	HM	3	4	2.5	1	75
45	1384	26379	HM	2.5	0	1.5	1	36
46	1385	26329	HM	3	4	2	3	8 5
51	1244	24692	LM	3	3	2.5	1	67
52	1280	25053	LM	3	2	2.5	1.5	64
53	1286	25080	LM	3.5	4	3	3	96
54	1336	25855	LM	2.5	0.5	2.5	0.5	43
55	1354	25995	LM	2.5	2.5	2.5	0.5	57
56	1363	26055	LM	3	2	2.5	0.5	57
57	1367	26107	LM	1	1	1	1.5	25
58	1390	26413	LM	1.5	2	2.5	1	50
62	1224	24653	C2	3	3	2.5	1	67
63	1264	24916	C2	2	3	1.5	0.5	50
64	1298	25201	C2	0.5	2	0.5	0	21
65	1310	25391	C2	2.5	2	2.5	0.5	54
66	1320	25564	C2	2	2	2	0.5	46
67	1350	25941	C2	2	2.5	2.5	0.5	54

After 4 Weeks Curative Therapy

40	1261	24892	HM	3	2.5	2.5	0.5	61
41	1339	25891	HM	3	2.5	3	0.5	64
42	1381	26247	HM	2	2	2.5	0.5	50
43	1411	26711	HM	1.5	3	2	1	54
44	1415	26830	HM	1	1	0	0.5	18
45	1432	26943	HM	2	2	2	0.5	46
46	1434	26951	HM	3	2	2.5	0.5	57
51	1266	24938	LM	3.5	3	2.5	0	64
52	1304	25303	LM	2.5	2.5	2.5	0.5	57
53	1308	25360	LM	0.5	1	0	1.5	21
54	1366	26097	LM	1	1	0	1.5	25
55	1378	26238	LM	1.5	2	2.5	1.5	54
56	1380	26246	LM	1	1.5	0	0.5	21
57	1388	26379	LM	1	1	1.5	1.5	36
58	1395	26508	LM	1.5	2	1	1	39
62	1265	24937	C2	2.5	4	2	0.5	64
63	1287	25087	C2	1.5	2	2	0.5	43
64	1309	25384	C2	2	0	1.5	0.5	29
65	1322	25579	C2	1	1.5	0	1	25
66	1337	25867	C2	1.5	2.5	2.5	0.5	50
67	1373	26170	C2	1.5	2	1.5	1	43

Continued/

6 Months After the Termination of Curative Therapy

PATIENT DATA				MORPHOLOGICAL SCORE				
P	EM	GI	D	S1	S2	S3	S4	MI%
40	Patient relapsed							
41	1446	27882	HM	2	1.5	2.5	0.5	46
42	1436	27614	HM	0.5	2	2	1	39
43	1461	28069	HM	1	2	1	1	36
44	1464	28198	HM	1	2	1	1.5	39
45	Patient relapsed							
46	Patient relapsed							
51	Patient relapsed							
52	1409	26667	LM	1	2	1	1.5	39
53	1399	26605	LM	2.5	2	2.5	1	57
54	Patient relapsed							
55	1430	27369	LM	0.5	2	2	1	39
56	1437	27704	LM	1	1.5	1	0.5	28
57	1433	27585	LM	3	2.5	1.5	1	64
58	Patient relapsed							
62	1452	27920	C2	2.5	3	2.5	1	64
63-66	All patients relapsed							
67	1435	27609	C2	3	2	2	0.5	50

Table XIII: Lists the thesis and hospital patient numbers (P;EM;GI) and numeric morphological data from biopsies obtained before, after 4 weeks therapy and 6 months after the end of treatment with either cimetidine(C2) or high-(HM) or low-dose (LM) misoprostol. D = Drug.

7.2.1. Juxta-DU and Scar Mucosal Morphology (MI)

The MI of the pre- and post-therapy specimens obtained from the 41 patients who healed after therapy were sorted in ascending order and plotted in Figure 20. The paired pre- and post-therapy MI data is listed in Table XIV

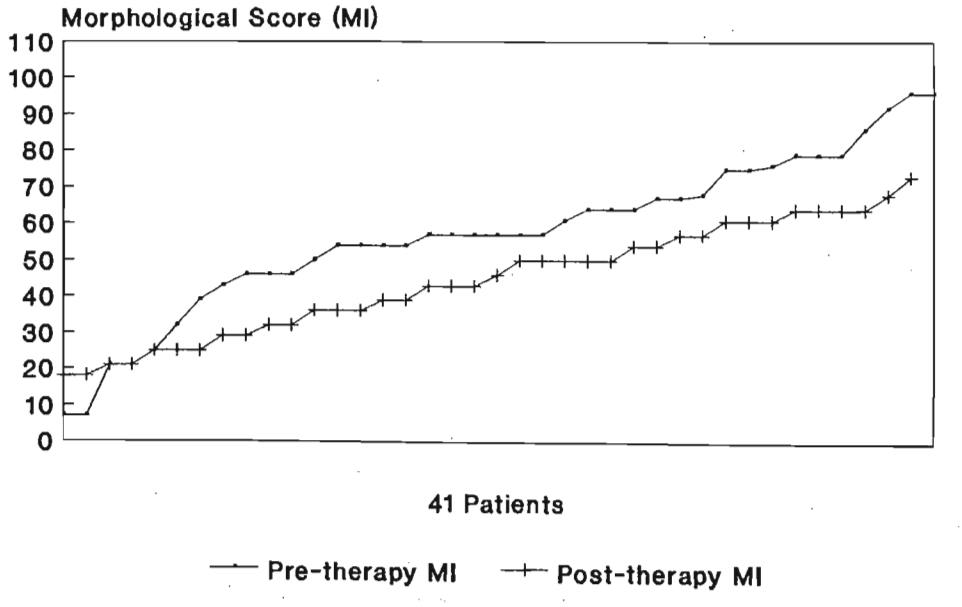


Figure 20: Pre- and post-therapy MI in juxta-DU and scar specimens from patients who healed after drug therapy.

There was a difference between pre- and post-therapy data. The difference was manifest as a significant reduction in mean MI from MI:56.2 before treatment to MI:43.9 after curative therapy (Mann-Whitney p = 0.0035; Variance p = <0.05). There was also a difference between the paired pre- and post-therapy data (Wilcoxon p = 0.0245).

The morphological scores of biopsies obtained prior to and after treatment from each of the 41 patients that healed were assigned to one of the 5 numeric bins. The data is summarised in Figure 21.

PAIRED MI DATA: BEFORE AND AFTER THERAPY

P.No.	1	2	3	4	6	11	12	13	41	42	43	44
Pre.T	79	54	57	75	92	86	76	61	79	21	57	75
Po.T	64	39	29	68	32	50	50	61	64	50	54	18
Drug	S	S	S	S	S	C1	C1	C1	LM	LM	LM	LM

P.No	52	53	55	56	57	62	67	45	46	58	65	66
Pre-T	64	96	57	57	25	67	54	36	85	50	54	46
Po.T	57	21	54	21	36	64	43	46	57	39	25	50
Drug	HM	HM	HM	HM	HM	C2	C2	HM	HM	LM	C2	C2

P.No.	5	7	8	9	10	14	15	16	17	18	19	20
Pre.T	68	32	57	7	39	79	7	46	46	54	64	57
Po.T	57	61	32	18	50	36	25	32	46	39	43	-
Drug	S	S	S	S	S	C1	C1	C1	C1	C1	C1	C1

P.No.	40	51	54	63	64
Pre-T	64	67	43	50	21
Po.T	61	64	25	43	29
Drug	LM	LM	HM	C2	C2

Mean Pre-T: **MI = 57.2** **SD = 21.6**
Mean Po.T : **MI = 44.4** **SD = 15.5**

Tests between Pre-T & Po.T

Wilcoxon **p = 0.0245**
Mann-Whitney **p = 0.0035**
Variance **p = <0.05**

Table XIV: The morphological scores (MI) of juxta-DU and scar biopsies from the same patients

P.No. = Patient number
Pre-T = Mean morphological score before therapy.
Po.T = Mean morphological score after curative therapy.
S = Sucralfate
C(1 or 2) = Cimetidine - 2 regimens (see methods)
LM = Low-dose Misoprostol
HM = High-dose Misoprostol

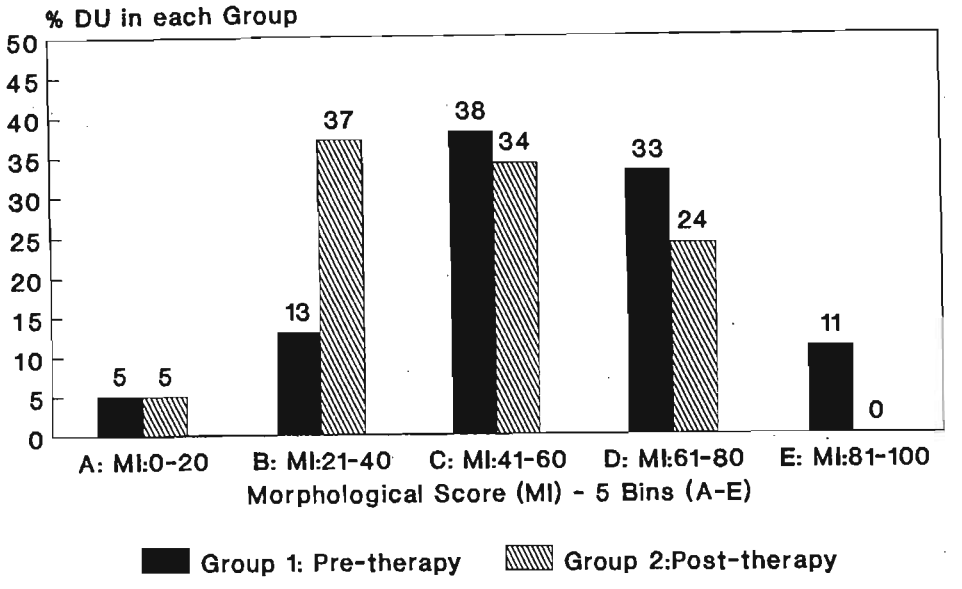


Figure 21: Percentage of juxta-DU and scar specimens in each numeric bin.

There was a significant difference in the distribution of pre and post-therapy specimens in each bin ($p < 0.01$). Particular differences were found in bins **B** (MI:21-40 - $p = 0.0035$) and **E** (MI:81-100 $p = 0.00$).

The pre-therapy data was sorted in ascending order and specimens were asigned to each of the 5 numeric bins. The mean juxta-DU MI of specimens in each bin was plotted with the mean scar MI from the same patients. The data is summarised in Figure 22. After healing, there was an increase in mean scar MI of specimens in bins **A** & **B** and a reduction in mean scar MI of specimens in each of bins **C,D** & **E** (variance $p = <0.01$). Prior to treatment there was a significant difference in variance between the means of specimens in each morphological bin ($p = <0.01$). After treatment, other than a difference in mean MI of scar specimens in bins **A** and **E** ($p = <0.05$) all other bin combinations were similar.

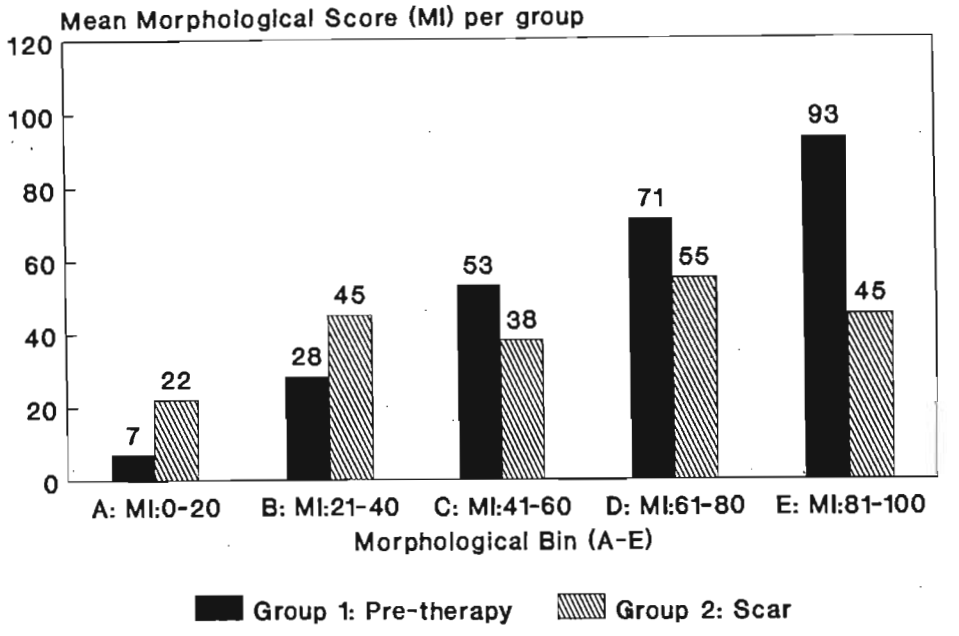


Figure 22: The mean morphological scores of juxta-DU specimens in each bin correlated with their scar counterparts.

7.2.2 Scar Mucosal Morphology Correlated with the Duration of Remission.

The duration of remission was deduced for each of the 41 healed patients (consult tables XII and XIII). The specimens were divided into 2 groups: group 1, from 19 patients experiencing remission for more than 6 months; group 2, from 22 patients who relapsed within 6 months after the termination of treatment. The paired juxta-DU and scar MI, means and standard deviation of group 1 are listed in table XVA and group 2 in table XVB. The pre- and post therapy MI of each specimen from group 1 were sorted and plotted in ascending order in Figure 23 and the data from group 2 sorted and plotted in a similar fashion in Figure 24.

There was a significant difference between the paired pre- and post-therapy data in both groups (group 1: $p = 0.004$; group 2: $p = 0.009$). The difference was manifest as a

P.No.	1	2	3	4	6	11	12	13	41	42	43	44
Pre.T	79	54	57	75	92	86	76	61	79	21	57	75
Po.T	64	39	29	68	32	50	50	61	64	50	54	18
Drug	S	S	S	S	S	C1	C1	C1	LM	LM	LM	LM

P.No	52	53	55	56	57	62	67
Pre-T	64	96	57	57	25	67	54
Po.T	57	21	54	21	36	64	43
Drug	HM	HM	HM	HM	HM	C2	C2

Mean Pre-T: MI = 64.7 SD = 19.0
Mean Po.T : MI = 46.3 SD = 15.5

Tests between Pre-T & Po.T

Wilcoxon	**p = 0.004**
Mann-Whitney	**p = 0.0025**
Variance	**p = <0.05**

Table XV A: Paired MI data: Morphological scores of biopsies obtained before and after therapy in patients experiencing remission from DU for more than 6 months.

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P.No.	5	7	8	9	10	14	15	16	17	18	19	20
Pre.T	68	32	57	7	39	79	7	46	46	54	64	57
Po.T	57	61	32	18	50	36	25	32	46	39	43	-
Drug	S	S	S	S	S	C1	C1	C1	C1	C1	C1	C1

P.No.	40	51	54	63	64	45	46	58	65	66
Pre-T	64	67	43	50	21	36	85	50	54	46
Po.T	61	64	25	43	29	46	57	39	25	50
Drug	LM	LM	HM	C2	C2	HM	HM	LM	C2	C2

Mean Pre-T: MI = 49.9 SD = 21.4
Mean Po.T : MI = 42.6 SD = 15.1

Tests between Pre-T & Po.T

Wilcoxon	**p = 0.009**
Mann-Whitney	**p = 0.01**
Variance	**p = <0.05**

Table XV B: Paired MI data: Morphological scores of biopsies obtained before and after therapy in patients experiencing remission from DU for less than 6 months.

P.No.	= Patient number
Pre-T	= Mean morphological score before therapy.
Po.T	= Mean morphological score after curative therapy.
S	= Sucralfate
C(1 or 2)	= Cimetidine - 2 regimens (see methods)
LM	= Low-dose Misoprostol
HM	= High-dose Misoprostol

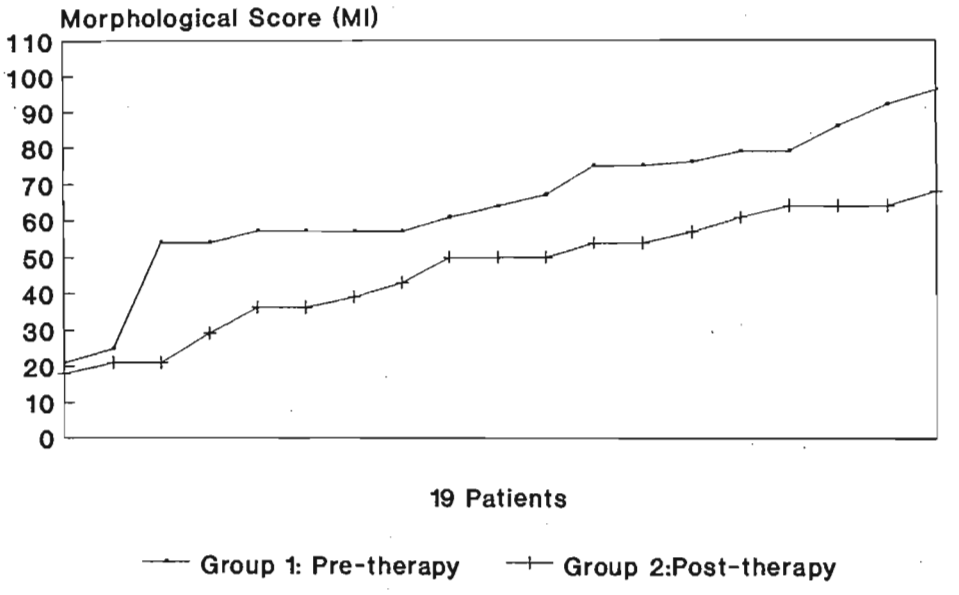

Figure 23: The pre- and post-therapy morphological scores of patients who remained in remission for longer than 6 months after the termination of therapy plotted in ascending order.

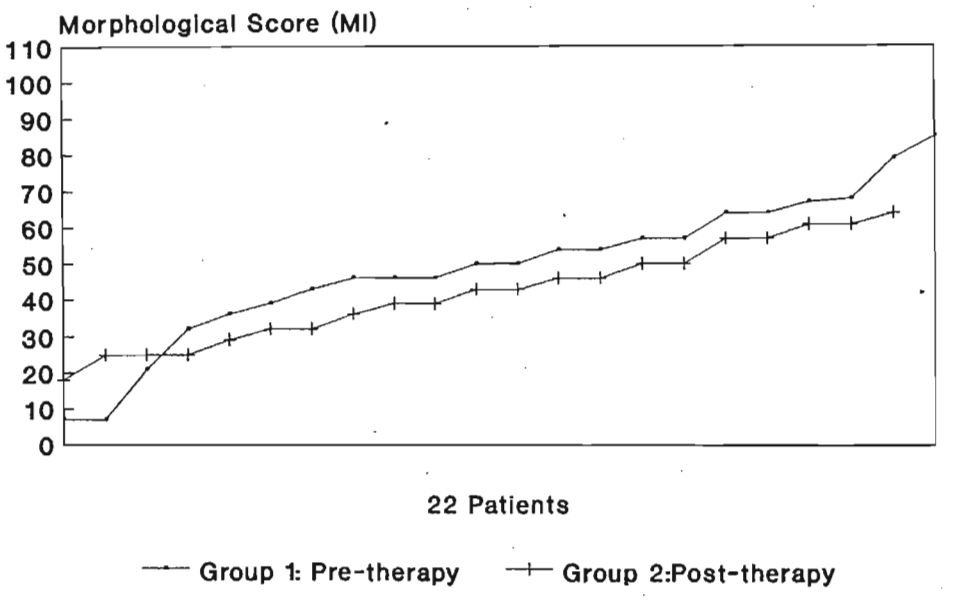

Figure 24: The pre- and post-therapy morphological scores of patients who relapsed within 6 months of the termination of therapy plotted in ascending order.

significant reduction in mean MI from MI:64.7 & MI:49.9 before treatment to MI:46.3 and MI:42.6 respectively after curative therapy ($p = 0.0025$ and 0.009; Variance $p = <0.05$).

The scar MI of each patient who remained in remission for more or less than 6 months after the termination of therapy is plotted in Figure 25. No statistical differences were found between the two groups.

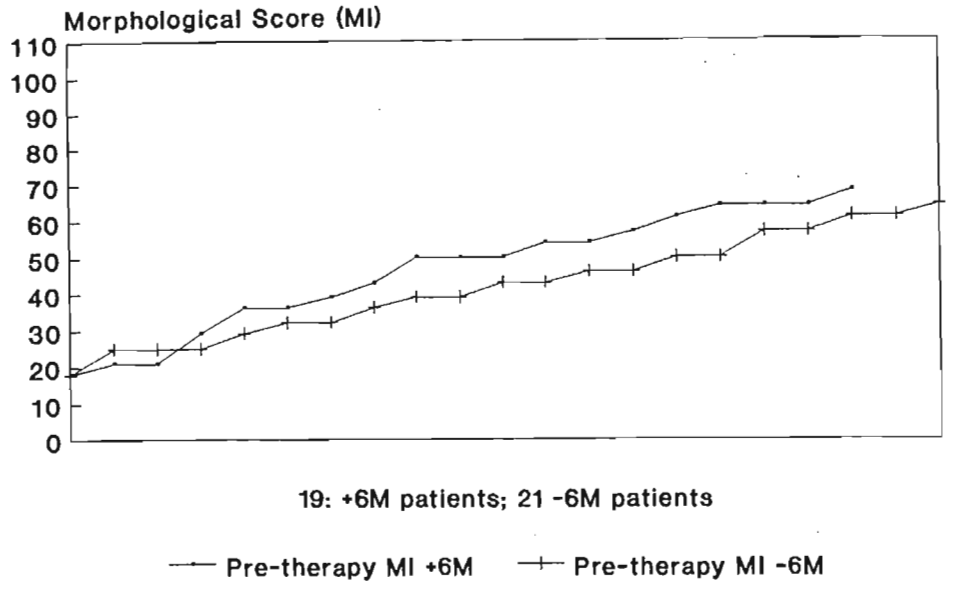

Figure 25: Scar data from patients in remission for more or less than six months after therapy.

Patients were divided into 2 groups; those who experienced remission for more or less than six months. The MI of scars from each group were placed into each of the numeric bins and the number of specimens in each bin recorded. The data from both groups of patients is summarised in Figure 26. There was no difference in the distribution of MI between groups ($p = 0.563$) and there were no significant differences in the number of specimens in each bin.

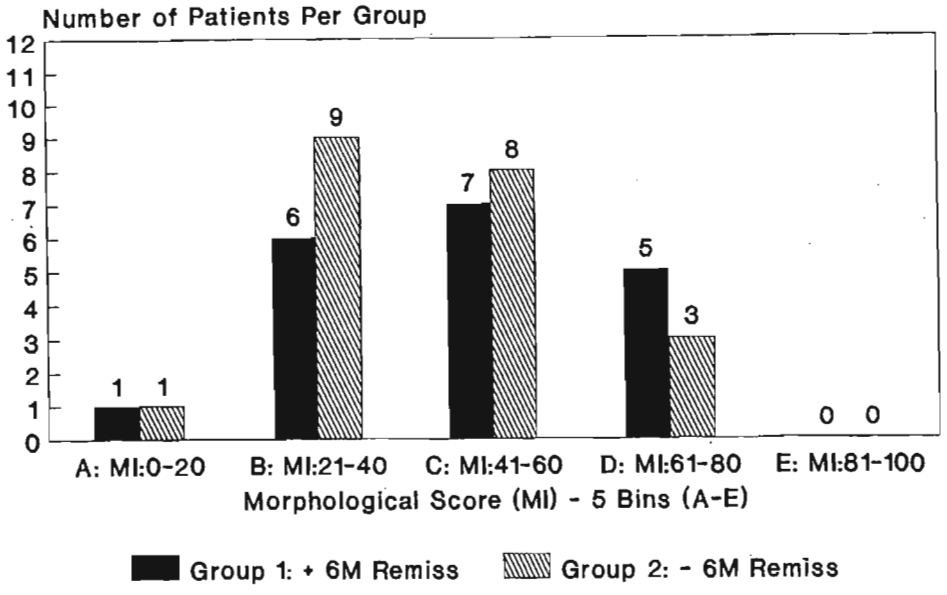

Figure 26: Comparison of data from scars in patients who were in remission for more or less than 6 months.

7.2.3. The Morphological Appearance Of Scar Mucosa During The Period Of Remission

Figure 27 and Table XVI detail the mean MI of biopsies obtained before and after therapy and at 13 week periods up to 1 year after treatment. Also shown in Table XVI are the results of comparative analyses made between various groups.

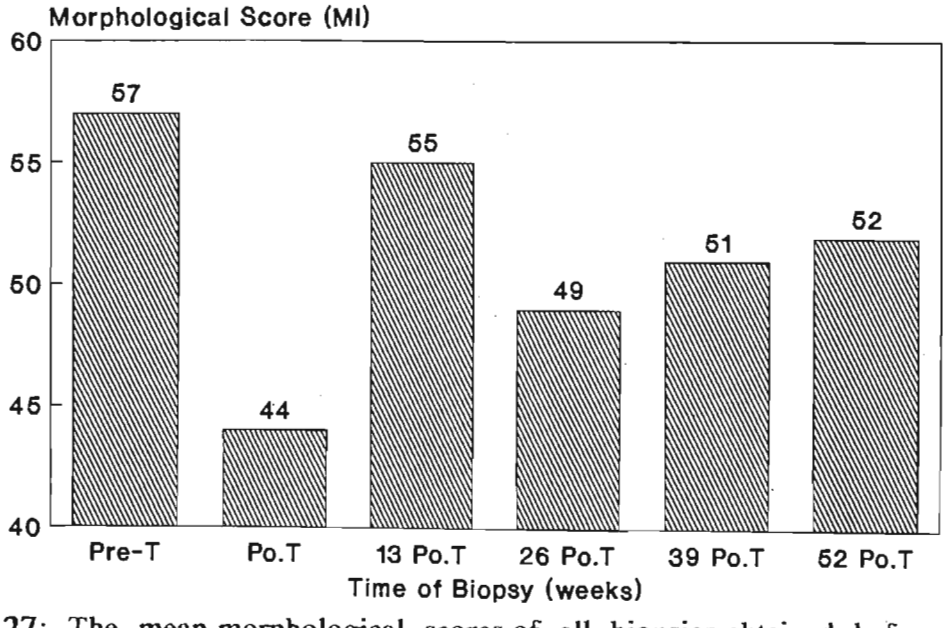

Figure 27: The mean morphological scores of all biopsies obtained before (Pre-T) after therapy (Po.T) and at 13, 26, 39 and 52 weeks after the termination of treatment.

Time	Pre	Post	13 Wk.	26 Wk.	39 Wk.	52 Wk.
Group	A	B	C	D	E	F
No.Pats	41	40	7	17	3	4
Mean MI	56.2	43.9	55.1	48.7	51.3	51.8
SD (MI)	20.9	14.5	10.7	10.2	1.9	5.6

Mann-Whitney		F-distribution (Variance)
A & B	p = 0.0035	F = 1.94 <0.05
A & D	p = 0.0438	F = 4.48 >0.05
B & C	p = 0.0473	F = 2.09 >0.05
B & D	p = 0.3214	F = 2.31 <0.05
D & E	p = 0.8	F = 28.8 <0.05

Table XVI: Mean morphological scores (MI) of biopsies obtained before and up to 1 year after the termination of therapy. Where significant differences were found between groups it is shown.

There was a difference in mean MI from MI:43.9 at the termination of treatment to MI:55.1, 13 weeks later (p = 0.0473). Other than some differences in the variance of mean MI in the later periods of remission (B&D & D&E - see Table XVII), there were no further differences in MI from 13 weeks to 1 year after treatment.

All specimens obtained from patients prior to and after treatment and from scars of those in remission from 13 weeks to 1 year were assigned to one or other of the 5 numeric bins. Table XVII lists the data. Figure 28 describes graphically the percentage of specimens obtained 13, 26, 39 and 52 weeks after therapy whose MI placed them in each bin.

	NUMERIC BINS									
	A:0-20		B:21-40		C:41-60		D:61-80		E:81-100	
	No.	%	No.	%	No.	%	No.	%	No.	%
Pre.Therapy	2	5	6	14	16	39	13	32	4	10
Po. Therapy	2	5	15	38	15	37	8	20	0	0
13 Wks	0	0	2	28	2	28	3	43	0	0
26 Wks	0	0	6	35	8	47	3	18	0	0
39 Wks	0	0	0	0	3	100	0	0	0	0
52 Wks	0	0	0	0	3	75	1	0	0	0

Comparison: Times of Biopsy	**Chi-squared**
Bins A-E Pre- & post-therapy	p = 0.046
Bins A-C Pre- & 13 weeks	p = 0.671
Bins A-E Post-therapy & 13 weeks	p = 0.717
Bins A-E Post-therapy & 26 weeks	p = 0.6
Bins A-E Post-therapy & 39 weeks	p = 0.16
Bins A-E Post-therapy & 52 weeks	p = 0.32
Bins A-E 13 weeks & 26 weeks	p = 0.43
Bins A-E 26 weeks & 39 weeks	p = 0.23
Bins A-E 39 weeks & 52 weeks	p = 0.893

Table XVII: The mean morphological data from all specimens obtained from patients prior to (pre-) and after (po.) treatment and from scars of those in remission from 13 weeks to 1 year were assigned to one or other of the 5 numeric bins. The numeric data was compared and the results shown.

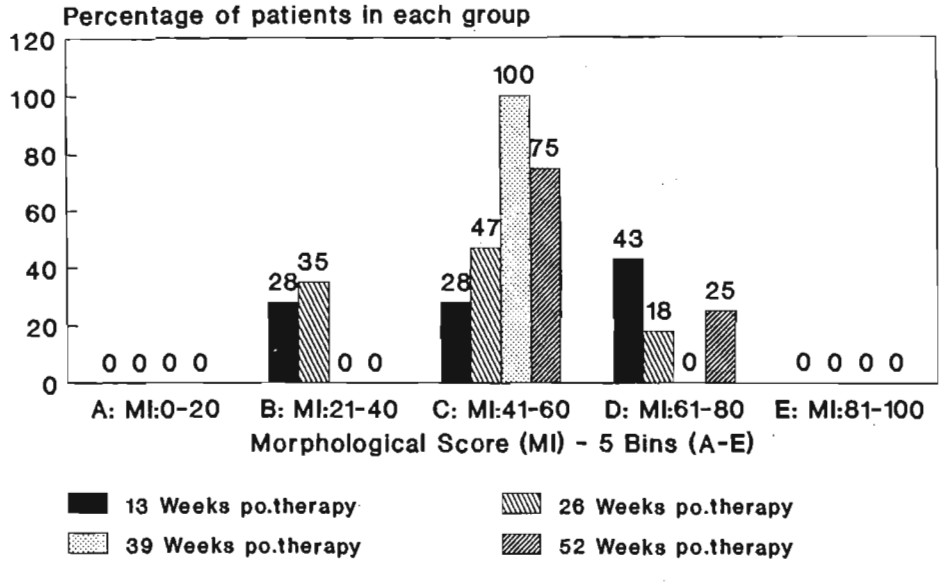

Figure 28: The type of mucosa in biopsies obtained from scars, 13, 26, 39 and 52 weeks after the termination of therapy. Note that no specimens have a mucosa that is particularly metaplastic (MI:81-100) or degeneratively non-metaplastic (MI:0-20).

These results show that after an initial post-therapy reduction in MI, by 13 weeks after treatment, there was a rise in MI to levels approximating those in pre-therapy tissue. This level of MI persisted in patients who remained in remission for up to 1 year. From MI scores alone, it would appear that the scar mucosa 13 weeks after treatment (MI:55) had regressed to mimic the morphology of tissue surrounding active DU (MI:57). However, Table XVII and Figure 28 show that from 13 weeks to 1 year after therapy, there were no specimens in either Bins A or E. The successfully healed mucosa, therefore, was confined to the moderately metaplastic bins B, C & D.

7.2.4. Summary Of Correlations Between Pre- and Post-Therapy Data And Duration of Remission

These results showed that scar mucosal morphology differed from juxta-DU morphology and confirmed that mucosa surrounding scars was abnormal. The data showed that prior to therapy, juxta-DU morphology varied extensively whereas after treatment, the spectrum of morphological variation near to scars was reduced. Most specimens from scars were moderately metaplastic. This type of mucosa persisted in patients experiencing extended remission. It is interesting to note that after the termination of treatment, the morphological appearance of scar mucosa (morphological "quality" of healing) did not appear to influence remission prognosis.

Section 2: The Influence Of Drug Therapy On DU Healing And Remission

7.3. Mucosal Morphology Before And After Curative Drug Regimens

The mean MI of all juxta-DU specimens from the groups of patients to be treated with cimetidine(1) or sucralfate are plotted in Figure 29. Also plotted are the mean post-therapy MI of patients in remission for up to 1 year after either curative regimen.

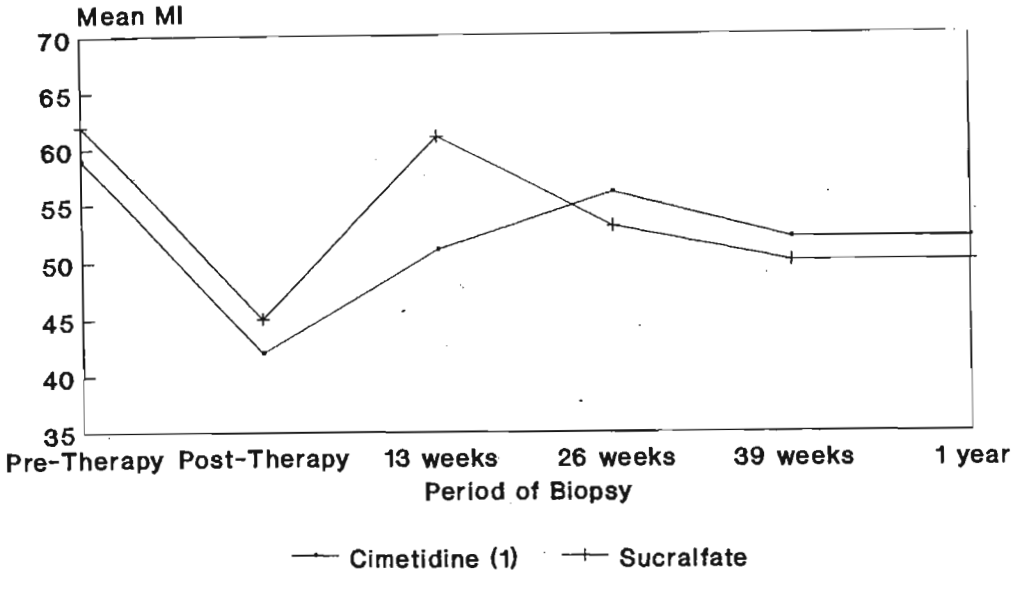

Figure 29: Plotted are the mean morphological scores of all juxta-DU and scar specimens before, at the end of and at 13 week periods up to 1 year after therapy with either sucralfate or cimetidine(1).

There is a reduction in MI after both regimens. At 13 weeks post-therapy, in both cases morphological scores had risen to near pre-therapy levels. By 26 weeks after therapy, the morphological scores from both groups had plateaued at approximately MI:50 and remained at this level for the period of study. The apparent difference in MI 13 weeks after treatment was not significant at $p < 0.05$.

The mean pre- and post therapy data for patients treated with either cimetidine(2), high or low-dose misoprostol is plotted in Figure 30.

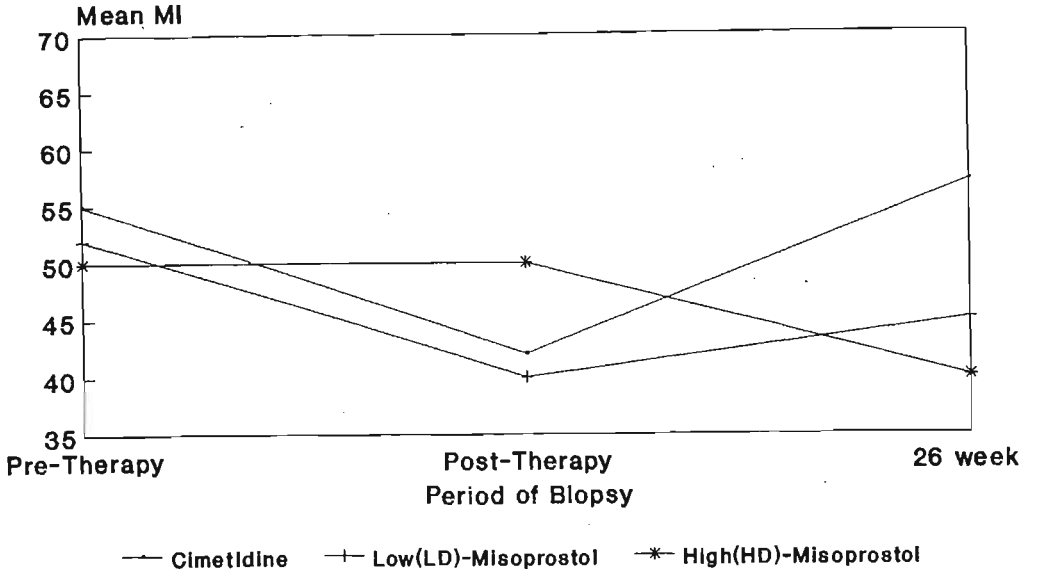

Figure 30: Plotted are the mean morphological scores of all juxta-DU and scar specimens before, at the end of and 6 months after treatment with either cimetidine(2), low- or high-dose misoprostol.

There is an apparent reduction in MI after treatment with cimetidine and low-dose misoprostol. In the case of high-dose misoprostol pre- and post-therapy MI are similar. Six months after therapy, cimetidine and low-dose misoprostol have returned to near pre-therapy levels whereas high-dose MI has dropped. The visual differences in mean MI were not significant at $p < 0.05$.

7.3.1. Incidence of Healing After Drug Therapy

The percentage healing after therapy with either of the 7 drug regimens is shown in Table XIII. Of the 84 patients treated for DU, 34 (40%) were not healed at the termination of treatment.

No.Pats	Drug Therapy	No.Healed	%
16	Cimetidine(1) 1g/6wks	11	63
10	Cimetidine(2) 300mg/4wks	6	60
9	Cimetidine(3) 800mg/4wks	5	56
16	Sucralfate 1g/day/6wks	10	63
11	LD Misoprostol	8	73
12	HD Misoprostol	7	58
10	Pirenzipine	3	30
Total 84		**50**	**60**

Figure XVIII: Incidence of healing after various types of drug therapy. The number of patients (No.Pats), type of drug therapy and the number (No.) and percentage of patients healed in each of the studies is shown.

The proportions test revealed a difference between the number of patients that healed after treatment with pirenzipine and low-dose misoprostol ($p < 0.05$) and pirenzipine and cimetidine(1) ($p < 0.05$).

7.3.2. The Juxta-DU Mucosa Before Drug Therapy

The morphological scores of all specimens obtained before and after therapy with each of the 7 drug regimens are listed in Table XIX. The Mann-Whitney and Variance tests were applied to all pre-therapy data. With the exception of comparisons between O & AP (Variance $p =< 0.05$), there were no significant differences in the numeric data between any of the 21 possible pre-therapy combinations.

The morphological scores of each juxta-DU specimen was assigned to one of 5 numerical bins. The number and percentage of specimens in each bin prior to treatment with either of 7 drug regimens are listed in Table XX.

Table XIX

Cimetidine (1): 1g/day/6 wks

P.No.	11	12	13	14	15	16	17	18	19	20	68	69	73	74	81
Pre-T	86	76	61	79	7	46	46	54	64	57	100	82	39	0	100
Po-T	50	50	61	36	25	32	46	39	43	*					
Heal ±	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Rem ±6M	+	+	+	-	-	-	-	-	-	-					

Mean Pre-therapy (All)	A: MI:59.4	SD: 27.7
Mean Pre-therapy (H+)	B: MI:57.6	SD: 21.2
Mean Pre-therapy (H-)	C: MI:62.3	SD: 35.9
Mean Pre-therapy (+6M)	D: MI:74.3	SD: 10.3
Mean Pre-therapy (-6M)	E: MI:50.4	SD: 20.6
Mean Post-therapy	D: MI:42.4	SD: 10.6
Mean Post-therapy (+6M)	E: MI:53.7	SD: 5.2
Mean Post-therapy (-6M)	F: MI:36.8	SD: 7.0

Cimetidine (2): 2 X 300mg/day/4 wks

P.No.	62	63	64	65	66	67	70	71	84	83	72
Pre-T	67	50	21	54	46	54	92	21	54	43	100
Po-T	64	43	29	25	50	43					
Heal ±	+	+	+	+	+	+	-	-	-	-	-
Rem ±6M	+	-	-	-	-	+					

Mean Pre-therapy (All)	G: MI:54.7	SD: 23.6
Mean Pre-therapy (H+)	H: MI:48.7	SD: 13.9
Mean Pre-therapy (H-)	I: MI:62.0	SD: 29.8
Mean Pre-therapy (+6M)	J: MI:60.5	SD: 6.5
Mean Pre-therapy (-6M)	K: MI:42.8	SD: 12.9
Mean Post-therapy	L: MI:42.3	SD: 13.0
Mean Post-therapy (+6M)	M: MI:53.5	SD: 10.5
Mean Post-therapy (-6M)	N: MI:36.8	SD: 10.2

Cimetidine (3): 2 X 400mg/day/4 wks

P.No.	21	21	23	24	25	26	27	28	29
Pre-T	71	43	68	68	50	29	79	50	29
Post-T	-	-	-	-	-	-	-	-	-
Heal ±6M	+	+	-	-	-	-	+	+	+

Mean Pre-therapy (All)	O: MI:54.1	SD: 17.3
Mean Pre-therapy (H+)	P: MI:54.4	SD: 18.3
Mean Pre-therapy (H-)	Q: MI:53.8	SD: 16.1

Pirenzipine: 50mg X2/day/4wks

P.No.	30	31	32	33	34	35	36	37	38	39
Pre-T	4	25	82	79	29	32	71	36	93	100
Post-T	-	-	-	-	-	-	-	-	-	-
Heal ±	-	-	-	+	+	-	-	-	-	+

Mean Pre-therapy (All)	AP: MI:55.1	SD: 31.8
Mean Pre-therapy (H+)	AQ: MI:69.3	SD: 29.7
Mean Pre-therapy (H-)	AR: MI:49.0	SD: 30.6

Continued/

Sucralfate: 1g/day/6wks

P.No.	1	2	3	4	5	6	7	8	9	10	80	76	77	78	79	75
Pre-T	79	54	57	75	68	92	32	57	7	39	93	53	53	46	85	100
Po-T	64	39	29	68	57	36	61	32	18	50						
Heal ±	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Rem ±6M	+	+	+	+	-	+	-	-	-	-						

Mean Pre-therapy (All)	R: MI:61.9	SD: 24.1
Mean Pre-therapy (H+)	S: MI:56.0	SD: 23.7
Mean Pre-therapy (H-)	T: MI:71.7	SD: 21.6
Mean Pre-therapy (6M+)	U: MI:71.4	SD: 14.2
Mean Pre-therapy (6M-)	V: MI:40.6	SD: 21.1
Mean Post-therapy	W: MI:45.4	SD: 16.1
Mean Post-therapy (6M+)	X: MI:47.2	SD: 15.7
Mean Post-therapy (6M-)	Y: MI:43.6	SD: 16.2

Misoprostol (LM): 50ug/day/4wks

P.No.	51	52	53	54	55	56	57	58	59	60	61
Pre-T	67	64	96	43	57	57	25	50	75	14	21
Po-T	64	57	21	25	54	21	36	39			
Heal ±	+	+	+	+	+	+	+	+	-	-	-
Rem ±6M	-	+	+	-	+	+	+	-			

Mean Pre-therapy (All)	Z: MI:51.7	SD: 23.6
Mean Pre-therapy (H+)	AA: MI:57.4	SD: 19.2
Mean Pre-therapy (H-)	AB: MI:36.7	SD: 27.3
Mean Pre-therapy (6M+)	AC: MI:59.8	SD: 22.6
Mean Pre-therapy (6M-)	AD: MI:53.3	SD: 10.1
Mean Post-therapy	AE: MI:39.8	SD: 15.9
Mean Post-therapy (6M+)	AF: MI:37.8	SD: 15.5
Mean Post-therapy (6M-)	AG: MI:43.0	SD: 16.0

Misoprostol (HM): 300ug/day/4wks

P.No.	40	41	42	43	44	45	46	47	48	49	50	82
Pre-T	64	79	21	57	75	36	85	18	96	11	25	36
Post-T	61	64	50	54	18	46	57					
Heal ±	+	+	+	+	+	+	+	-	-	-	-	-
Rem ±6M	-	+	+	+	+	-	-					

Mean Pre-therapy (All)	AH: MI:50.3	SD: 28.1
Mean Pre-therapy (H+)	AI: MI:59.6	SD: 21.8
Mean Pre-therapy (H-)	AJ: MI:37.2	SD: 30.5
Mean Pre-therapy (6M+)	AK: MI:58.0	SD: 22.9
Mean Pre-therapy (6M-)	AL: MI:56.0	SD: 5.9
Mean Post-therapy	AM: MI:50.0	SD: 14.3
Mean Post-therapy (6M+)	AN: MI:46.5	SD: 17.2
Mean Post-therapy (6M-)	AO: MI:54.6	SD: 6.3

Table XIX: The mean morphological scores (MI) of juxta-DU and scar biopsies before and after therapy with each regimen. Patient number (P.No.). mean pre-therapy (Pre-T), post-therapy (Post-T) MI data are shown together with indications whether the patient healed (H±) and whether the patient experienced remission for more or less than 6 months (Rem±6M).

	PRE-THERAPY									
DRUG	BIN:A		BIN:B		BIN:C		BIN:D		BIN:E	
	No	%	No	%	No	%	No	%	No	%
(C)imetidine1	2	12	1	7	4	27	4	27	4	27
(C)imetidine2	0	0	2	18	5	46	1	9	3	27
(C)imetidine3	0	0	2	22	3	33	4	44	0	0
(S)ucralfate	1	6	2	13	6	38	3	19	4	25
(LD) Miso.	1	9	2	16	4	33	3	25	2	17
(HD) Miso.	2	17	4	33	1	8	3	25	2	17
(P)irenzipine	1	10	4	40	0	0	2	20	3	30

Table XX: The type of morphology in juxta-DU specimens before therapy with either cimetidine (1, 2 or 3), sucralfate, high or low-dose misoprostol or pirenzipine. The mean morphological data from all specimens obtained from patients prior to each regimen were assigned to one or other of the 5 numeric bins. BIN:A (MI:0-20); BIN:B (MI:21-40);BIN:C (MI:41-60); BIN:D (MI:61-80); BIN:E (MI:81-100)

A comparison of all pre-therapy data showed no significant difference between the overall distribution of specimens from each therapy group that were placed in each numeric bin (Chi.squared p >0.05). There were, however, fewer specimens from patients to be treated with either pirenzipine or HD misoprostol in bin C than in the combined cimetidine(1, 2 and 3) data (p <0.05).

7.3.3. Juxta-DU And Scar Morphology Before And After Therapy

Mean MI data from all patients prior to each type of therapy were correlated with mean scar MI after treatment and plotted in Figure 31. With the exception of HD misoprostol, there was a consistant reduction of mean MI after healing. The reduction was significant (<0.05) after treatment with cimetidine(1) (B and D) and LD misoprostol (AA and AE - see Table XIX).

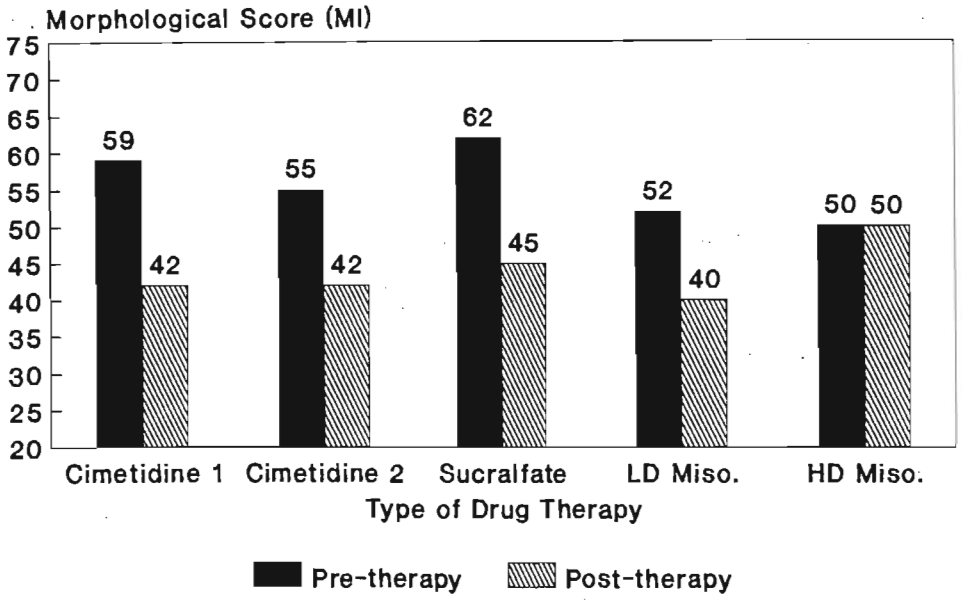

Figure 31: Mean pre-therapy morphological scores (MI) correlated with mean scar MI after treatment with either cimetidine (1 or 2), sucralfate, low-dose(LD) or high-dose(HD) misoprostol.

7.3.4. Scar Morphology After Curative Therapy

The individual and mean morphological scores (MI) of specimens obtained from scars after therapy with either cimetidine(1 or 2), sucralfate, low-dose and high-dose misoprostol are listed in Table XIX. The Mann-Whitney and Variance tests were applied to all post-therapy data from each therapy group. There were no significant differences in the data between any of the 10 possible post-therapy combinations.

The morphological scores from each scar specimen were assigned to one of the 5 numeric bins. The number and percentage of specimens in each bin after treatment with either of 5 drug regimens are listed in Table XXI

DRUG	BIN:A		BIN:B		BIN:C		BIN:D		BIN:E	
	No	%	No	%	No	%	No	%	No	%
Cimetidine(1)	0	0	4	44	4	44	1	12	0	0
Cimetidine(2)	0	0	2	33	3	50	1	17	0	0
(S)ucralfate	1	10	4	40	2	20	3	30	0	0
(LD) Miso.	1	17	0	0	2	33	3	50	0	0
(HD) Miso.	0	0	4	57	2	29	1	14	0	0

Table XXI: The type of morphology in specimens from near scars after successful therapy with either cimetidine (1 or 2), sucralfate, low-dose or high-dose misoprostol. before each type of therapy. The mean morphological data from all specimens obtained from patients after treatment were assigned to one or other of the 5 numeric bins. BIN:A (MI:0-20); BIN:B (MI:21-40); BIN:C (MI:41-60); BIN:D (MI:61-80); BIN:E (MI:81-100).

A comparison of all post-therapy data showed a difference in the distribution of scar morphology between cimetidine(1) and sucralfate (Chi.Sq. p =<0.0435). This is shown graphically in Figure 32. No significant differences in the distribution of scar morphology were found between specimens from any other therapy group.

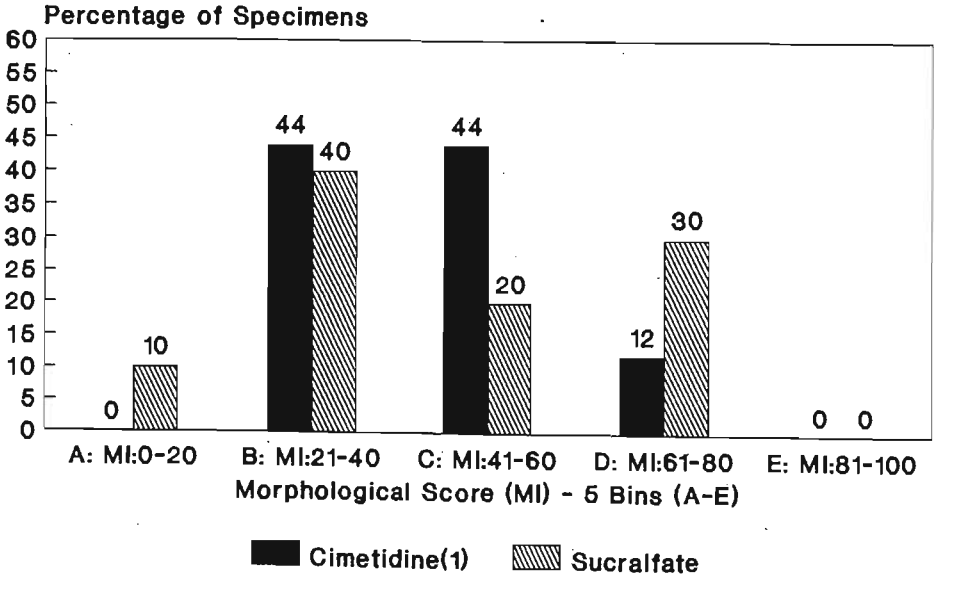

Figure 32: Comparison between the distribution of scar morphology after treatment with cimetidine(1) or sucralfate.

7.3.5. DU Healing Correlated With Juxta-DU MI Scores.

The mean morphological scores from the juxta-DU mucosa of patients who healed and did not heal after each of the 7 drug regimens is shown in Figure 33.

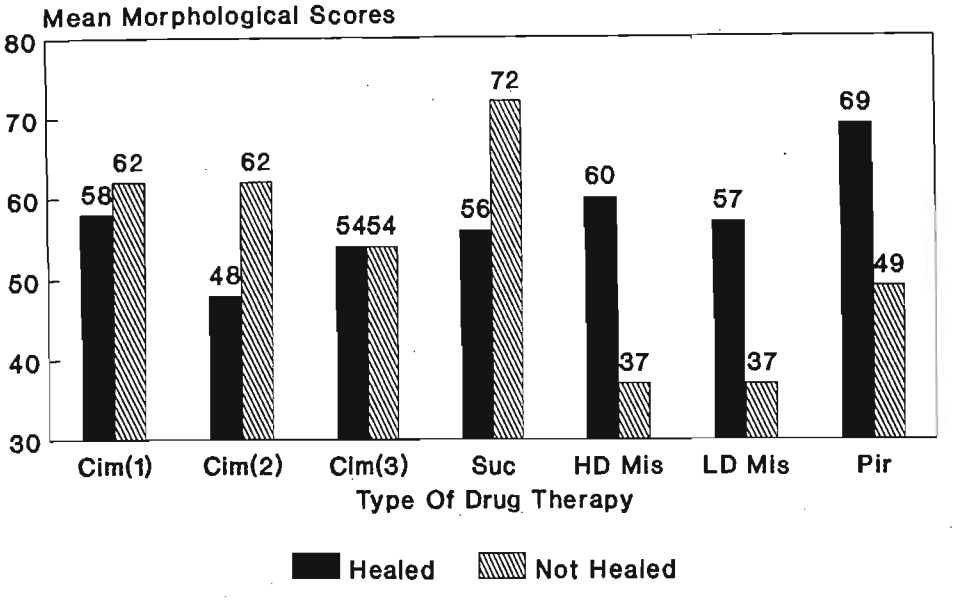

Figure 33: The mean morphological scores (MI) of juxta-DU specimens from patients that did or did not heal after therapy with cimetidine (1, 2 or 3), sucralfate, high-dose or low-dose misoprostol or pirenzipine.

In patients treated with each of the cimetidine regimens and sucralfate, the mean pre-therapy MI in patients who healed was lower than in those that did not. The difference, however, was only significant in the case of sucralfate ($p < 0.05$ - S and T - see table XIX). Patients who did not heal after either pirenzipine, high- or low-dose misoprostol, had lower mean morphological scores than those who did heal. Although in the cases of high- and low-dose misoprostol the differences in mean pre-therapy MI were substantial, they were not significant at $p < 0.05$.

A comparison of pre-therapy data from patients who healed showed no significant differences in MI between therapy

groups. In patients who did not heal after treatment with cimetidine(1) or sucralfate, mean pre-therapy MI was higher than that of patients to be treated with high-dose misoprostol ($p < 0.05$: C and AJ, T and AJ - see Table XIX).

These results show that each curative regimen was able to heal DU primarily circumscribed by a moderately metaplastic mucosa. Where DU were surrounded by particularly metaplastic tissue, sucralfate was associated with a lower healing rate. Pirenzipine and especially misoprostol, however, appeared less able to heal non-metaplastic DU.

7.3.6. Juxta-DU MI Correlated With Endoscopic Severity And Incidence Of Healing In Patients Treated With Cimetidine

The small number of patients treated with each regimen made it difficult for statistical analyses to give significance to morphologic, endoscopic and healing correlations of data obtained before or after a particular type of drug therapy. A total of 35 patients were treated with cimetidine, albeit with different doses for 4 or 6 weeks. The sum of data reported and described in 7.4 and in chapter 8 below suggests that cimetidine heals in a particular manner irrespective of dose or time of treatment. To afford enough data for statistical analysis to be performed on material from patients treated with one drug, the data from DU exposed to cimetidine regimens 1, 2 or 3 were combined. Prior to therapy, DU in patients later treated with either cimetidine 1,2 or 3 were graded by endoscopy as either severe (Group 1) or moderate (Group 2).

The morphological scores (MI) and incidence of healing was recorded for each specimen. The mean MI, standard deviation of the mean and the percentage of patients that healed

(Group 1A and 2A) and did not heal (Group 1B and 2B) were recorded and compared (Table XXII).

Group 1 (Endoscopically severe DU. SL:4;3;2)

P.No.	68	72	81	70	69	12	13	25	73	20	28	18	23	17	22	29
Pre-T	100	100	100	92	82	76	61	50	39	57	50	54	68	46	43	25
Heal ±	-	-	-	-	-	+	+	-	-	+	+	+	-	+	+	+
SL	4	4	4	4	4	4	4	4	4	3	2	3	2	2	2	2
Reg.	C1	C2	C1	C2	C1	C1	C1	C3	C1	C1	C3	C1	C3	C1	C3	C3

	Group 1A (Healed DU)	Group 1B (Not-healed DU)
Mean MI	51.5	78.9
SD.	13.8	22.5
% Pats.	50	50

Group 2 (Endoscopically moderate DU. SL:1;0)

P.No.	67	14	21	24	66	64	15	74	83	11	27	62	19	65	16	71	26	63	84
Pre-T	54	79	71	68	46	21	7	0	43	86	79	68	64	54	46	21	29	50	54
Heal ±	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+
SL	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1
Reg.	C2	C1	C3	C3	C2	C2	C1	C1	C2	C1	C3	C2	C1	C2	C1	C2	C3	C2	C2

	Group 2A (Healed DU)	Group 2B (Not-healed DU)
Mean MI	55.6	32.2
SD.	21.2	22.6
%Pats.	74	26

Table XXII: The morphological and endoscopic scores of patients treated with either of the cimetidine regimens correlated with DU healing. Lesions were divided into two groups: severe or moderate as determined by endoscopy and correlated with incidence of healing. The mean MI of healed and non-healed patients in each group were compared. SL = endoscopic severity; Reg. = cimetidine regimen 1, 2 or 3; SD = standard deviation of the mean; %Pats = percentage of patients in each sub-group.

In group 1, healed patients had a lower juxta-DU MI (MI:51.5) than those that did not (MI:78.9; p = >0.05). In group 2, those that healed had a higher MI (MI:55.6) than those that did not (MI:32.2; p = >0.05). There was no difference in MI between DU that healed in either endoscopic severity group (p = >0.05). There was, however, a significant difference in MI between those that did not heal

in each group (p = <0.01). There was an equal number of healed and non-healed patients in group 1 whereas most patients healed (74%) in group 2. These data are graphically described in Fig.34.

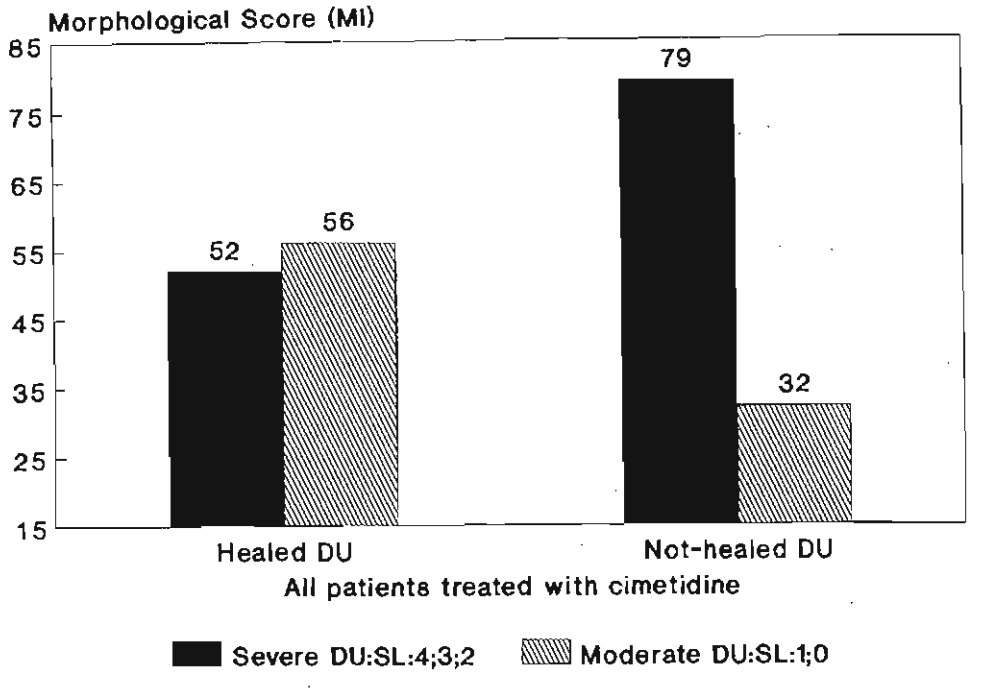

Figure 34: Combined data from patients treated with cimetidine(1,2 or 3). The mean morphological scores (MI) of juxta-DU specimens correlated with severity of DU as determined by endoscopy and incidence of healing.

These results show that irrespective of the size of DU, lesions surrounded by a moderately metaplastic mucosa are likely to heal. When large lesions do not heal, they are often surrounded by a well differentiated metaplastic mucosa whereas when small lesions persist, they are surrounded by non-metaplastic tissue. These data support the hypothesis that there are at least 2 different types of DU which may be prognostically and morphologically distinct (see Chapter 8).

7.3.7. Scar Morphology Correlated With The Duration of Remission

Figure 35 shows the mean morphological scores from patients who experienced more or less than six months remission after successful therapy with either cimetidine (1 or 2), sucralfate or high-dose or low-dose misoprostol.

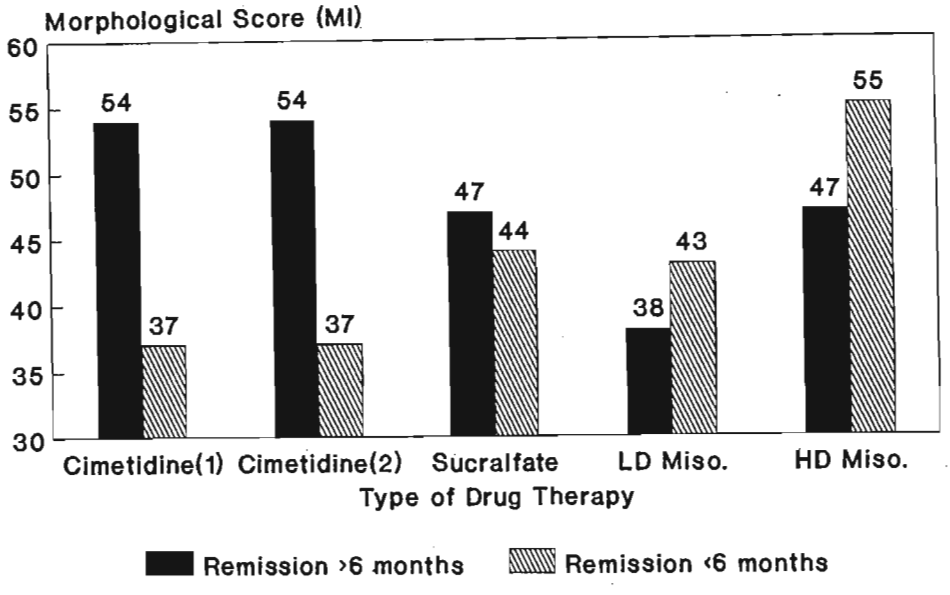

Figure 35: The mean morphological scores (MI) of scars from patients successfully treated with either cimetidine (1 or 2), sucralfate, high-dose or low-dose misoprostol, who remained in remission for more or less than 6 months.

There were no detectable differences in scar morphology between remission groups after treatment with sucralfate, low-dose or high-dose misoprostol. There were, however, significant differences in scar morphology between patients who relapsed within six months or remained in extended remission after both cimetidine regimens ($p < 0.05$). Patients whose scars had low morphological scores experienced early relapse. In the case of cimetidine, therefore, the type or quality of morphological healing did influence remission prognosis.

7.3.8. Juxta-DU MI Correlated With Duration Of Remission

The juxta-DU MI of patients treated with either cimetidine regimen 1 or 2 was correlated with duration of remission (more or less than 6 months). The morphological scores are shown in Table XXIII.

Group 1: Remission > 6 months

P.No.	11	12	13	62	67
Pre-T MI:	**86**	**76**	**61**	**68**	**54**
Reg.	C1	C1	C1	C2	C2

Mean MI:69
SD MI:11.2

Group 2: Remission < 6 months

P.No.	14	15	16	17	18	19	20	63	64	65	66
Pre-T MI:	**79**	**7**	**46**	**46**	**54**	**64**	**57**	**50**	**21**	**54**	**46**
Reg.	C1	C1	C1	C1	C1	C1	C1	C2	C2	C2	C2

Mean MI:47.6
SD MI:18.6

Table XXIII:Patients treated with cimetidine regimens 1 or 2. Juxta-DU MI (Pre-T MI:) correlated with duration of remission (>6 months - group 1; < 6 months - group 2). Reg. = cimetidine regimen 1 or 2. SD standard deviation of the mean.

The juxta-DU mucosa surrounding DU that healed and remained in remission for more than 6 months had a significantly higher MI (MI:69) than tissue from patients who relapsed within 6 months (MI:47.6; $p = 0.0272$). This suggests that patients with metaplastic DU, when healed, have a better probability of staying in extended remission.

7.3.9 Drug Mediated Alterations In The Cytological Composition Of Scar Mucosa

The results suggested that the efficacy of a particular drug to heal DU may vary with the morphological class of lesion to be healed. In addition, in the case of cimetidine, but not of sucralfate or misoprostol, the quality of morphological healing appeared to influence remission prognosis. These data suggested that pharmaceutical preparations may preferrentially change mucosal morphology and by so doing, may alter remission prognosis. Light microscopic morphometry was employed to investigate this possibility. The number of goblet cells/100um (GC/100um) of villous epithelium in normal, non-metaplastic juxta-DU and scar mucosa before and after curative therapy with either sucralfate or cimetidine is listed in Table XXIV. The non-metaplastic specimens that were included in this evaluation are highlighted and underlined in Table II.

GROUP A			**GROUP B**			**GROUP C**			**GROUP S**		
GC	**Dist**	**Ave**	**GC**	**Dist**	**Ave**	**GC**	**Dist**	**Ave**	**GC**	**Dist**	**Ave**
300	12633	2,4	185	4021	4,6	31	2079	1,6	77	2403	3,2
196	10665	1,8	103	2512	4,0	47	2087	2,1	95	3072	3,1
195	9843	2,0	123	3331	3,7	33	2184	1,5	58	2524	2,3
212	11990	1,8	85	2362	3,6	45	2831	1,6	106	3392	3,1
246	12350	2,0	65	1842	3,5	53	2245	2,4	89	3605	2,5
-	-	-	77	2047	3,7	34	2091	1,8	-	-	-
-	-	-	85	2362	3,6	-	-	-	-	-	-
-	-	-	130	4019	3,2	-	-	-	-	-	-
-	-	-	84	2688	3,1	-	-	-	-	-	-
-	-	-	72	2423	3,1	-	-	-	-	-	-
Mean		**2,0**			**3,7**			**1,8**			**2,8**
SD		**0,2**			**0,4**			**0,4**			**0,3**

Table XXIV: Drug mediated differences in scar morphology. The number of goblet cells (GC) per length of mucosal surface evaluated (Dist) and the average number of GC per unit length of mucosa (Ave) are tabulated. Data was obtained from normal volunteers (Group A), before therapy from the non-metaplastic mucosa of patients with DU (Group B) and after therapy with either cimetidine(1) (Group C) and sucralfate (Group D). The average number of GC per 100um of surface mucosa (Mean GC/100um) together with the standard deviation (SD) of each mean is shown.

There was an increase in the number of goblet cells in the juxta-DU mucosa from 2GC/100um in normal biopsies to 3.7GC/100um in untreated ulcerated tissue (Wilcoxon p <0.01). After therapy with cimetidine, goblet cell numbers were reduced to near normal levels (1.8GC/100um) whereas after treatment with sucralfate, goblet cell numbers remained elevated (2.8GC/100um). There was a difference between the number of goblet cells in scar mucosa after treatment with sucralfate or cimetidine (p <0.02). The mean GC/100um data before and after therapy with sucralfate or cimetidine(1) are summarised graphically in Figure 36.

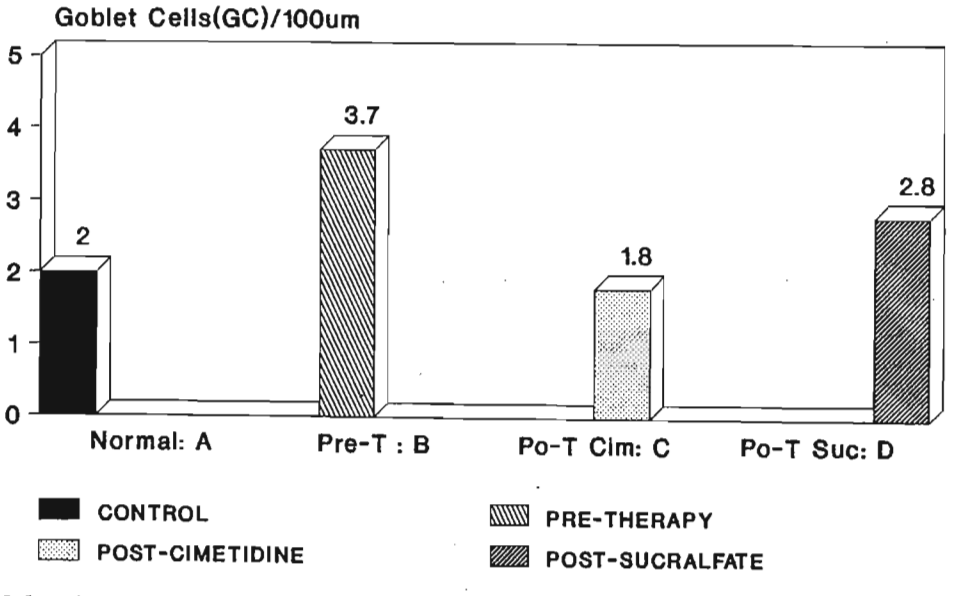

Figure 36: The number of goblet cells per 100um of surface mucosa (GC/100um) in the non-metaplastic mucosa before and after treatment with either sucralfate or cimetidine(1). Note that from Table XXIII there were significant differences between A and B ($p < 0.01$), C and D ($p < 0.02$) and A and S ($p < 0.02$).

These data suggest that the type of drug therapy does influence post-therapy mucosal morphology.

Section 3: Prediction of DU Prognosis By Means Of Morphological Analysis

The results showed that irrespective of the type or duration of curative regimen, the size of DU and the morphological class of the mucosa surrounding DU were important factors that influenced the probability of healing. Furthermore, it appeared that the morphological appearance of the scar mucosa after curative therapy with cimetidine could be correlated with the duration of remission. Mucosal morphology, therefore, was an important factor in healing and perhaps remission prognoses. These data suggested that prediction of healing and/or remission prognoses may be possible by a morphological evaluation of either juxta-DU or scar mucosa. This premise is investigated below.

7.4. Predicting DU Healing With Cimetidine Therapy

The morphological appearance (expressed as mean MI) of juxta-DU and scar specimens, before, after and up to 6 months after the termination of treatment with cimetidine (1) and (2) is shown in Figure 37.

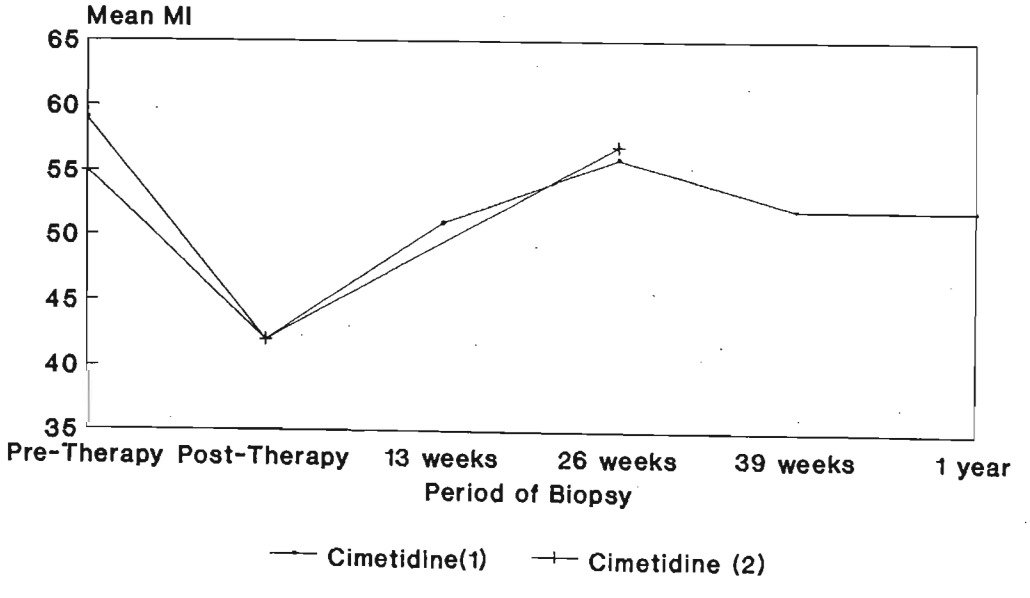

Figure 37: The mean morphological scores before and up to 1 year after treatment with cimetidine(1) are plotted with pre- and post-therapy MI data from patients treated with cimetidine(2).

Both regimens healed morphologically indistinguishable groups of DU (7.3.2. Table XIX, p.180) and produced scars with similar morphology (7.3.3. Figure 31, p.182). The classes and morphological scores of DU that did not heal within the prescribed times were virtually identical with both regimens (Table XIX). Furthermore, correlations between scar morphology and remission prognosis were similar ($p < 0.05$) after both cimetidine regimens (7.3.7. Fig.35 p. 188). These data suggested that, irrespective of dose or time, cimetidine performed in a uniform way.

The following section describes how discriminant formulae that identify morphological parameters associated with healing and non-healing were derived from the combined pre-therapy data from both cimetidine studies. It continues by showing how the formulae were employed to predict healing/non-healing in patients to be treated with cimetidine in a third study.

7.4.1. Precepts For Prediction Of DU Healing

The predictability of DU healing with a particular drug requires that the following precepts be accepted:

1. That endoscopic severity and the type of juxta-DU mucosal morphology are predictive factors that influence the probability of DU healing.
2. That although the mechanism of action of differing drug regimens may vary, the therapeutic action of a particular drug is constant causing mucosal alteration and DU healing to be effected in a predictable manner.

The sum of data reported in this thesis to date suggests that these precepts are valid.

7.4.2. Discriminant Analysis

This technique finds appropriate weighting co-efficients for each of the separate scores awarded to the endoscopic and morphological criteria (SL; S1-S4). The co-efficients were applied to the data in such a way as to create a single discriminant function that maximally separates the two categories: healed and not healed DU.

The linear discriminant analysis procedure was applied to all the numeric data (SL; S1-S4) from the juxta-DU specimens prior to therapy with cimetidine(1 & 2). The unstandarised discriminant function coefficients were:

Criterion	**Coefficient**
SL	-0.39786
S1	1.23246
S2	-0.48526
S3	0.00883
S4	-1.15045
Constant	0.41708
Chi.sq p = 0.01045	

The formula for calculating healing prognosis is:
SL X Cf + S1 X Cf + S2 X Cf + S3 X Cf + S4 X Cf + Constant
Cf = coefficient. If the resultant score is + a patient should heal

With a highly significant separation (p = 0.01045), the formula did not predict correctly for 7 (27%) of the 26 specimens. These poor results suggested that some factor or factors were interfering with the separation of data.

The results reported in 7.1.3. to 7.1.6. and in 7.3.6. above describe two endoscopically and morphologically dissimilar classes of DU that were difficult to heal. In extremis, the one class of DU was larger and surrounded by a metaplastic mucosa, the other smaller and often delimited by

degenerative non-metaplastic epithelium. Perhaps cimetidine was more or less efficaceous in healing one or other class of DU. If, as suspected, there were two morphologic classes of DU; each with its own prognostic characteristics, then this may explain why the discriminant formula predicted inaccurately in 27% of cases. Using correlations between percentage healing and morphological scores (MI) the following identifies and numerically delimits two morphological class of DU.

7.4.3. Numerical Delimitation Of Morphological Classes Of DU

The juxta-DU MI of all 84 specimens were sorted in ascending order and correlated with the incidence of healing. The specimens were placed in groups according to pre-therapy MI and the percentage healing per group recorded. Figure 38 and Table XXV show the correlation between mean bin MI and percentage healing.

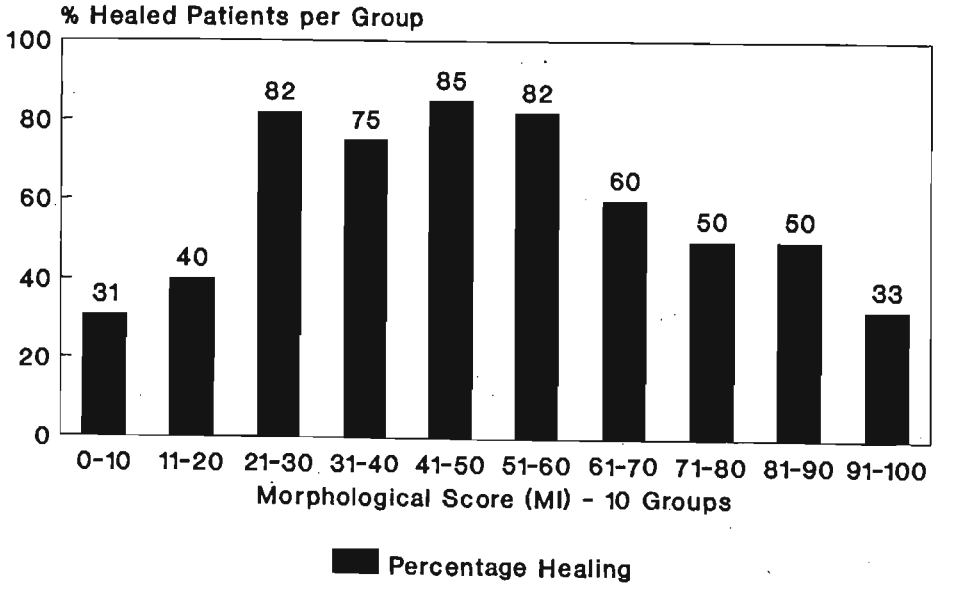

Figure 38: Correlation between morphology and incidence of healing. The morphological scores (MI) from each juxta-DU specimen were sorted in ascending order. Specimens were grouped according to MI (MI:0-10 X 10 - 100). The percentage DU healing in each group is shown.

If, as suspected, two morphologic classes of lesions exist, MI:0 and MI:50 and MI:100 and MI:50 represent the worst and best prognoses for each class respectively. Specimens with morphological score of MI:<50 were called class N and those with scores MI:>51 class M. Two regression analyses were performed using the correlation between mean group MI and percentage healing in class N and M specimens. The regression output is as follows.

Regression Output	**ClassN**:	Constant	29.223
		X Coefficient	1.031083
	ClassM:	Constant	171.2
		X Coefficient	-1.43771

The actual and corrected MI/percentage healing correlations are shown in Table XXV.

	Class N						Class M			
Group	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
MI	**0-10**	**11-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**	**91-100**
MnMI	4	15	24	35	47	56	66	76	84	96
%H	33	50	50	60	82	85	75	82	40	31
C.V.	33	45	54	65	78	91	76	62	50	33

Table XXV: Correlation between mean morphological scores (MnMI) and percentage healing (%H) in each of 10 equal numeric groups (A - J). Also shown are the corrected value (C.V.) of each MnMI as per regression analysis.

The corrected mean MI data were plotted in Figure 39. Note that the two regression lines meet at MI:56. For the purpose of the following discriminant analyses, all specimens with an MI >56 were considered metaplastic class M and all specimens <55 non-metaplastic class N.

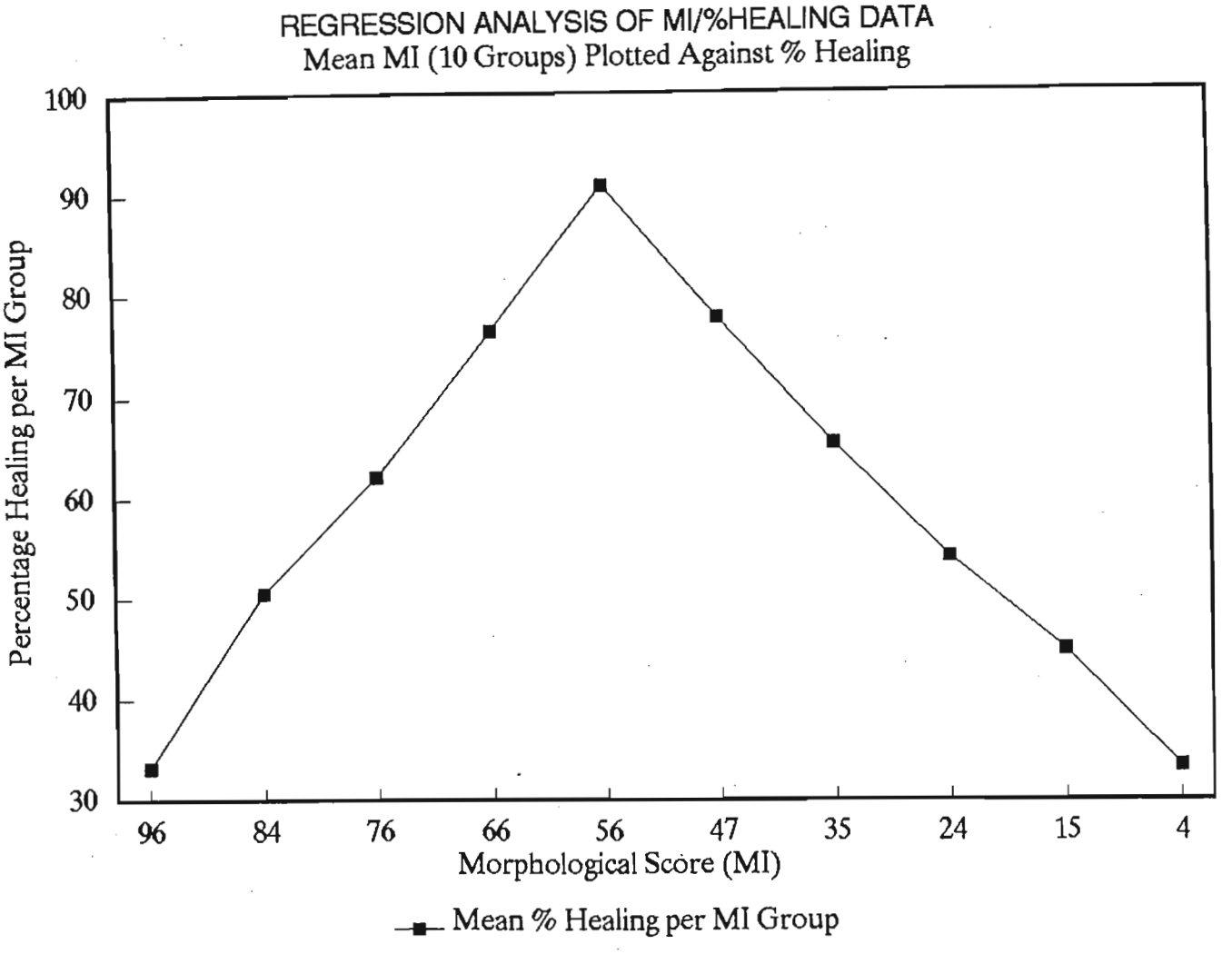

Figure 39: Regression lines correlating corrected MI data with incidence of healing for class N and class M specimens.

7.4.4. Prediction Of DU Healing With Cimetidine

The juxta-DU data prior to treatment with cimetidine (1 and 2) were combined and based on their morphological scores, separated into metaplastic (M) and non-metaplastic (N) groups. Table XXV lists the 12 group M and 14 group N data. Discriminant analyses were performed on both sets of data. The unstandarised discriminant function coefficients for group M and N were:

Discriminating Formula For (M)etaplastic Specimens (> MI:56)
SL X -0.55287 + S1 X 3.23502 + S2 X -2.38994 + S4 X -1.57182 + 1.912
Chi.Sq. p = 0.01036

P	D	SL	S1	S2	S3	S4	MI	H	DA	Correct
11	C1	0	3.5	3	2.5	3	85	+	+	+
12	C1	4	3.5	4	2.5	0.5	75	+	+	+
13	C1	4	2.5	2	2.5	1.5	60	+	+	+
14	C1	1	3.5	3	2.5	2	78	+	+	+
19	C1	0	2.5	4	2	0.5	64	+	-	-
20	C1	3	2.5	3	2.5	0	57	+	+	+
68	C1	4	4	4	3	3	100	-	-	+
69	C1	4	3	3	2.5	3	82	-	-	+
81	C1	4	4	4	3	3	100	-	-	+
62	C2	0	3	3	2.5	1	67	+	+	+
70	C2	4	3	4	2	1	92	-	-	+
72	C2	4	4	4	3	3	100	-	-	+

Formula For (N)on-metaplastic Specimens < MI:55)
S1 X 1.01058 + S2 X 0.39742 + S4 X -1.51353 -0.35
Chi.Sq. p = 0.01847

P	D	SL	S1	S2	S3	S4	MI	H	DA	Correct
15	C1	1	0.5	0.5	0	0	7	+	+	+
16	C1	0	2	2	1.5	1	46	+	+	+
17	C1	2	2.5	0.5	2.5	1	46	+	+	+
18	C1	3	3	2	2.5	0	53	+	+	+
73	C1	4	0.5	2	1.5	1.5	39	-	-	+
74	C1	1	0	0	0	0	0	-	-	+
63	C2	1	2	3	1.5	0.5	50	+	+	+
64	C2	1	0.5	2	0.5	0	21	+	+	+
65	C2	0	2.5	2	2.5	0.5	54	+	+	+
66	C2	1	2	2	2	0.5	46	+	+	+
67	C2	1	2	2.5	2.5	0.5	54	+	+	+
71	C2	0	1	1	0	1	21	-	-	+
83	C2	1	1.5	0.5	2.5	1.5	43	-	-	+
84	C2	1	2	2.5	2.5	0.5	54	-	-	+

Table XXVI: The M and N discriminant formulae applied to the SL (endoscopic severity level) and S1 - S4 morphological data derived from juxta-DU biopsies before treatment with either cimetidine (1 or 2).
P = thesis patient number, D = drug regimen, H = whether DU actually healed, DA = prediction as per discriminating formula (+) should heal (-) should not heal. Correct = Was the formula correct in its prediction: + = yes, - = no.

(M)ETAPLASTIC	
Criterion	**Coefficient**
SL	-0.55287
S1	3.23502
S2	-2.38994
S4	-1.57182
Constant	1.912
Chi.sq p = 0.01036	

(N)ON-METAPLASTIC	
Criterion	**Coefficient**
S1	1.01058
S2	0.39742
S4	-1.51353
Constant	-0.35
Chi.Sq p = 0.01847	

In the case of group M, S3 was found to be superfluous to good discrimination; in group N, SL and S3 were superfluous. The significance of both sets of coefficients is very good (group M: p = 0.01036; group N: p = 0.01847).

The formula that found the best separation between healed and not-healed DU in group M specimens is:

-SL X 0.55287 + S1 X 3.23502 - S2 X 2.38994 - S4 X 1.57182 + 1.912

The formula that found the best separation in group N is:

S1 X 1.01058 + S2 X 0.39742 - S4 X 1.51353 - 0.35

If the resultant score is positive (+) the formula predicts healing, if negative (-), DU should persist. Table XXVI shows that the formula was able to accurately separate 11 out of 12 metaplastic cases into healed or not healed and all non-metaplastic cases. The overall accuracy of separation into healed and not-healed groups was 96%.

7.4.4.1. Accuracy of Prediction

Having created formulae that can use morphological criteria to separate lesions into those that heal and do not heal, the same formulae when applied to similar data from patients to be treated with the same drug should be able to predict

outcome. In order to test the accuracy of both formulae in predicting healing outcome, they were applied to data from the juxta-DU mucosa of patients prior to treatment with cimetidine(3). Table XXVII shows that the formulae predicted correctly in 8 out of 9 cases (89%)

P	D	SL	S1	S2	S3	S4	MH	H	DA	DA	Correct
					M NM						
21	C3	1	3.5	4	1.5	1	71	+	+		+
22	C3	2	1.5	2	1.5	1	43	+		+	+
23	C3	2	3	3	2.5	1	68	+	+		+
24	C3	1	3	4	1.5	1	68	-	-		+
25	C3	4	1.5	2	2	1.5	50	-		-	+
26	C3	0	1	1.5	0	1.5	29	-		-	+
27	C3	0	3.5	4	2.5	1	79	+	+		+
28	C3	2	2.5	2	1.5	1	50	+		+	+
29	C3	2	1	1	0	1.5	25	+		-	-

Table XXVII: The M and N discriminant formulae applied to juxta-DU specimens from patients to be treated with cimetidine(3).
H+ = States whether the patient healed.
DA:M = M Formula applied to M class DU (>MI:56).
DA:NM = N Formula applied to N class DU (<MI:55).
In both **DA:M & DA:NM**, + predicts healing; - predicts non-healing.
Correct = States whether prediction was correct (+ or -).

7.5. Predicting The Duration Of Remission

The results (7.2.4) suggested that if a DU should heal after treatment with cimetidine, the morphological appearance of the scar mucosa may indicate whether a patient could expect to remain in remission for more or less than 6 months. Discriminant analysis was employed to create a formula that may predict remission prognosis after curative therapy with cimetidine.

7.5.1. Predicting Duration Of Remission From Scar Morphology

The linear discriminant analysis procedure was applied to the numeric data (S1-S4) from scar specimens in patients that healed after curative cimetidine therapy. The purpose was to find the best separation between data from patients that remained in remission for more or less than six months after therapy. The unstandarised discriminant function coefficients are:

Criterion	Coefficient
S1	1.49847
S2	0.69225
S4	3.06976
Constant	-6.3
Chi.sq p = 0.0028	

Parameter S3 did not improve the separation and reduced the significance. The formula that found the best separation after curative therapy with cimetidine is:

S1 X 1.49847 + S2 X 0.69225 + S4 X 3.06976 - 6.3

Table XXVIII shows that the formula was able to use the morphological data to separate patients into those that experienced extended remission or relapsed within 6 months. The significance of the separation is very high ($p = 0.0028$).

7.6. Summary Of Predictive Results

The substantial improvement in the accuracy of separating patients into healed/not-healed using the M and N discriminant formulae supports the premise that two morphological classes of DU exist, each with its own prognostic identity. The discriminating formulae for metaplastic and non-metaplastic specimens are able to

predict healing prognosis in 90% of patients treated with cimetidine. These and the formula derived from the scar data may prove useful in predicting outcome for patients who are to be or have been treated with cimetidine.

Discriminant Formula: S1 X 1.49847 + S2 X 0.69225 + S4 X 3.06976 - 6.3

P	D	S1	S2	S3	S4	MI	R	DA	Correct
11	C1	2	2	2	1	50	+	+	+
12	C1	1.5	1.5	2.5	1.5	50	+	+	+
13	C1	2.5	2	2.5	1.5	61	+	+	+
14	C1	1.5	0.5	2.5	0.5	36	-	-	+
15	C1	2	0	1.5	0	25	-	-	+
16	C1	1.5	1	1.5	0.5	32	-	-	+
17	C1	2.5	1	2.5	0.5	46	-	-	+
18	C1	2.5	0.5	2.5	0	39	-	-	+
19	C1	1.5	2.5	1.5	0.5	43	-	-	+
62	C2	2.5	4	2	0.5	64	+	+	+
63	C2	1.5	2	2	0.5	43	-	-	+
64	C2	2	0	1.5	0.5	29	-	-	+
65	C2	1	1.5	0	1,5	25	-	-	+
66	C2	1.5	2.5	2.5	0.5	50	-	-	+
67	C2	1.5	2	1.5	1	43	+	+	+

Table XXVIII: Discriminant analysis applied to the S1-S4 scar data after therapy with cimetidine. The formula was able to find good separation between patients in remission for more or less than 6 months.
P.No. = Patient number; D = Drug; S1-S4 = Morphological criteria
MI = Morphological index; R = remission >(+) or <(-) than 6 months
DA = prediction according to discriminant formula (+) >6M (-) <6M.
Correct = Whether the DA prediction was accurate: (+) predicted correctly; (-) failed.

7.7. Comparison Of Drug Efficacy Employing Morphological Analysis

Most studies designed to compare the performance of various drugs in promoting DU healing or extending remission do so on a purely numeric basis. That is, the relative healing efficacy of therapeutic regimens is estimated by noting the percentage of healed patients at the termination of a fixed period of time. The relative efficacy of a particular drug to influence the duration of remission is determined by calculating the average period of time before relapse. Such analyses presuppose that prior to treatment, all DU are equal with regard healing and remission prognoses and that any differences in healing or remission are a consequence of the drug. The results reported in this study suggest that the endoscopic severity, juxta-DU morphology and the appearance of scar mucosa after curative therapy may influence healing and remission prognoses. Drug therapy, therefore, is but one, albeit important factor, that mediates for DU healing. In the following section, evidence for DU causing disparities in the apparent efficacy of curative regimens is investigated and a solution that incorporates the results described in this thesis proposed.

7.7.1. Observations On The Relative Healing Efficacy Of Drugs Employed In This Study

Table XVIII shows the percentage of healed patients after each of 7 curative regimens. There are 2 regimens that bear investigation. Only 30% of DU healed after pirenzipine therapy. The apparent efficacy of healing with pirenzipine was significantly worse than with either cimetidine(1) or LD misoprostol ($p = <0.05$). In the case of low and high-dose misoprostol, the lower dose had a better rate of healing than the higher. In both instances these data are in conflict with prior expectation (Nicholson 1985, Carmine

1985, Brand et al. 1985, Fich et al. 1985). Could a disproportionate number of DU with naturally poor prognoses populate these therapy groups and could they be the reason why these drugs have a poor healing rate?

Prior to treatment with pirenzipine or cimetidine(1,2 and 3), based on their morphological scores (MI), each juxta-DU specimen was asigned to one of 5 numerical bins (see table XX p.181). The percentage of specimens in each bin is shown in Figure 40.

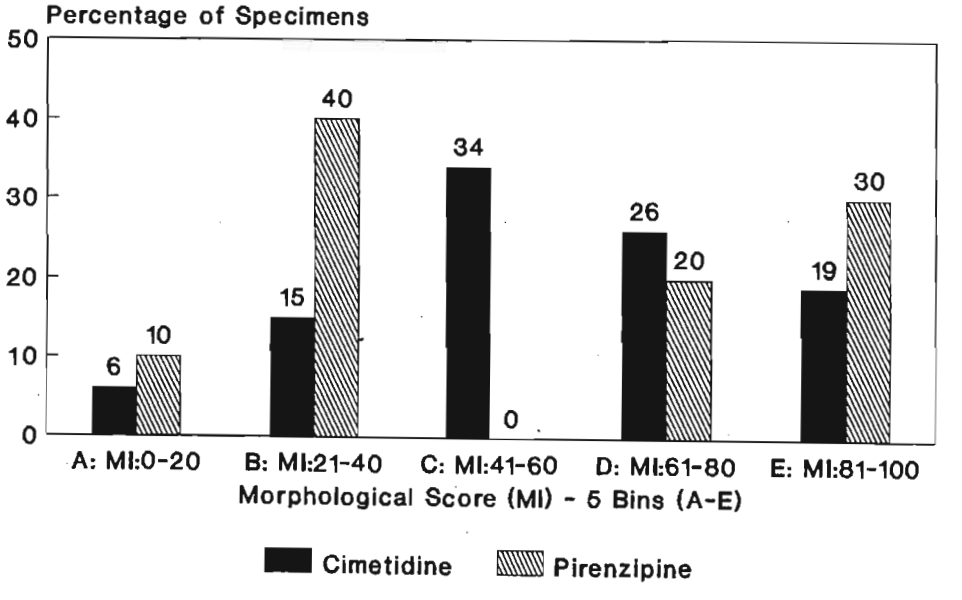

Figure 40: Comparison between the distribution of MI in juxta-DU specimens from patients to be treated with pirenzipine or cimetidine(1, 2 or 3).

There is a significant difference in the number of specimens in bin C ($p = <0.02$). There are no specimens in prognostically favourable bin C in the group of patients to be treated with pirenzipine.

Figure 41 compares the distribution of MI in specimens from patients to be treated with high- and low-dose misoprostol.

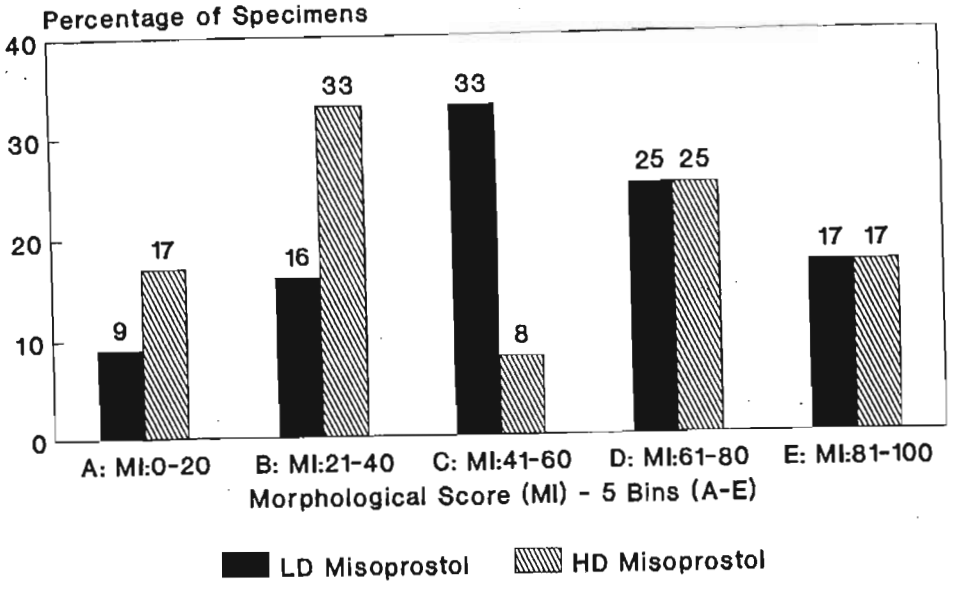

Figure 41: Comparison between the distribution of MI in juxta-DU specimens from patients to be treated with high-dose misoprostol or low-dose misoprostol.

There are fewer patients in prognostically favourable bin C in the group to be treated with high-dose misoprostol than with low-dose misoprostol. These data confirm that both pirenzipine and high-dose misoprostol had to contend with groups of patients with fewer DU with morphologically good prognoses.

7.7.2. Quantitative Morphological Analysis As A Means Of Estimating Drug Performance

The discriminant formulae can be used as independent standards against which the healing efficacy of any potentially curative regimen can be compared. By calculating whether a patient should heal after treatment with cimetidine and then comparing the actual with the predicted results, the relative performance of a curative regimen can be ascertained.

Comparisons are made using the following rationale:

> **If a patient with a particular morphological type of DU was treated with cimetidine, would the patient be expected to heal? By comparing the actual number of patients that healed after a particular type of drug therapy with the number predicted to heal with either the N or M cimetidine formulae, the relative efficacy of the experimental drug can be ascertained.**

The actual incidence of healing with sucralfate, pirenzipine and high and low-dose misoprostol together with that predicted with the M and N cimetidine formulae are summarised in Table XXIX. The full results are listed in Table XXX.

Sucralfate		Pirenzipine		LD Miso.		HD Miso.	
Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.
10	6	3	3	8	7	7	5

Table XXIX: Actual (Act.) and predicted (Pred.) healing of patients treated with sucralfate, pirenzipine and low- and high-dose misoprostol.

There were no statistical differences between the actual and predicted results. This was possibly a consequence of the small numbers in each group. However, based on the above rationale, there appeared a trend for cimetidine to perform less well than either low-dose and high-dose misoprostol and especially sucralfate. Note that given the same group of patients, cimetidine was not predicted to perform better than pirenzipine. This lends credence to view that the group of patients to be treated with pirenzipine were particularly difficult to heal.

Sucralfate

P.No	EM.No	Gl.No	D	H+	MI	DAM	DANM	Correct
1	898	20079	S	+	78	-		-
2	928	20772	S	+	53		-	-
3	1061	22399	S	+	57	-		-
4	1068	22519	S	+	75	+		+
5	1097	22727	S	+	67	+		+
6	1146	23530	S	+	92	-		-
7	875	19543	S	+	32		-	-
8	894	20009	S	+	57	-		-
9	901	20171	S	+	18		-	-
10	1077	22667	S	+	39		+	+
75	877	19538	S	-	100	-		+
76	1096	22726	S	-	53		+	-
77	869	19608	S	-	53		+	-
78	939	20957	S	-	46		+	-
79	890	19829	S	-	85	-		+
80	882	19749	S	-	93	-		+

Pirenzipine

30	1443	27757	P	-	4		-	+
31	1462	28071	P	-	25		-	+
32	1466	28419	P	-	82	+		-
33	1481	28762	P	+	79	-		-
34	1482	28835	P	+	29		+	+
35	1534	29042	P	-	32		-	+
36	1489	29174	P	-	71	+		-
37	1492	29248	P	-	36		-	+
38	1493	29250	P	-	93	-		+
39	1501	29428	P	+	100	-		-

Continued/

High-dose Misoprostol

P.No	EM.No	Gl.No	D	H±	MI	DA M	DA NM	Correct
40	1225	24657	HM	+	64	+		+
41	1318	25551	HM	+	79	+		+
42	1357	26013	HM	+	21		-	-
43	1391	26429	HM	+	57	+		+
44	1396	26510	HM	+	75	-		-
82	1335	25813	HM	-	36		+	-
45	1384	26379	HM	+	36		+	+
46	1385	26329	HM	+	85	-		+
47	1389	26412	HM	-	18		-	+
48	1248	24769	HM	-	96	-		+
49	1241	24709	HM	-	11		-	+
50	1370	26130	HM	-	25		-	+

Low-dose Misoprostol

51	1244	24692	LM	+	67	+		+
52	1280	25053	LM	+	64	+		+
53	1286	25080	LM	+	96	-		-
54	1336	25855	LM	+	43		+	+
55	1354	25995	LM	+	57	+		+
56	1363	26055	LM	+	57	+		+
57	1367	26107	LM	+	25		-	-
58	1390	26413	LM	+	50		+	+
59	1223	24189	LM	-	75	-		+
60	1359	26031	LM	-	14		-	+
61	1245	24722	LM	-	21		+	-

Table XXX: The M and N discriminant formulae applied to the juxta-DU data of biopsies from patients treated with sucralfate, prienzipine or high- and low-dose misoprostol.

H± = Indicates whether the patient healed (+) or not (-).

MI = Morphological score

DA M = Class M (>MI:56) discriminating formula to predict healing: Healing predicted (+); DU persistance predicted (-)

DA NM = Class N (<MI:55) discriminating formula to predict healing.

Correct = Describes whether the prediction was correct (+) or incorrect (-).

7.7.2.1. Differences In Drug Healing Potential

In 7.7.2. above, the number of patients in each therapy group that actually healed was compared with the number predicted to heal. No cognisance was taken with regards accuracy of prediction for individual patients. The M and N formulae appear able to predict outcome after treatment with cimetidine with a considerable degree of accuracy. The following examines the accuracy of the formulae in predicting outcome where individuals were treated with sucralfate, pirenzipine or misoprostol. Table XXXI summarises the data in Table XXX and shows the number of patients in each therapy group, the outcome for whom the cimetidine formulae predicted correctly.

Sucralfate			**Pirenzipine**			**LD Miso.**			**HD Miso.**		
No.P	**Correct**		**No.P**	**Correct**		**No.P**	**Correct**		**No.P**	**Correct**	
	N	**%**		**N**	**%**		**N**	**%**		**N**	**%**
16	6	37	10	6	60	11	8	73	12	9	75

Table XXXI: Accuracy of the M and N discriminant formulae in predicting outcome of therapy for patients treated with sucralfate, pirenzipine, high-dose or low-dose misoprostol.

No.P = Number of patients treated with drug.
Correct N = Number of patients whose outcome was predicted correctly by the cimetidine formulae.
Correct % = Percentage of patients whose outcome was predicted correctly by the cimetidine formulae.

The formulae only predicted correctly for 6/16 patients (37%) treated with cimetidine ($p < 0.05$). While the formulae predicted particularly badly for patients treated with sucralfate they predicted better for both misoprostol regimens (73% and 75%).

A breakdown of the data shows that some DU that healed after treatment with either sucralfate, pirenzipine or misoprostol were not predicted to heal with cimetidine and vice versa. Figure 42 shows the number of patients that healed with sucralfate, pirenzipine, low-dose or high-dose misoprostol compared with the number of patients that the formulae predicted would heal if each patient were treated with cimetidine.

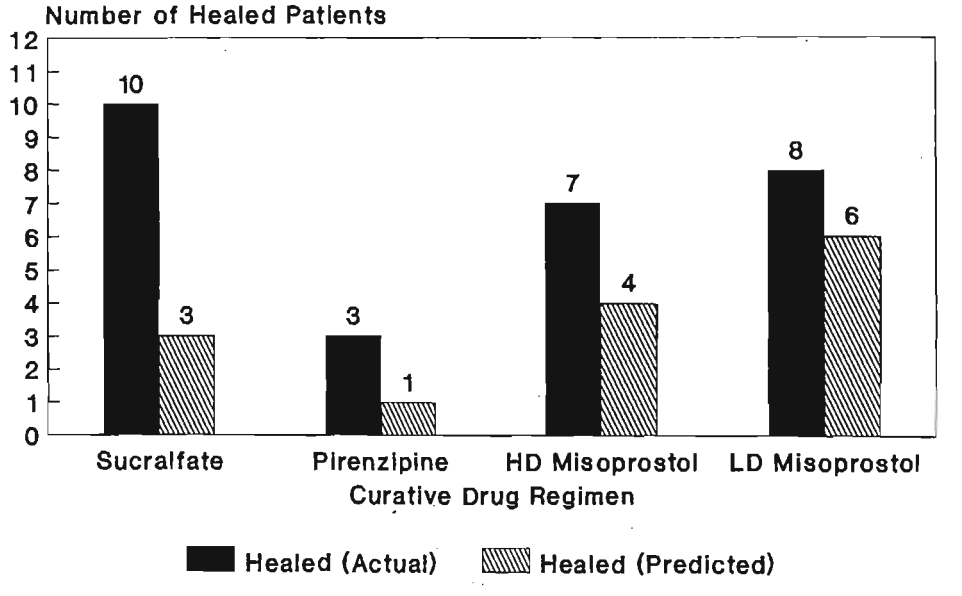

Figure 42: The number of patients that healed with sucralfate, pirenzipine, low-dose or high-dose misoprostol are compared with the accuracy of prediction.

Of the 10 patients that healed with sucralfate, only 3 (30%) were predicted to heal if treated with cimetidine. Of the 3, 7 and 8 patients that healed with pirenzipine, high- or low-dose misoprostol, 1 (33%), 4 (57%) and 6 (75%) respectively were predicted to heal should the same patients be treated with cimetidine.

A similar analysis was performed with patients that did not heal after treatment with each of the drugs. Figure 43 shows that of the 6, 7, 5 and 3 patients that did not heal after therapy with sucralfate, pirenzipine, high- or low-dose

misoprostol, 3 (50%), 2 (29%), 1 (20%) and 1 (33%) DU respectively were predicted to heal if treated with cimetidine.

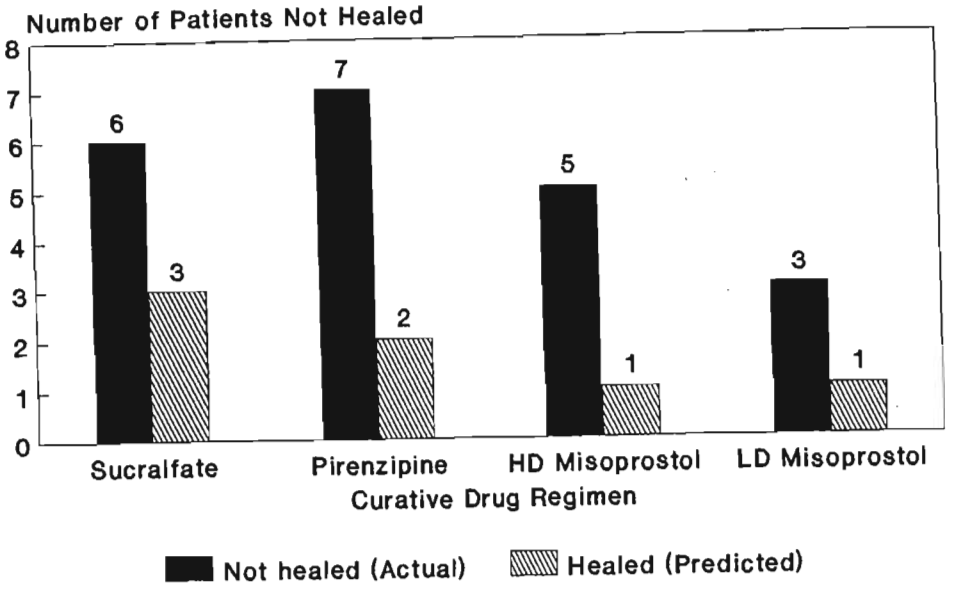

Figure 43: The number of patients that did not heal with sucralfate, pirenzipine, low-dose or high-dose misoprostol are plotted with the number of patients predicted to heal if treated with cimetidine.

Although differences were perceived, statistical methods when applied to the small groups of data showed no significance at $p < 0.05$. The trends shown, however, suggest that different drugs may not heal the same DU and that particular drugs may be more or less effective in healing morphologically distinct lesions.

7.7.3. Prediction Of DU Remission After Curative Therapy

The excellent prediction of duration of remission (more or less than 6 months) with the cimetidine formula suggested that as scar morphology did not differ markedly after different types of treatment, the formula would be able to correctly predict remission prognosis irrespective of the therapy employed. Table XXXII lists actual duration of

Formula: S1 X 1.49847 + S2 X 0.69225 + S4 X 3.06976 - 6.3

P	D	S1	S2	S3	S4	MI	R	DA	Correct
				Sucralfate					
1	S	3	3	2	1	64	+	+	+
2	S	1.5	1	1.5	1.5	39	+	+	+
3	S	1.5	1	0	1.5	29	+	+	+
4	S	3	3	2.5	1	68	+	+	+
5	S	2	2	2	2	57	-	+	-
6	S	1.5	2	0	1.5	36	+	+	+
7	S	3	2.5	2	1	61	-	+	-
8	S	2	0.5	2	0	32	-	-	+
9	S	1	1	0	0.5	18	-	-	+
10	S	2	2	1.5	1.5	50	-	+	-
				High-dose Misoprostol					
40	HM	3	2.5	2.5	0.5	61	-	+	-
41	HM	3	2.5	3	0.5	64	+	+	+
42	HM	2	2	2.5	0.5	50	+	-	-
43	HM	1.5	3	2	1	54	+	+	+
44	HM	1	1	0	0.5	18	+	-	-
45	HM	2	2	2	0.5	46	-	-	+
46	HM	3	2	2.5	0.5	57	-	+	-
				Low-dose Misoprostol					
51	LM	3.5	3	2.5	0	64	-	+	-
52	LM	2.5	2.5	2.5	0.5	57	+	+	+
53	LM	0.5	1	0	1.5	21	+	-	-
54	LM	1	1	0	1.5	25	-	+	-
55	LM	1.5	2	2.5	1.5	54	+	+	+
56	LM	1	1.5	0	0.5	21	+	-	-
57	LM	1	1	1.5	1.5	36	+	+	+
58	LM	1.5	2	1	1	39	-	+	-

Table XXXII: Remission discriminant formula applied to the scar data after therapy with sucralfate or high or low-dose misoprostol.
P = Patient number; D = Drug; S1-S4 = Morphological criteria
MI = Morphological index;
R = remission >(+) or <(-) than 6 months
DA = prediction of remission according to discriminant formula (+) >6M (-) <6M.
Correct = Whether the DA prediction was correct (+) or incorrect (-).

remission after treatment with sucralfate, high- or low-dose misoprostol together with that predicted with the cimetidine remission formula. The data is summarised in Table XXXIII.

Sucralfate		LD Misoprostol		HD Misoprostol	
Act.	Pred.	Act.	Pred.	Act.	Pred.
5	8	4	4	5	6

Table XXXIII: Actual and prediction of extended remission after therapy with sucralfate, low-dose or high-dose misoprostol.

Of the 8, 4 and 6 patients that were predicted to remain in >6 months remission if healed with cimetidine, 3 (38%), 2 (50%) and 3 (50%) relapsed less than six months after curative therapy with sucralfate, LD & HD misoprostol respectively. This is shown to good effect in Figure 43.

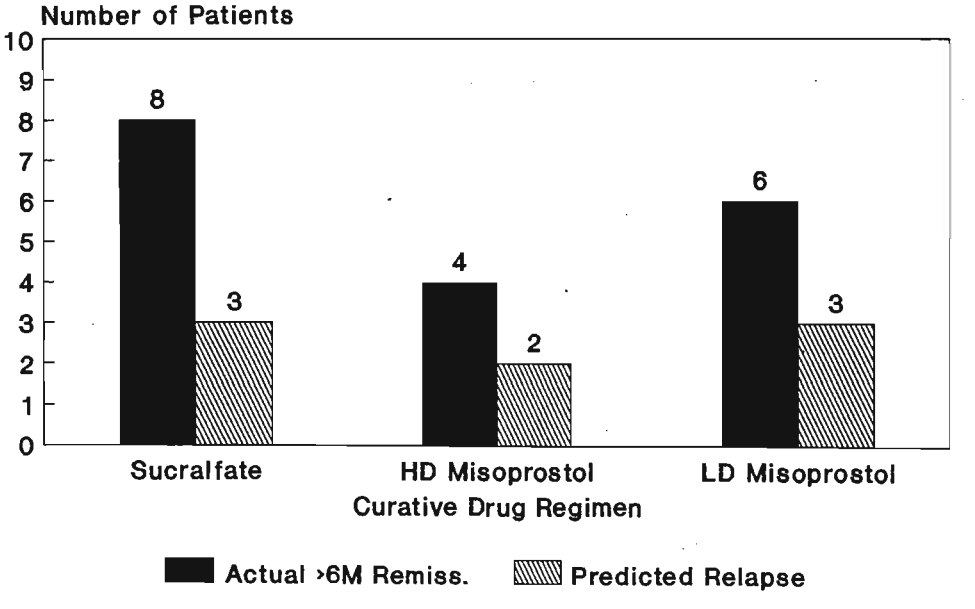

Figure 44: Accuracy of predicting duration of remission. Of patients treated with either sucralfate, high or low-dose misoprostol, 8, 4 and 6 were predicted to experienced remission for more than 6 months after therapy. Of these, 3, 2 and 3 respectively relapsed within 6 months.

This showed that irrespective of the apparent morphological similarity of scar tissue after the various types of treatment, scar morphology, per-se, was not predictive. This

in turn suggested that each drug may exert a unique, perhaps long-term influence over remission prognosis.

7.8. Summary

The results presented in this chapter show that the class of DU in any therapy group may influence healing statistics. This is of particular importance where small groups of patients are used to compare the relative efficacy of drug regimens. By using the cimetidine standards it may be possible to improve the accuracy of such studies. Of particular interest are the observations that particular types of curative therapy appear to be more efficaceous in healing some DU than others. Classification of DU may help clinicians select appropriate drugs for intransigent lesions. The morphological quality of healing is only predictive of remission prognosis after cimetidine therapy. The formula is not applicable to other types of chemotherapy. In addition, the results suggest that drugs may exert a hitherto unexpected long-term influence over remission prognoses. These and other important observations will be discussed in Chapter 8.

PART IV: CHAPTER 8

DISCUSSION

This study was designed to answer two questions: Does the severity of duodenal ulcer morphology influence healing prognosis; and does the quality of mucosal healing correlate with the duration of remission. Although seemingly straightforward, these questions were particularly difficult to answer because of the several variables involved. It may well be asked what constitutes morphological severity of DU and good or bad quality of healing in scar tissue? Also, to what extent did the size and depth of a particular lesion influence the probability of healing within the period of therapy? From the inception of this project, it was realised that the only way these questions could be addressed would be to correlate DU size and morphology with the incidence of healing, and scar morphology with duration of remission. Therefore, it was important to quantify the size of lesions and establish the morphological appearance of the mucosa near DU or scars.

8.1. Endoscopic Evaluation

Fibre-optic endoscopy made objective, in-situ, evaluation of DU possible. The size, depth, position and mucosal appearance of individual lesions was recorded and a similar appraisal of scars was made after the prescribed period of treatment. A simple grading system was devised that enabled the endoscopic severity of each DU to be scored. After treatment, and in accord with the guidelines set by Spitaels (1983), scores were awarded to the endoscopic features of the scar or residual DU. Few DU were larger than 20mm and none less than 5mm in diameter. By

correlating pre-therapy severity with incidence of healing, it became apparent that smaller lesions healed more readily than larger or deeper DU (Fig.7). This was not unexpected and did not preclude the possibility that the larger lesions would heal should the period of therapy be extended.

Of interest were the observations that eight patients with smaller lesions had not healed (Fig.8). If size of the ulcer was the only constraint on healing, why had these not healed? Furthermore, a number of DU did not improve during the period of therapy. If extended therapy would eventuate in healing, why were these DU not smaller? These data suggested that although the physical characteristics of lesions influenced prognosis, they were not the only factors that mediated for healing.

8.2. Morphological Evaluation

Quantifying the macro-physical characteristics of DU was a relatively uncomplicated procedure but to quantify mucosal morphology proved a far more difficult exercise. At first, the morphological appearance of juxta-DU biopsies appeared so varied that comparative or correlative analyses would be impossible. In addition, there appeared to be little or no difference in the appearance of juxta-DU biopsies or scar tissue. It did become apparent, however, that in both DU and scar specimens, the mucosa was comprised of one or more of seven morphologically identifiable cell types (Fig.3). This extensive variation in cell and tissue morphology represented various secretory phases and differentiative stages of the metaplastic gastric surface mucus secreting cells (MSC) that populated the villous epithelia of most DU and scar specimens. The remaining specimens exhibited many degenerative, non-metaplastic features.

These important observations made it possible to divide the specimens into two morphological types: metaplastic and non-metaplastic. As has been shown, the degree of metaplastic differentiation and degeneration of non-metaplastic epithelial cells allowed the specimens to be further subdivided in to well (MA) and moderately (MB) differentiated metaplastic mucosa or severe (NM) and moderately (ND) degenerative non-metaplastic tissue. Such classification enabled specimens to be grouped and correlated with the incidence of healing and duration of remission.

When the morphology of specimens from near DU were correlated with outcome, it became apparent that metaplastic mucosa was associated with a greater probability of healing than non-metaplastic tissue (Fig.9). Futhermore, DU circumscribed by a moderately metaplastic mucosa were very likely to heal while DU surrounded by less metaplastic or degenerative non-metaplastic tissue were less likely to do so (Figs. 9 and 10). When specimens from near scars were examined, the majority had a moderately metaplastic mucosa (Fig.11). In only two cases did the mucosa approach normality, and these also exhibited minimal metaplastic changes. In terms of morphological severity, therefore, metaplasia appeared to be a favourable prognostic criterion, moderate metaplasia a particularly positive sign and non-metaplasia a negative indicator for probability of healing.

The classification of specimens into four grades of morphology allowed order to be made from apparent disorder. Simple correlations between macro- and micro-morphology and healing statistics enabled some interesting deductions to be made. Size of the lesion and the type of juxta-DU mucosal morphology did have a bearing on DU healing. The

imprecise methodology, however, although useful for characterising general morphology, lacked the precision necessary to differentiate between specimens with disparate morphology in each grade. This was especially apparent when analysing the data from scars. Although differences in the degree of metaplasia could be perceived, most specimens were simply categorised as being moderately metaplastic (MB). Without further subdivisions within the grade, no correlations could be made between morphology and duration of remission. These observations showed that a more accurate means of quantifying mucosal morphology had to be devised.

8.3. Quantitative Morphological Analysis

The concept of a morphological index, based on the positive and negative criteria associated with DU healing, was promulgated from the preceding results. The scoring method was biased in a such a way as to accentuate differences between prognostically favourable gastric metaplasia on the one hand and unfavourable degenerative non-metaplasia on the other. Four primary morphological criteria were used and the scores were expressed as a percentage. The morphological index enabled gradations of metaplasia and non-metaplasia to be recorded and the prognostic profile of particular lesions to be quantified.

The morphological index was applied to all specimens entered into this study. The scores clearly showed that moderate metaplasia (MI:41-80) was a particularly favourable prognostic phenomenon (86% healing - Fig.14), whereas degenerative non-metaplasia (MI:0-40) was associated with DU persistence (43% healing). An unexpected phenomenon was the observation that well differentiated gastric metaplasia (MI:81-100) often correlated with DU

persistence for healing occured in only 33% of patients with this type of lesion (Tables IX and X). If moderate metaplasia correlated with DU healing, why should severe metaplasia be associated with DU persistence. This was a particularly important question that had to be answered since by showing that gastric metaplasia was not necessarily a favourable prognostic criterion this observation threatened to destroy the rationale on which the morphological key was based.

Correlations between the severity of DU as determined by endoscopy and the morphological scores of individual lesions showed that large, deep DU were often surrounded by well differentiated metaplastic tissue (Figs.17 and 19). Endoscopically severe DU had been shown to be less likely to heal within the period of therapy than their smaller counterparts (Fig.7). This physico-morphological association between the endoscopic severity of DU and well differentiated metaplasia suggested that size of lesion rather than metaplasia, per-se, was the reason that these DU were less likely to heal. Juxta-DU gastric metaplasia, therefore, may still be considered a favourable prognostic criterion and may be used as the basis of the morphological key.

Irrespective of the morphological type of DU or the endoscopic quality of healing, the mucosa surrounding scars remained abnormal. This accords with the findings of Pillay et al. (1977) and Moshal et al (1979). The most consistent feature was the reduction in the range of morphological variation in scar tissue (Fig.22). Prior to therapy, some juxta-DU specimens were populated almost exclusively by metaplastic cells while others showed little evidence of metaplastic transformation. This was reflected in their morphological scores that ranged from MI:7 to MI:93. After

therapy, no specimen could be regarded as being particularly metaplastic (MI:81-100) and only two specimens had a mucosa with a score <MI:20 (Table XVA and B). Moderate metaplasia (MI:40 to MI:60) persisted in scar mucosa of all patients who remained in remission for more than six months (Fig.27). Although there was a general uniformity in scar morphology, there were identifiable differences between scars derived from metaplastic and non-metaplastic lesions.

In DU surrounded by metaplastic tissue, healing was always characterised by a progressive reduction in the degree of metaplasia during and after the healing period (Gregory et al. 1982**a,b**), a phenomenon also reported by Tovey et al (1989**a,b**). The scars formed from such lesions, however, retained many metaplastic characteristics (Table VI). In the case of lesions surrounded by degenerative non-metaplastic tissue, although there was a marginal increase in mean scar MI on healing, most healed with a score <MI:40 (Fig.22). This showed that scars retained many of the primary morphological characteristics of the parent DU. As DU heal by an overgrowth of the epithelium lateral to the crater, this phenomenon was not unexpected (Poulson and Szabo 1977, Giampaolo et al. 1978).

The results showed that patients with DU surrounded by a metaplastic mucosa have a good probability of healing. Should these lesions heal and remain healed with a moderately metaplastic scar, patients have a good chance of remaining in remission for more than six months. Conversely, patients with non-metaplastic lesions have less chance of healing and a greater probability of early relapse. These data show that a critical evaluation of ulcerative mucosa can indicate remission and healing prognoses.

8.4. Mucosal Morphology And Drug Therapy

The results presented here show that the type of mucosa surrounding DU may indicate healing prognosis. The deductions, however, were made from the combined data before and after the use of 7 disparate therapeutic regimens. The observations, therefore, may have been biased by one or more of the regimens. To determine whether one or other drug had preferentially modified the overall results, the morphological scores (MI) before and after each type of therapy were correlated with the information regarding healing and remission.

The drug regimens covered both primary mechanisms of action: acid inhibition (cimetidine and pirenzipine) and cytoprotection (sucralfate). Also, two groups of patients were treated with a prostaglandin analogue (misoprostol), a substance that both reduces acid secretion and improves mucosal protection.

8.4.1. Drugs And DU Healing

There were no group differences in the range of morphology (Table XIX) or mean MI prior to each type of drug therapy (Fig.31). Similarly, there were no apparent drug mediated differences in the range or mean scar MI after treatment. In every instance, metaplastic differentiation of each group was reduced after DU healing (Fig.31). In spite of the apparent similarity in mucosal morphology before and after therapy with each regimen, there were some interesting drug-specific anomalies that emerged when correlating juxta-DU morphology and healing, and scar morphology and duration of remission.

Each drug was equally efficacious in healing lesions surrounded by a moderately metaplastic mucosa (Fig.33). Irrespective of dosage or duration of therapy, cimetidine was equally efficacious in healing metaplastic and less metaplastic DU. Sucralfate appeared less effective in healing DU surrounded by well differentiated metaplastic mucosa whereas pirenzipine and especially misoprostol appeared less effective in healing DU circumscribed by degenerative non-metaplastic tissue. Perhaps the difference in healing relates to the aetiology of each lesion and the mechanism of action of each drug.

Cimetidine, pirenzipine and misoprostol in various ways reduce gastric acid secretion and promote DU healing by neutralising duodenal chyme (Aadland and Berstad 1978, Wolf and Soll 1988, Schmueli and Record 1992). Sucralfate has no effect on intraluminal pH, but promotes healing by forming a protective paste over the ulcer crater (Samloff 1983). The paste is thought to bind pepsin and impede the passage of hydrogen ions to the mucosal surface (Rees 1991). Most lesions that did not heal after sucralfate therapy had a particularly high juxta-DU MI indicating a high degree of gastric metaplasia. If this reflects a milieu of particular hyperacidity (pp.35 - 36), sucralfate would appear less effective in the presence of low pH chyme. It was, however, equally effective in healing less-metaplastic lesions as cimetidine, misoprostol and pirenzipine.

In the case of misoprostol and pirenzipine, most DU that healed had a high morphological score. The trend for these drugs to heal metaplastic lesions suggested that they were particularly effective in treating lesions with an acid-based aetiology in a low pH milieu. These results suggest that the efficacy of a drug to heal DU may be related to their mechanism of action and the physiological environment in which the ulceration has occurred.

8.4.2. The Effect Of Drugs On Scar Mucosa And The Duration Of Remission

After treatment with each drug, scar morphology was correlated with the duration of remission. No significant correlations were determined between scar morphology and duration of remission with either sucralfate or misoprostol. In both cimetidine studies, however, moderately metaplastic scars with higher MI were associated with extended remission (>6 months) whereas less metaplastic (low MI) tissue was correlated with early relapse (Fig.35). Moderate metaplasia persisted in the scars of patients during their periods of remission irrespective of the regimen employed to effect healing (Figs. 29 and 30).

Cimetidine, sucralfate or misoprostol have not been shown to extend long-term control over acid or pepsin or to maintain cytoprotection once treatment ceased. After therapy, therefore, the ulcerogenic factors responsible for the original lesion could resurface and the mucosa may relapse. After treatment, a moderate level of gastric metaplasia in the scar mucosa appears to protect the patient against relapse.

In this study there were no significant differences in the duration of remission after each type of treatment. There are, however, reported differences in the duration of remission after various types of therapy (Eichenberger et al 1982, Tovey et al. 1989**a**, Rauws and Tytgat 1990, Hui et al. 1992). If drugs do not regulate ulcerogenic factors in the post-therapy period, how might they influence remission prognoses? During the healing process, drugs may possibly alter the cytoprotective properties of the mucosa in such a way as to create a scar that was more or less susceptible

to the ulcerogenic factors responsible for relapse. Although no obvious drug-mediated differences in scar morphology were detected, this did not preclude the possibility that subliminal changes existed. Quantitative light microscopic morphometry was employed to investigate this possibility.

Light microscopic morphometric evaluation of non-metaplastic areas of mildly metaplastic and non metaplastic specimens before therapy showed that goblet cells increased in number from 2GC/100um of mucosal surface in normal specimens to 3.7GC/100um in the juxta-DU mucosa (Table XXIV and Fig.36). After therapy with cimetidine, there were 1.8GC/100um whereas after treatment with sucralfate there were 2.8GC/100um of mucosa ($p = <0.02$). The difference in goblet cells after sucralfate and cimetidine treatment showed that drug therapy could influence scar morphology. In this case, secretions from an increased number of goblet cells in sucralfate treated patients may offer better cytoprotection against renewed acid attack. These findings offer an explanation for the clinical observations made in some studies that sucralfate is associated with a more prolonged remission of DU than patients treated with H^2-receptor antagonists (Marks et al. 1981, Lam et al. 1987, Tovey et al. 1989a, Gregory et al. 1991a).

In summary, correlations between juxta-DU morphology and lesion healing after each type of therapy were similar to those determined by analyses of the combined data and confirmed that the macro- and micro-morphologic appearance of the juxta-DU mucosa indicated probability of healing. The results also showed that in the case of cimetidine, the morphological appearance of the scar indicated the duration of remission, a phenomenon that had been masked by the combined data. Drugs were shown to alter scar morphology

and to be more or less efficaceous in healing specific morphological classes of DU. If different types of therapy were more effective in healing identifiable DU, perhaps this information may be used both to direct treatment and predict its outcome.

8.5. Prediction Of Outcome After Drug Therapy

Cimetidine was prescribed in this study at two dosages. Correlations with scar morphology, healing and remission outcome were similar in both cases. If drugs effect repair of morphologically distinct DU in a consistent way, then prediction of healing before and perhaps remission after a particular type of drug therapy may be possible.

Discriminant analysis was employed to predict healing. This method finds appropriate weighting co-efficients for SL and S1 to S4 to best separate data from healed and not-healed patients. The method was also applied to the S1 to S4 scar data to separate patients into those who experienced extended remission and early relapse. Should inherent differences in the data enable the method to separate the groups effectively, then the numeric data, coefficients and constant (expressed as a discriminant formula) should predict the probable outcome when applied to similar data from other patients treated with the same drug. The already proven uniformity of healing after both cimetidine regimens made this the test drug of choice.

When applied to the data derived from scars, discriminant analysis enabled 100% separation of patients in extended remission and those who experienced early relapse. Only S1, S2 and S4 morphological criteria were required for the separation ($p = 00028$). The best separation of healed and

not-healed patients that could be achieved from the juxta-DU data using all 5 criteria was 73%. Seven specimens were misclassified. Such unsatisfactory separation suggested that the discriminant formula was being used to separate discordant data.

Large lesions surrounded by a metaplastic mucosa and characterised by a high MI and small non-metaplastic DU with a low MI rarely healed (Table XXII and Fig.34). This suggested the existence of at least two morphological classes of DU, each with their prognostic determinants. If two prognostic class of DU exist, a single discriminant formula designed to separate the combined data would be less effective than might be two formulae designed to separate the data form each class. For the analysis to succeed, it was important to define the numeric boundaries of each class of DU.

Using correlations between MI and percentage healing in each of 10 equal MI bins, it was found that healing least often occurred at MI:0-10 and MI:90-100; and most often between MI:40 and MI:60. The pre-therapy specimens were roughly separated into two categories: metaplastic (M) and non-metaplastic (N) at MI:50. Regression analyses performed on the two sets of data showed that both classes of DU healed most often when MI = 56 and that class N healed least at MI = 4 and class M at MI = 96. Based on these results, class N DU were characterised by having MI:<55 and class M, MI:>56.

Having defined the numeric range of the two classes of DU, discriminant analyses was performed on the data from each. Using criteria SL, S1, S2 and S4, separation was successful in 100% of cases with class N specimens ($p = 0.01036$) and

using S1, S2 and S4, 86% of class M specimens ($p = 0.01847$). The overall accuracy of separation of juxta-DU specimens prior to treatment with cimetidine was 92%

To test whether the two formulae could accurately predict healing, they were applied to the data from class N and M lesions derived from patients treated with cimetidine(3). The formulae predicted correctly in 8/9 (89%) cases. These results confirmed that prediction of healing with cimetidine is possible. No opportunity has been afforded to test the predictive accuracy of the cimetidine remission formula. Further work on this aspect of the morphological index is indicated, particularly in view of the excellent separation of data that was obtained.

8.6. Comparing The Efficacy Of Drug Regimens

The results have shown that the morphological appearance of the juxta-DU mucosa could indicate healing prognosis. All DU, therefore, appear not to be equal with regards probability of healing. Most drug studies designed to determine the relative efficacy of two or more curative regimens simply compare the number of healed patients at the end of a fixed period of time (Bardhan et al. 1986, Lipsey et al. 1990, Marks et al. 1991). Such analyses presuppose that all DU are equal with regards probability of healing and that differences in the number of patients healed at the end of treatment describes the relative efficacy of the drug.

If large numbers of patients are entered into different therapeutic groups, then variations in the healing potential of individual DU may be balanced by randomisation. However, in small groups, such randomisation

can not be guarranteed and any therapeutic group may include a disproportionate number of easy or difficult "healers". This phenomenon was found to occur in some therapeutic groups used in this study.

Pirenzipine has been shown to effect healing in up to 87% of cases (Nicholson 1985, Carmine 1985) and is reported to be at least as efficaceous as the other drugs employed in this study. In two studies performed in the USA that employed misoprostol at low (50ug/day) and high (200ug/day) doses for four weeks, in both cases the higher dose healed significantly more patients than did the lower dose within the prescribed time (Brand et al. 1985, Fich et al. 1985). In this study, the reverse was the case. An evaluation of the juxta-DU mucosa in the groups of patients treated with pirenzipine or both doses of misoprostol revealed a significant reduction in the number of moderately metaplastic DU (easy healers) in the pirenzipine group and a concommitant increase in the number of low MI and high MI difficult healers (Fig.40). A similar distortion in the distribution was observed between the low and high-dose misoprostol with the high-dose group having fewer easy healing DU (Fig.41). These data showed that for the statistics of comparative drug trials to truly reflect the efficacy of a regimen, especially when patients per therapy group are low, then some accommodation should be made for the prognostic differences inherent in DU.

8.6.1. Determination Of Drug Efficacy Using The Discriminant Formulae

The excellent predictive properties of the N and M discriminant formulae were used to accommodate for prognostic differences in individual DU (Gregory and Simjee 1986). Comparisons between the relative efficacy of

different drugs could be made by:

i) Using the formulae to determine whether a DU would heal if treated with cimetidine

ii) Determining the number of patients that healed after treatment with the experimental regimen.

By comparing the actual number of patients that healed after a particular regimen with the number predicted by either the N or M cimetidine formulae, the relative efficacy of an experimental drug or curative regimen could be ascertained.

In this context, the discriminant formulae were used as independent standards to compare drug efficacy. The results proved the merit of the system. In the case of the patients treated with pirenzipine, only 30% of patients were predicted to heal. With low-dose misoprostol 64% and high-dose misoprostol 42% of patients were predicted to heal if treated with cimetidine. This supported the earlier premises that the groups of patients treated with pirenzipine and high-dose misoprostol had DU that were indeed difficult to heal and that these patients would have fared as poorly had they been treated with cimetidine. The discriminant formulae, therefore, provide a useful tool for levelling the playing field in drug trials with small numbers of patients.

8.7. Certain Drugs For Certain DU?

In 8.5. above, the possibility that certain drugs may be more efficacious in healing lesions of a particular morphological type was postulated. Using the discriminant formulae, a critical evaluation of the results exposed some interesting associations between predicted and actual healing/non-healing that supported this premise.

There was a significant difference in the number of patients that healed or did not heal with sucralfate when compared with prediction of healing should the same patients be treated with cimetidine. Of the ten patients that healed after treatment with sucralfate, only three were predicted to heal if treated with cimetidine (Fig.42). Of the six patients who did not heal with sucralfate, three were in fact predicted to heal should they be treated with cimetidine (Fig.43). Similar anomalies, but to a lesser extent were observed with data from pirenzipine, as well as low- and high-dose misoprostol.

Sucralfate is a cytoprotective drug that does not alter luminal pH, while cimetidine effects healing by reducing gastric acid output. Although in this study both drugs healed approximately 60% of patients, sucralfate healed patients that were predicted not to heal with cimetidine and vice-versa. The possible differences in the local luminal environment together with the substantially different pharmacokinetic mechanisms of action may explain this phenomenon. If as seems probable, certain drugs are particularly good at healing morphologically distinct lesions, it may be possible in difficult cases to tailor a particular drug regimen to a particular DU. More important, perhaps, such insights may enable ulcers that are difficult to heal or which may quickly relapse to be more rapidly identified, thereby allowing the option of surgery to be considered earlier.

8.8. Drugs And Remission Prognosis

The discriminant formula derived from scars after treatment with cimetidine correctly indicated remission outcome in 100% of cases. The ability of the formula to predict remission outcome suggested that the morphological

appearance of the scar and therefore, perhaps the morphological quality of healing influenced remission prognosis. If this were the case, a morphologically healthy scar would be expected to extend remission irrespective of the curative regimen. This was not found to be the case. Although there was little difference in the number of patients in each therapy group who actually remained in remission when compared with the cimetidine prediction (Table XXXIII), the actual patients experiencing remission were often not those predicted to do so (Fig.44). This showed that the formula was not able to predict remission outcome in individual patients treated with drugs other than cimetidine. It also showed that the morphological appearance of scar mucosa did not inevitably indicate remission prognosis. As patients with similar scar morphology but treated with different drugs experienced different periods of remission, it appears possible that even after treatment has ended, drugs may exert some hitherto unknown long-term influence on remission prognosis.

8.9. Juxta-DU Morphology And DU Prognosis

This project has shown that a morphological appraisal of the juxta-DU mucosa enables healing to be predicted should patients be treated with cimetidine. Further, once healed with cimetidine, it may be possible to predict the duration of remission from scar morphology. Of particular interest were the observations that drugs preferentially healed some lesions but not others. Furthermore, drugs had the capacity to alter scar morphology, a phenomenon that may explain how drugs influence remission long after treatment has ceased. All these observations were deduced by correlating mucosal morphology, expressed as numeric MI, with clinical results. Why should juxta-DU morphology indicate healing or remission prognosis?

In this study, the absence, presence and level of juxta-DU metaplasia and the degree of degenerative non-metaplasia were the corner-stones on which the quantitative analysis of endoscopic specimens was based. The analyses showed that irrespective of the type of drug therapy, the morphological appearance of the mucosa surrounding DU indicated healing prognosis. In order to understand how juxta-DU morphology may indicate outcome of therapy it is necessary to review current concepts on why and how the mucosa surrounding DU should differ from that found elsewhere in the ulcerated and non-ulcerated duodenum.

8.9.1. Gastric Metaplasia And The Ulcerated Duodenum

The presence of MSC near DU is controversial. Patrick et al. (1974) suggested that MSC arose by transformation and migration of cells from the ducts of Brunner's glands, while James (1964) and Gregory (1982) considered them to be derived from the undifferentiated stem cells at the base of the crypts of Lieberkuhn. The latter premise was supported by the results of this study. In well differentiated metaplastic mucosa, MSC lined both the villi and the crypts. Stem cells undergoing mitosis contained secretory granules and were in close proximity to MSC in various phases of metaplastic diferentiation (Plate 52). Rather than differentiating into goblet and absorptive cell precursers, stem cells appeared to develop directly into immature MSC, which during migration towards the villus, matured into typical well differentiated, actively secreting MSC. Further support for a crypt rather than Brunner's gland origin were the variations in epithelial cell morphology in metaplastic tissue and the progressive changes that took place during the process of healing.

Well differentiated MSC were typified by their short and

sparse microvilli, thin, often discontinuous glycocalyx and large quantities of mucus in the supranuclear position. During and after healing, and at increasing distances from the lesion, there was a reduction in the number of mucus granules in MSC. Reduced mucus synthesis appeared to be associated with an apparent concommitant increase in the number of small osmiophilic crinophagic vesicles, length and number of microvilli and thickness of the glycocalyx. Near-normal absorptive cells in metaplastic epithelium contained few osmiophilic vesicles and had normal microvilli. The sum of morphologic data suggested a continuum between well differentiated MSC (type a cells) and near-normal absorptive cells (type dii cells). This in turn supported crypt stem cells as being the precurser of the seven morphologically identifiable cell types commonly found in the ulcerated mucosa.

8.9.2. Juxta-DU Gastric Metaplasia

In the ulcerated duodenum, well differentiated MSC were localised to within 8mm of the edge of DU. There was a progressive reduction in the degree of differentiation of metaplastic cells up to 20mm from the DU crater with the epithelium between 20mm and 50mm from lesions appearing morphologically normal or near normal (Gregory and Spitaels 1982). Gastric metaplasia is reported to develop in the duodenum as a response by the intestinal mucosa to unusually high levels of acid and pepsin in the chyme (Rhodes 1964, James 1964, Hoedemaker 1970, Johansen and Hansen 1973). Foci of well differentiated MSC at the periphery of DU suggest a localised reduction in pH at the ulcer site. Such a premise supports a theory by Mann (1925) who suggested that DU may be caused by jets of corrosive stomach fluid being directed at the anterior wall of the duodenum via an incompetent pylorus. Although an acid jet aetiology for DU is possible, there is more convincing

evidence to suggest that in patients with DU, the entire first part of the duodenum is exposed to low pH fluids (Lam et al. 1982, Malagelada et al. 1977). If this is the case, gastric metaplasia should occur throughout the first part of the duodenum and not be localised near DU. Although low pH in the duodenum predisposes to metaplasia, it may not be the only factor that causes this phenomenon to occur near the edge of DU.

Gastric metaplasia in the juxta-DU position has been described as a natural protective mechanism evoked by the mucosa to promote healing (Patrick et al. 1974, Gregory et al. 1982a). These authors proposed that the secretion of copious ammounts of neutral gastric mucosubstance from numerous well differentiated MSC at the periphery of DU may more effectively protect the ulcer crater from the corrosive effects of acidic chyme than the sulphated acid-mucosubstance secreted by the relatively few goblet cells in the non-ulcerated and ulcerated non-metaplastic mucosa. While gastric mucosubstance provides a barrier behind which DU have an opportunity to heal, the soma of metaplastic enterocytes circumscribing a lesion may prevent further mucosal erosion and DU enlargement (Gregory et al. 1982a). The combined effect of mucus protection and lesion delimitation provides a mileu in which natural healing can take place.

From the above, although metaplastic transformation may be dependent on a lower than normal luminal pH, mucosal damage in the ulcerated mucosa may be the phenomenon that triggers a focal protective response at the forming face of developing DU. Perhaps villous erosion during the early stages of ulcerogenesis exposes stem cells in nearby crypts of Lieberkuhn to low pH luminal fluids, thereby preferentially stimulating differentiation into MSC rather

than absorptive and goblet cells. This may explain why in the ulcerated duodenum, gastric metaplasia often occurs near DU and less often elsewhere.

8.9.3. Variations In Metaplastic Cell Morphology

In this study, the degree of metaplastic differentiation varied within and between juxta-DU and scar biopsies. Such variation may represent different phases in the natural history of duodenal ulcerogenesis and healing (Gregory et al. 1987). Duodenal ulcers form, regress are contained and some heal naturally (Poulson and Szabo 1977, Scheurer et al. 1977, Giampaolo et al. 1978, Zoli et al 1984). Each phase in the diathasis of duodenal ulcerogenesis and healing may be characterised by a particular type of juxta-DU morphology.

Migration of cells from the base of the crypts of Lieberkuhn to the tips of villi takes from 4 to 6 days (MacDonald 1964). Bearing this time factor in mind, some non-metaplastic specimens may be derived from the juxta-DU mucosa of developing ulcers whose crypt stem cells had not had time either to differentiate into MSC or migrate to the villus. Specimens exclusively populated with well differentiated MSC may be derived from older lesions in which the MSC have had time both to differentiate fully and migrate to all parts of juxta-DU villi. These specimens may have been obtained from near DU that were contained laterally by peripheral MSC and were in a mucoprotective phase of healing. Specimens populated with MSC in intermediate phases of differentiation may be from the edge of enlarging DU in intermediary phases between regression and containment. Alternatively, as healing and scar mucosa are characterised by moderate metaplasia, these specimens may be from DU in phases between containment and healing.

If the latter premise is correct, these lesions may be in various stages of spontaneous healing (Scheurer et al. 1977, Zoli et al. 1984)

Although the extent of metaplastic differentiation may describe phases of ulcerocenesis and healing, it is possible that in some cases the degree of metaplastic differentiation may reflect the pH of the luminal content at or near the time of biopsy. Well differentiated juxta-DU metaplasia may indicate a low pH chyme whereas less well differentiated MSC may indicate a less acidic luminal milieu. Measurement of luminal pH and correlation with juxta-DU morphology was beyond the scope of this study making confirmation or rejection of this postulate impossible at the present time. However, the possibility that juxta-DU morphology may indicate luminal pH warrants further investigation.

8.9.4. Juxta-DU Morphology As A Possible Indicator Of DU Aetiology

The results presented in earlier chapters show that juxta-DU morphology can be used to good effect in predicting DU healing. Many DU's, however, probably would not heal without drug intervention. This implies that morphology was not directly responsible for DU healing, but rather indicates the manner in which the mucosa had responded to whatever factors had caused ulcers to develop. This suggests that juxta-DU morphology may provide some clues to the aetiology of individual DU.

Juxta-DU metaplasia suggests a DU that has developed in an acid environment. The mucosa surrounding such DU are exhibiting a healthy protective response to low pH chyme.

Non- or poor juxta-DU metaplasia may be associated with either acid based ulcers whose protective mucosal responses were defective or lesions whose aetiology were other than low pH chyme. The degenerative features most often observed in non-metaplastic tissue were akin to the necrotic and oedematous changes associated with cellular ischaemia in other tissues (Goldstein 1979, Gregory and Mars 1991, Gregory and Mars 1992). Perhaps, these DU also had an ischaemic aetiology. Ischaemia has been postulated as a cause of duodenal ulceration since the middle of the last century (Virchow 1853, Wilkie 1911). More recent studies have shown that mucosal ischaemia of both the stomach (Svanes and Varhaug 1982) and duodenum (Piasecki 1974, Piasecki 1977, Linder and Lenz 1978) is an important factor in the development of some lesions. Whether the DU reported on in this thesis had a predominantly ischaemic or acid-based aetiology, or whether such a distinction is reasonable is not known for the recording of mucosal blood flow, gastric and/or duodenal luminal pH and pepsin levels was beyond the scope of this study. Nevertheless, such work is in progress.

In summary, juxta-DU gastric metaplasia is probably a protective response by the mucosa to low pH ulcerative factors in the chyme. Well differentiated metaplasia in the juxta-DU mucosa is correlated with poor healing prognosis. Well differentiated MSC were regularly associated with larger lesions designated by endoscopy as severe. Large size, regressive phase of ulcerogenesis and/or an inherently low luminal pH either generally or focally at the site of the DU, are all factors that may militate against healing.

Moderately metaplastic specimens may describe a lesion developing in a predominantly acid mileu that is in either

a regressive phase of ulcerogenesis or phase of healing. The majority of moderately metaplastic lesions heal, suggesting that at the time of biopsy many were in the process of healing and may well have healed without pharmaceutical intervention. The lower level of metaplasia may reflect a more normal luminal pH at the time of biopsy - also a factor that would militate for healing.

In the case of non-metaplastic specimens, they may characterise DU with an acid-based aetiology where the natural processes of mucosal metaplastic cytoprotection were defective. Alternatively they may describe such DU in the early phases of regression or DU whose aetiology predominantly involved other factors such as ischaemia. These lesions may be small or large and many do not heal. I would suggest that most non-metaplastic DU that heal are "young" acid-based lesions while those that do not heal have a predominantly ischaemic aetiology.

As mentioned above, precise interpretation of the significance of morphological features is not possible within the bounds of the present study. Nevertheless, such speculation raises important therapeutic questions that require further investigation.

8.10. Morphological Indices

The results of this study were based on data derived from an index conceived in 1981 and reported in 1982 (Gregory et al. 1982**c**) and modified in 1985 (Gregory and Simjee 1985). Why should this index prove successful whereas another was less so (Tovey et al. 1989**a,b**)? The difference between these indices lies in the morphological key and in the way that scores awarded to morphological criteria were biassed.

Tovey's group did not investigate correlations between juxta-DU mucosa and DU healing; they were more concerned with identifying changes in scar mucosa after sucralfate and cimetidine therapy. Their primary concern was to explain recorded differences in remission outcome between the therapy groups. Their key was based on the assumption that juxta-DU morphology was quintessentially bad and that both necrosis and well differentiated metaplasia represented the most severe expressions of DU pathomorphology. Well differentiated metaplasia and non-metaplastic degeneration were placed together at one end of the pathomorphological spectrum while normal and near-normal, minimally metaplastic tissue were at the other. Whereas the index used in this study maximally separated prognostically favourable metaplasia from unfavourable degenerative non-metaplasia, the index used by Tovey et al. (1989) numerically overlapped both types of DU morphology. Furthermore, rather than using electron microscopy to complement light microscopy and give an overall numeric description of the ulcerative mucosa, Tovey separated the two analyses, thereby possibly reducing both the accuracy of the data.

Tovey was able to detect a numeric, morphological difference between the high scoring degenerative and metaplastic juxta-DU mucosa and the lower scoring moderately metaplastic tissues surrounding scars. They were, however, unable to correlate their light and electron microscopic findings with duration of remission; nor were they able to confirm that metaplasia was a favorable prognostic criterion.

8.11. Glycocalyceal Bodies

Apart from revealing the predictive capability of the ulcerative mucosa, other interesting morphological observations were made that may have relevance to DU ulcerogenesis and cytoprotection.

Glycocalyceal bodies (GB), first named as such by Ozzello et al. (1977), invested the glycocalyx of poorly differentiated metaplastic (type b cells) and non-metaplastic type di) and dii) cells in approximately 40% of moderately metaplastic (MB) and 90% of non-metaplastic (ND) specimens (Gregory et al. 1986). Glycocalyceal bodies, although reported to occur in many parts of the human gut and in intestinal neoplasia (Marcus 1981) previously had not been described in association with DU. Although the morphogenesis of these structures is unconfirmed, some authors have speculated that they may arise from budding of the plasmalemma (Misch et al. 1980) or from multivesicular bodies (Lusk et al. 1977). In this study, the glycocalyceal bodies appeared to be derived from multivesicular bodies within type b) di) and dii) enterocytes.

There appeared to be an inverse relationship between the integrity of the glycocalyx and number of glycocalyceal bodies near the cell surface. Where large numbers of GB were present, the glycocalyx was fragmented, discontinuous or absent. The precise role of glycocalyceal bodies is unknown. However, the results of recent studies suggest that GB contain glycoprotein specific hydrolases (Murayama et al. 1991), enzymes that are known to be involved in glycocalyx turnover (Ito 1974, Misch et al. 1980).

The glycocalyx, as well as being involved with the

digestion of proteins is perfused with and mucus and bicarbonate may help bind these substances to the cell surface (Ito 1974). An overproduction of glycocalyceal bodies and subsequent release of hydrolase may irreversibly damage the glycocalyx. This in turn may negatively affect the stability of the unstirred mucus layer and bicarbonate interface and thereby expose the cell surface to ulcerogenic low pH fluids. Glycocalyceal bodies, therefore, may be an important factor in the aetiology of DU.

8.12. Helicobacter Pylori

During the period that the experimental work for this study was carried out, the possible importance of H. pylori in duodenal ulcerogenesis and relapse was not known. However, in describing all morphological features associated with DU and scars, the presence of "bacteria" was noted (Appendix B). These bacteria in retrospect, were found to exhibit the spiral morphology of H. pylori.

Bacteria were found by electron microscopy(EM) in approximately 40% of all juxta-DU biopsies. Exclusive reliance on EM derived data to determine the presence of an organism is unsatisfactory because the area evaluated is inevitably small. There is a high probability that areas of infection may be missed. In an attempt to better determine the number of infected samples, and more accurately correlate the numbers of infected patients with healing and remission outcome, the larger histological sections were demounted and restained with the Warthin Starry silver method for spirochetes. The restaining method described by Offerhaus et al. (1990) was applied to the histological material without success. My inability to unequivically demonstrate the presence or absence of H.pylori in each sample made accurate correlation between infection, healing

or remission impossible. The presence or absence of H.pylori was, therefore, not included in any analysis.

Bacteria were found in most juxta-DU specimens exhibiting well differentiated metaplasia and also in the mucosubstance secreted by and adhering to the surface of some MSC in less well differentiated phases. They were usually found in juxta-DU specimens whose MI ranged from approximately MI:60 to MI:100. H.pylori were never found in degenerative non-metaplastic specimens nor were they found in scar tissue.

There is evidence that H. pylori damage microvilli and the cytoskeletal web and cause mucus depletion in gastric mucus cells (Goodwin and Armstrong 1986). Most authors, however, consider that H. pylori damages the mucosa by destabilising the unstirred surface mucus layer, thereby permitting passage of cytotoxic low pH fluids to the cell surface (Slomiany et al. 1987, Sarosiek et al. 1987). In this study, no particular pathomorphological changes were seen in MSC populating infected specimens. However, in some cases H.pylori had disrupted intercellular junctions and were found in the intercellular spaces between well differentiated MSC. Disruption of the intercellular junctions between surface mucus cells in the stomach has been reported by Chen et al. (1986) but to my knowledge a similar phenomenon has not been described in the duodenum. By severing the intercellular connections and destroying the integrity of the mucosa, H.pylori might allow luminal fluids to reach the unprotected lateral and basal aspects of enterocytes. This together with a concentration of their toxic waste-products (Murakami et al. 1987) in the intercellular spaces may cause cell death and create potential sites for ulceration.

Various authors have shown that H. pylori-associated gastritis is present in up to 100% of patients with DU (Rauws et al. 1988, Sipponen et al. 1989). The organism has been reported to infect the mucus associated with MSC from between 17% and 55% of endoscopic biopsies from patients with DU (Offerhaus et al. 1990, Collins et al. 1990). Many have concluded that H. pylori are a causative factor for DU (Rathbone et al.1986, Megraud and Lamouliatte 1992) and most are convinced that erradication of H.pylori extends the period of remission (McKinlay 1990, Hui et al. 1991). Irrespective of whether H. pylori cause DU or influence the duration of remission, they are undoubtedly an important factor in healing and/or remission prognoses. Although the morphological index was devised before the importance of H. pylori was known, the morphological key was created to quantify the degree of gastric metaplasia and degenerative non-metaplasia in mucosal biopsies. As H. pylori can only survive in the duodenum in association with the mucus secreted by gastric MSC (Wyatt and Rathbone 1989), the index not only describes mucosal morphology, but quantifies the host environment. Such data may prove useful as a means of assessing the potential for H. pylori survival after various curative regimens.

8.13. Summary

To summarise, duodenal ulcers are not all the same. There appears to be at least two morphological types, metaplastic and non-metaplastic, each with their own characteristics. Lesions surrounded by well differentiated MSC are generally large while non-metaplastic DU are usually smaller, but commonly both types often do not heal within the period of chemotherapy. The majority of specimens exhibited metaplastic characteristics between these two extremes. These DU ranged in size from large to small, were surrounded by moderately metaplastic tissue and generally

healed irrespective of the type of therapy. When healed, the scars from metaplastic DU retained many metaplastic characteristics and patients often remained in remission for more than six months whereas scars from non-metaplastic lesions often relapsed within this period. Metaplasia was generally a favourable prognostic criterion whereas non-metaplastic cytology was often associated with poor-healing or early relapse.

The numeric data showed that cimetidine, irrespective of dosage or period of therapy, did or did not heal morphologically identifiable DU in a predictable manner. This phenomenon was employed to create discriminant formulae which, when applied to morphologic data from another cimetidine study, predicted outcome correctly in 89% of cases. The results showed that DU were not prognostically equal. A preponderance of good or bad "healers", especially in small therapy groups could influence the apparent efficacy of drugs in comparative trials. The discriminant formulae were employed to predict probable outcome of therapy if patients with DU were treated with cimetidine.

Comparative analyses showed that DU surrounded by certain types of mucosal morphology were not healed with some drugs but healed with others, and vice-versa. These data suggested that the mechanism of action of some drugs may be more effective in healing identifiable DU than others. This information may help clinicians in the treatment and management of the disease. The morphological quality of healing correlated with the duration of remission after cimetidine treatment but was not consistant after other regimens. This suggested that drugs may exert specific and long-term influences over either the factors which cause ulceration or post-therapy mucosal morphology. Support for

the latter premise was afforded by the data that showed that drugs could alter the cytological composition of scar mucosa.

Glycocalyceal bodies were found near the surface of cells with defective glycocalyx and may be a factor in duodenal ulcerogenesis. H. pylori, present in approximately 40% of juxta-DU specimens sometimes disrupted the lateral cell junctions of MSC. The intercellular perfusion of luminal fluids together with a concentration of bacterial waste products in the intercellular spaces may cause cell death and create potential sites for ulceration.

This study shows that the morphological analysis of biopsy specimens from near DU may be useful in the clinical management of duodenal ulcer disease. Further, such analyses may help to unravel the mechanisms involved in duodenal ulcerogenesis and healing.

CHAPTER 9

CONCLUSIONS

This study was designed to examine two premises: that the morphological severity of duodenal ulcers (DU) may influence the incidence of healing and that the morphological quality of healing after curative therapy may influence the duration of remission.

In order to determine whether the morphological appearance of the ulcerative mucosa influenced healing and/or remission prognoses, it was necessary to correlate juxta-DU and scar morphology with incidence of healing and duration of remission. Mucosal gastric metaplasia was correlated with DU healing and extended remission while degenerative non-metaplasia was associated with non-healing and early relapse. While showing that the appearance of the mucosa surrounding DU and scars influenced prognoses, the methodology was too vague to enable correlative analysis to determine the probable prognosis of individual DU. To remedy this, an index was devised that numerically described the morphological appearance of each DU and scar.

As with other indices, the morphological index employed in this study was formulated about the positive (gastric metaplasia) and negative (degenerative non-metaplasia) criteria of the phenomena (healing and remission prognoses) to be studied. Scores were awarded to each of four morphological parameters (S1-S4). High scores were given to features associated with gastric metaplasia and low scores to degenerative non-metaplasia. The sum of scores, expressed as a percentage (MI), described the extent of gastric metaplasia and degree of metaplastic

differentiation or the severity of degenerative non-metaplasia in each specimen. The numeric MI enabled correlations to be made between the morphology extant in individual and small groups of DU and healing and remission data.

The quantitative results confirmed that the morphological appearance of the juxta-DU mucosa indicates the probability of healing. While indicating prognosis, morphology, per-se, does not only describe the severity of a lesion but probably also indicates DU aetiology, the manner in which the mucosa has responded to the causative factors, stage of ulcerogenesis and/or the phase of natural healing. In summary, the data suggests that there are at least two prognostic types of lesion: the one surrounded by gastric metaplastic tissue and likely to heal, the other by degenerative non- or less-metaplastic tissue and likely to persist. Large lesions surrounded by well differentiated metaplastic tissue may not heal within the prescribed period of therapy.

Drugs employed to heal DU were cimetidine, sucralfate, misoprostol and pirenzipine. The drug regimens covered both primary mechanisms of action: acid inhibition (cimetidine and pirenzipine) and cytoprotection (sucralfate). Misoprostol both reduces acid secretion and improves mucosal protection. No significant differences in the range of scar morphology were found after each type of therapy.

Irrespective of the type of therapy, the duodenal mucosa does not return to normal after treatment but retains a moderate level of metaplasia. This is especially the case where DU were metaplastic. Persistent moderate metaplasia is a pre-requisite for extended remission. In the case of

cimetidine treatment, the morphological quality of healing influences the extent of remission for there was a significant difference between MI scores of scar mucosa from patients who experienced extended remission and those who relapsed within six months of treatment.

Cimetidine was prescribed at two dosages. Correlations with scar morphology, healing and remission outcome were similar in both cases. The consistant manner in which cimetidine healed morphologically distinct DU suggested that prediction of healing was possible. Discriminant analysis was employed to predict healing with cimetidine in a separate study. This method finds appropriate weighting coefficients for the size of DU (SL) and morphological parameters of the index (S1-S4) to best separate data from healed and non-healed patients. The coefficients when applied to the numeric data derived from patients to be treated with cimetidine were found to predict outcome in 92% of cases.

The morphological index thus enables prognostic classes of DU to be identified and prediction of outcome after treatment with cimetidine to be made. Prediction of remission, however, has yet to be confirmed. Where small numbers of patients are included in comparative drug efficacy trials, discriminant formulae obtained from the cimetidine trials can be employed as standards to accommodate for inequalities in the number of easy and difficult "healers" in therapy groups.

The small numbers of patients in therapy groups made it impossible to confirm other interesting observations, however they are worthy of comment. The four drugs used in this study do not necessarily heal the same type of lesion.

This was especially the case with sucralfate and cimetidine. Many lesions that healed with sucralfate were not predicted to heal with cimetidine. Also, after treatment with sucralfate or cimetidine, there appeared to be a difference in the duration of remission in patients that healed with morphologically similar scars. This suggested that drugs may have the capacity to exert a long-term influence over either the mucosa or the factors that caused ulceration. Certainly the results of this study show that sucralfate and perhaps cimetidine have the capacity to alter mucosal morphology during healing, but how they may modify the extent of remission is unknown.

Only one other group has employed a morphological index to quantify mucosal morphology in duodenal ulceration (Tovey et al. 1989a,b). Their attempts to correlate scar morphology with remission outcome was unsuccessful. A possible reason for their failure was inappropriate scoring of morphological parameters. While Toveys' morphological key was based on the assumption that well differentiated gastric metaplasia and degenerative non-metaplasia were prognostically bad (both awarded high scores), the index employed in this study maximally separated these criteria (high and low scores).

As with most studies, the results pose more questions than they provide answers. Does juxta-DU morphology indicate lesion aetiology? Are some drugs more proficient in healing identifiable DU than other? Is it possible to identify intransigent DU where surgery may be more appropriate than chemotherapy? Is it possible to predict healing and duration of remission with drugs other than cimetidine? Do drugs influence remission prognoses? These and other questions can be addressed now that the foundations for quantitative morphological evaluation of ulcerative mucosa

have been laid. The present study has clearly demonstrated the valuable role that morphological analysis based on both light and electron microscopical studies can play in the planning, management and evaluation of duodenal ulcer therapy in the future.

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APPENDIX A

Examples of the Gastrointestinal Unit endoscopy report forms include those for Mr G ... (20175) and for Mr Neville ... (15831). In the former case the position of the DU and size of lesion are recorded. In the latter instance the position from where biopsies were taken from a normal volunteer are shown. The consent form for Mr Neville ... is included.

GASTROINTESTINAL UNIT

UNIVERSITY OF NATAL / KING EDWARD VIII HOSPITAL

GASTROINTESTINAL ENDOSCOPY REPORT : 902

NUMBER : UPPER D 175
COLON

DATE 21/04/21

DEPARTMENT : MED/SURG. WARD : HOSP. No : I/P
NAME : G SURGEON : O/P
A : 32 R : I S : M/C PHYSICIAN : ADDRESS :

CLINICAL : PREVIOUS SURGERY : FOLLOW-UP : FINAL DIAGNOSIS :
Main Sympt. Date :
Length : Type : Date :

BARIUM MEAL : Date : Radiologist : X-RAY No

RESULT :

X-Ray Removed :

SITE :	OESOPH	STOM	DUOD	SIGM	DESC	ASC	ILEUM
Reached							
Major Lesion							
Biopsies							
Photographs							

Circle Reason not entering Site :
A : Blocking Lesion B : Food/Faeces C : Unruly D : Dangerous Lesion E : Failure

INSTRUMENT USED :
GF Type B
GIF - D
JF - B
CF - LB
Other (1) ✓

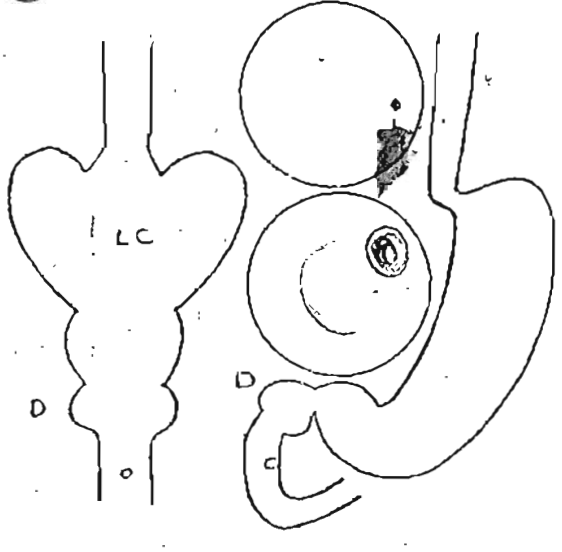
MEDICATION :

	YES	NO		YES	NO
Anaesthetic Throat Spray	✓		Valium IV. 10/20 mg		✓
Atropine IM: 0,6 mg		✓	Probanthine 30 mg IV/IM		✓

OTHER/ Gravol 2 x 1/cc

INSERTION : EASY / DIFFICULT

REPORT :



Oesophagus normal. O.G.J 40cm
Stomach normal
duodenal cap deformed inflamed and 1½ cm ulcer
crater duodenal ulcer with distortion of apex.

Δ Chronic duodenal ulcer

POST-ENDOSCOPY INSTRUCTIONS : (Except for Colonoscopy)

1. Not to eat or drink until gag reflex returns.
2. Any neck swelling report to Doctor and contact Gastroenterology Staff

UNIVERSITY OF NATAL / KING EDWARD VIII HOSPITAL

GASTROINTESTINAL ENDOSCOPY REPORT :

NUMBER : UPPER 15837
COLON

DATE 26. 11. 79

DEPARTMENT : MED./SURG.
NAME : Neil
A: W S: M.
WARD :
SURGEON :
PHYSICIAN :
HOSP. No : I/P Staff
O/P
ADDRESS :

CLINICAL (Dysphagia)
PREVIOUS SURGERY :
Date :
Type :
FOLLOW-UP : FINAL DIAGNOSIS :
Date :

BARIUM MEAL : Date : Radiologist : X-RAY No
Report :

X-Ray Removed :

SITE :	OESOPH	STOM	DUOD	SIGM	DESC	ASC	ILEUM
Reached							
Major Lesion							
Biopsies							
Photographs							

Ref. Reason not entering Site :
A : Blocking Lesion B : Food/Faeces C : Unruly D : Dangerous Lesion E : Failure

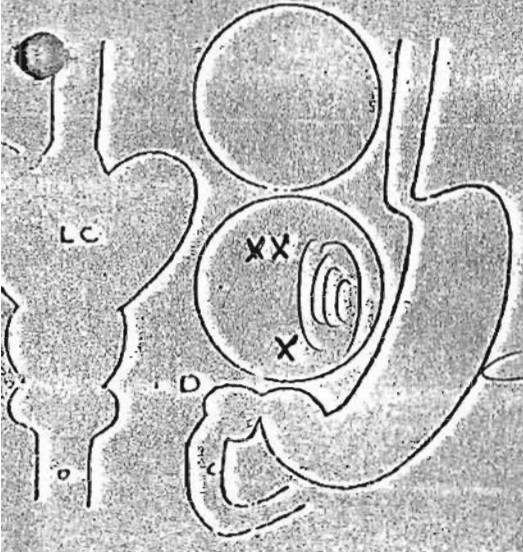
INSTRUMENT USED :
GF Type B
GIF - D
JF - B
CF - LB
Other GIF-K

MEDICATION :

	YES	NO		YES	NO	OTHER
Anaesthetic Throat Spray	☑	☐	Valium IV. 10/20 mg	☐	☑	Scopex 1cc I.V.
Atropine 1/1: 0,6 mg	☐	☑	Probanthine 30 mg IV/IM	☐	☑	

INSERTION : EASY / DIFFICULT

REPORT :



Oesophagus :
G.O. junction 43 cm.
Stomach :
"état mamelonné" antrum.
Central ulcer.
Wide duodenal cap :
3 Biopsies taken.

ENDOSCOPY INSTRUCTIONS : (Except for Colonoscopy)
Treat as drug until proven otherwise

TO WHOM IT MAY CONCERN

I, the undersigned, do hereby give consent for an upper endoscopy with duodenal biopsy, to be performed on myself for research purposes.
In the event of complications occurring, which I understand are unlikely, I will not hold the Endoscopist liable.

SIGNED: .....

DATE: 26/11/1979.....

APPENDIX B

METHOD 1

Schedule for the preparation of specimens for histology

After appropriate fixation (see page 47), the biopsies were washed in 0.2M cacodylate buffer prior to being dehydrated through increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. The processing time schedule was standardised for all specimens by using an Elliot automatic tissue processor with a the following fixed programme.

1.	Wash in cacodylate buffer (pH.7.4) at RTP	30 minutes
2.	70% ethanol	1.5 hours
3.	90% ethanol	1.5 hours
4.	Absolute ethanol I	30 minutes
5.	Absolute ethanol II	45 minutes
6.	Absolute ethanol III	1 hour
7.	Absolute ethanol IV	1 hour
8.	Xylene I	45 minutes
9.	Xylene II	1 hour
10.	Wax bath I (56C-58C melting point)	1.5 hours
11.	Wax bath II " "	1.5 hours
	TOTAL TIME	**11.5 hours**

The specimens were embedded in labelled polythene moulds.

METHOD 2

Procedure for haematoxylin and eosin stain

The wax was removed and the sections brought to water. They were then stained with haematoxylin, differentiated, blued and stained with eosin. The sections were then dehydrated, cleared and mounted in DPX. Staining was as per following schedule.

1.	Xylene I	1 minute
2.	Xylene II	2 minutes
3.	Absolute ethanol I	1 minute
4.	Absolute ethanol II	2 minutes
5.	70% ethanol	2 minutes
6.	Water	5 minutes
7.	Stain in Ehrlich's haematoxylin (Drury and Wallington 1980)	20 minutes
8.	Wash in running tap water	3 minutes
9.	Differentiate in 1% HCl in 70% ethanol (acid alcohol) until nuclei are selectively stained).	
10.	Stop differentiation and "blue" in running tap water (if alkaline).	2 minutes
11.	Stain in 1% aqueous eosin	2 minutes
12.	Wash off surplus stain with water	30 seconds
13.	Check stain with microscope	
14.	70% ethanol	30 seconds
15.	Absolute ethanol III	30 seconds
16.	Absolute ethanol IV	1 minute
17.	Xylene III	1 minute
18.	Xylene IV	2 minutes
19.	Mount in DPX (Dystrene Plasticizer - tricresyl phosphate - Xylene).	

Results:

Nuclei - blue/purple.

Cytoplasm, connective tissue - pink.

METHOD 3

Procedure for periodic acid Schiff stain (PAS)

1.	Xylene I	1 minute
2.	Xylene II	2 minutes
3.	Absolute ethanol I	1 minute
4.	Absolute ethanol II	2 minutes
5.	70% ethanol	2 minutes
6.	Water	5 minutes
7.	Oxidise in 1% aqueous periodic acid	5 minutes
8.	Wash in running tap water	5 minutes
9.	Place in Schiff's reagent (Drury and Wallington 1980 p239).	15 minutes
10.	Wash in running tap water	10 minutes
11.	Stain nuclei with iron haematoxylin	3 minutes
12.	Wash in running tap water	30 seconds
13.	Check stain with microscope	
14.	70% ethanol	30 seconds
15.	Absolute ethanol III	30 seconds
16.	Absolute ethanol IV	1 minute
17.	Xylene III	1 minute
18.	Xylene IV	2 minutes
19.	Mount in DPX	

Results:

PAS positive substances (epithelial mucins) - red or magenta.
Nuclei - blue.

METHOD 4

Procedure for Alcian blue/PAS stain

1.	Xylene I	1 minute
2.	Xylene II	2 minutes
3.	Absolute ethanol I	1 minute
4.	Absolute ethanol II	2 minutes
5.	70% ethanol	2 minutes
6.	Water	5 minutes
7.	Stain in Alcian blue solution (pH2.5) (Drury and Wallington 1980 p.246)	20 minutes
8.	Wash in running tap water	5 minutes
9.	Wash in distilled water	2 minutes
10.	Place in Schiff's reagent	15 minutes
11.	Wash in running tap water	10 minutes
12.	Check stain with microscope	
13.	70% ethanol	30 seconds
14.	Absolute ethanol III	30 seconds
15.	Absolute ethanol IV	1 minute
16.	Xylene III	1 minute
17.	Xylene IV	2 minutes
18.	Mount in DPX	

Results:

Acid mucosubstances - blue/purple
Neutral mucosubstances - red

METHOD 5

Procedure for Southgate's mucicarmine stain

1.	Xylene I	1 minute
2.	Xylene II	2 minutes
3.	Absolute ethanol I	1 minute
4.	Absolute ethanol II	2 minutes
5.	70% ethanol	2 minutes
6.	Water	5 minutes
7.	Stain with Mayer's haematoxylin (Drury and Wallington 1980 p.139)	4 minutes
8.	Wash in running tap water	3 minutes
9.	Differentiate in 1% HCl in 70% ethanol (acid alcohol) until nuclei are selectively stained).	
10.	Stop differentiation and "blue" in running tap water (if alkaline).	2 minutes
11.	Stain with Southgate's solution (Drury and Wallington 1980 p.245)	45 minutes
12.	Rinse in distilled water	30 seconds
13.	Check stain with microscope	
14.	70% ethanol	30 seconds
15.	Absolute ethanol III	30 seconds
16.	Absolute ethanol IV	1 minute
17.	Xylene III	1 minute
18.	Xylene IV	2 minutes
19.	Mount in DPX	

Results:

Mucins - red
Nuclei - blue

METHOD 6

Schedule for the manual preparation of biopsies for transmission electron microscopy

After 10 minutes fixation (see page 47), biopsies were minced into 1 mm cubes. The tissue was re-immersed in fixative for a further one hour prior to be being washed, osmicated, dehydrated, cleared and embedded in Araldite epoxy resin. All specimens were processed at RTP as follows.

1.	Wash in 0.2M cacodylate buffer (pH7.4)	30	minutes
2.	Post-fix/stain with 1% osmium tetroxide in 0.2M cacodylate buffer at pH7.4 (in dark).	1	hour
3.	Wash in 0.2M cacodylate buffer (pH7.4)	30	minutes
4.	70% ethanol	30	minutes
5.	90% ethanol	30	minutes
6.	Absolute ethanol I	30	minutes
7.	Absolute ethanol II	30	minutes
8.	Dry absolute ethanol III (over anhydrous copper sulphate)	30	minutes
9.	Propylene oxide	30	minutes
10.	Propylene oxide/araldite (50/50)	30	minutes
11.	Araldite I (50C)	2	hours
12.	Araldite II (50C)	2	hours
13.	Embed specimens in fresh araldite in polythene moulds (60C)	24 to 48	hours.

There were a minimum of 4 blocks made from each specimen.

APPENDIX C

The histological, light and electron microscopic appearance of each specimen were recorded in rough while visualising the tissue. Observations on pathomorphology, mucosynthesis and other phenomena that may have had relevance to the study and/or may have lead to a better understanding of ulcerogenesis or DU healing were recorded while viewing the specimens. Light and electron micrographs were taken of each sample and measurements made of important features. After evaluating the micrographs, this data together with the observations recorded earlier were collated and reported on specially designed forms. The following are examples of the final reports from 4 patients whose morphological data were included in this study.

Note in "ANALYSIS OF CASE" that scoring was made for MI:1, MI:2 and MI:3. MI:1 describes the overall appearance of the mucosa with regards presence and degree of metaplasia/degenerative non-metaplasia and is the index used throughout this study. MI:2 (S1 and S3) and MI:3 (S1 and S4) were experimental indexes that described the presence and degree of either metaplasia or degenerative non-metaplasia in each specimen. The latter indexes did not improve correlations between morphology and prognosis and were discarded.

Different nomenclature was used on the forms to those used in this thesis:

MF No (1,2,3,4) = S No. (S1: S2; S3; S4).
G.A.M. = S1
G.C.M. = S2
M.C. = S3
N.M.C. = S4
POINTS = Score for each S value. Note that 4 3 = 3.5 points
MAX = Maximum score (MI:1 14 points)
SCORE = Actual score.

DUODENAL ULCER THERARY

DATE: 20/3/84

G.I. NO: 29248

E.M. NO: 1492

PATIENT'S NAME: G Segadu LENGTH OF TREAT: Pre-treat

ENDOSCOPY REPORT: PYRENZIPINE. Two DU; 2mm X 2mm + 12mm DU. (2)

HISTOLOGY: PAS, Alcian-Blue/PAS, H&E

Stains Employed -

REPORT:

The full thickness of the mucosa including some Brunner's glands was present. The specimen was obliquely cut but contained quite long-finger shaped villi. The mucosa appeared essentially normal and contained absorptive and PAS positive goblet cells. There were occasional cells that contained some alcian blue+ granules. These were not typical MSC.

C. Slides No. 2 C. Print No. 4 B & W Print No. 4

1u TOLUIDINE BLUE RESIN EMBEDDED SECTION

No of Blocks cut:- 3

REPORT: There was a quite well formed villus and two obliquely cut villi in the field of view. The mucosa was populated with apparently normal absorptive and goblet cells. No obvious metaplastic cells were seen. The majority of absorptive cells have a well defined, thick brush border. There are quite large intercellular spaces in some areas. Many of the cells contain Tol. Blue positive inclusions, and what appear to be vacuoles in the approximate region of the Golgi apparatus. In some well demarcated areas, the brush border becomes irregular, thin and/or absent. No obvious cytoplasmic irregularities were observed. Goblet cells are normal in number. and many are in the process of secretion. Mucus granules often contained Tol. Blue positive inclusions. There were many 'pale' cells in the mucosa, also quite a lot of lymphocytes were present.

5

B & W PRINTS NO: .....

ANALYSIS OF ELECTRON MICROGRAPHS

Where absorptive cells were normal, microvilli were approx 1,2 um long and there were 8:9MV/um. The glycocalyx is thick (224nm) and continuous, Goblet cells in these regions are ±10um in diameter and contain normal mucous granules which, near to the surface were 1,6 x 1,5um in size. In some regions, absorptive cells had dilated RER, mitochondria were swollen and electron lucent and the nuclei were more crenated and had a swollen nucleolemma. Microvilli were 1,1um long and there were 6:7MV/um. The glycocalyx was discontinuous, fragmented or absent. In some regions absorptive cells had a more metaplastic appearance. Such cells contained either pale or dark secretory granules near to their luminal plasmalemma. The general appearance of the cells suggested that they had activated their protein synthetic machinery. Microvilli were approx 0,6um long and there were 5MV/um. Occasional glycocalyceal bodies were present in these regions. There was a rapid and immediate transition between these and the more normal absorptive cells. Numerous abnormal goblet cells were observed in some regions.

ANALYSIS OF CASE

		POINTS (MI1/2)	MI3
G.A.M: MF No	1	4 3 2 1 0	4 3 2 1 0
G.C.M: "	2	4 3 2 1 0	- - - - -
M.C: "	3	- 3 2 1 0	- - - - -
N.M.C: "	4	- 3 2 1 0	3 2 1 0

M.F. No	MI 1 1+2+3+4	MI 2 1+3	MI 3 1+4
MAX	14	7	7
SCORE	5	1,5	4,5
PERCENTAGE	36	21	64

COMMENTS

Two moderately severe DU presented for therapy. After treatment 2 DU 5mm X 8mm + 3mm X 3mm remained. Endo. score G, our score 1.

ELECTRON MICROSCOPY

BLOCKS CUT: 2

GRIDS MADE: 4

STAIN: PbC + UA

REPORT: The greater part of the surface mucosa appears normal. Absorptive cells are morphologically normal and have long densely packed microvilli from which projects a thick, continuous glycocalyx. Goblet cells were present, generally contained normal mucus droplets and were often seen in the process of secretion. There are areas, however, where absorptive cells exhibit abnormal features. The rough endoplasmic reticulum is swollen and envelopes pale mitochondria in such cells. The microvilli are often short and sparse and the glycocalyx is discontinuous., often being associated with individual microvilli.There are often small groups of glycocalyceal bodies in the inter-microvillous spaces.In some instances, secretory granules were seen in the apical regions of cells. These osmiophylic entities were, in some cases, seen to be exocytosed into the luminal space. These may represent an early or late phase in metaplastic differentiation. Goblet cells in these regions often contained mucus droplets, within which were osmiophylic inclusions. There was also larger intercellular spaces in these regions. Lymphocytes, polymorphs were commonly observed in the sub-mucosa and in the epithelium in places.

NEG NOS: 12900 - 12910

NO. OF PRINTS: 11

DUODENAL ULCER THERAPY

DATE: 3/4/84

G.I. NO: 29369

E.M. NO: 1500

PATIENT'S NAME: D Sunder ..... LENGTH OF TREAT: Pre-treat .....

ENDOSCOPY REPORT: CIMETIDINE 1cm X 1cm recurrent DU. (2)

HISTOLOGY:

Stains Employed - PAS, Alcian-Blue/PAS, H&E

REPORT:

The mucosa appeared obliquely cut and only a few Brunner's glands were seen. Five villi were present but appeared shorter than in control specimens. Metaplastic patches containing MSC with various quantities of PAS+ mucus were situated near the tips of 2 villi. There was a small patch of well differentiated MSC near the mid region of a third villus. Apparently normal absorptive and goblet cells populated the greater part of each villus.

C. Slides No. ..1.... C. Print No. ..3.... B & W Print No. ..6....

1µ TOLUIDINE BLUE RESIN EMBEDDED SECTION

No of Blocks cut:- 2

REPORT: A single obliquely cut villous was observed. A large part of the mucosal surface is populated with actively secreting metaplastic gastric surface mucous cells and GMC in various stages of differentiation. Occasional goblet cells were observed in this region. Other areas were populated with darkly staining absorptive cells. These cells had a thick, continuous brush border. There were large intercellular spaces between these cells. No goblet cells were present in this region. Approximately 30% of this specimen was populated with what appeared to be morphologically normal goblet and absorptive cells. Occasional argentaffin cells were present in some parts of the epithelium.

B & W PRINTS NO: .....

ELECTRON MICROSCOPY

BLOCKS CUT: 2

GRIDS MADE: 4

STAIN: PbC + UA

REPORT: The epithelial cells could be divided into 2 distinct morphological types.
Type 1 - Intermediate metaplastic cells. These cells had numerous short microvilli with no discernible glycocalyx. Such cells had numerous small osmiophylic inclusions in the luminal plasmalemmal region. Some of these cells had dilated rough and smoothe endoplasmic reticular and Golgi systems. Goblet cells were present, but often contained mucin within which were osmiophylic inclusions. In some 'intermediate' cells were ?secretory/ lysosomal granules. Some of the absorptive cells had a typical 'ischaemic' appearance with extensively vacuolated reticular and mitochondria. Occasional glycocalyceal bodies were present, especially in 'ischaemic' regions. Quite a few 'spiral bacilli' were seen in the duodenal lumen in close proximity to the epithelial cell plasmalemma of intermediate MSC
Type 2 - Metaplastic gastric surface mucous cells. These cells were not always typical of GMC. They had reduced quantities of mucin, but were observed to be capable of active secretion. They often contained large ?'crinophagic' vacuoles and other vacuoles which contained vesicles and cytoplasmic moities. Occasional spiral bacilli were observed close to the luminal surface of these cells.

NEG NOS: 12964 - 12973

NO. OF PRINTS: 10

ANALYSIS OF ELECTRON MICROGRAPHS

This is a peculiar specimen. The cells give the impression that they are in 3 intermediate phases of metaplastic differentiation. In phase 1, cells contained quite large quantities of moderate sized secretory granules that appear to be in tha process of cytoplasmic reabsorption. These cells contained large ? autophagosomes or crinophagic vacuoles. Some of the cells were observed in the process of secreting mucosubstance into the lumen. The microvilli of such cells were approx. 0,3um long and there were 3:4MV/um. No glycocalyx was seen. Type 2 cells appeared to be in a slightly later phase of dedifferentiation. These cells contained numerous small osmiophylic or electron lucent vesicles near to their terminal web. It appeared that the Golgi apparatus was still synthesising small numbers of secretory granules which, as they moved to the surface, were gradually degraded to become the luminal vesicles. These cells had microvilli approx. 0,72um long and there were 5:6MV/um. These cells had a fragmentary glycocalyx within which were occasional glycocalyceal bodies. In the remaining phase (phase 3), cell morphology was generally similar to that of phase 2. Primary differences were the substantial dilaton in rough endoplasmic reticulum , reductions in the numbers of electron dense and

ANALYSIS OF CASE

		POINTS (MI1/2)	MI3
G.A.M: MF No	1	4 3 2 1 0	4 3 2 1 0
G.C.M: "	2	4 3 2 1 0	- - - - -
M.C: "	3	- 3 2 1 0	- - - - -
N.M.C: "	4	- 3 2 1 0	3 2 1 0

M.F. No	MI 1 1+2+3+4	MI 2 1+3	MI 3 1+4
MAX	14	7	7
SCORE	7	4	3,5
PERCENTAGE	50	57	50

COMMENTS

electron lucent periplasmalemmal vesicles and a substantial increase in the numbers of multivesicular bodies. Microvilli were approx. 0,7um long and 6:7MV/um. The glycocalyx was thin (76nm) but generally continuous. There was a fully developed goblet cell with abnormal mucin granules. This cell was 11um in diameter. Abnormal mucous granules ranged in size from 0,8 - 1,1um.

Quite severe DU prior to therapy. After treatment just a clear scar remained.

DUODENAL ULCER THERAPY

DATE: 20/3/84

G.I. NO: 29250

E.M. NO: 1493

PATIENT'S NAME: N Naidoo LENGTH OF TREAT: Pre-treat

ENDOSCOPY REPORT: P/RENZIPINE . 15mm in diameter DU. (2)

HISTOLOGY:

Stains Employed - PAS, Alcian-Blue/PAS, H&E

REPORT: The mucosa contained no normal villi. The villi were "stubby" and in some areas the surface epithelium lined an "undulating" sub-mucosa. The epithelium was primarily populated with well differentiated MSC interspersed with MSC containing smaller quantities of mucosubstance. No normal absorptive cells were seen and only a few goblet cells were present. The MSC appeared to stretch to the base of Crypts. Very few Brunner's glands were seen - possibly a consequence of the biopsy procedure (shallow biopsy).

C. Slides No. ...0... C. Print No. ...2... B & W Print No. ...4.....

1µ TOLUIDINE BLUE RESIN EMBEDDED SECTION

No of Blocks cut:- 2

REPORT: The mucosa is abnormal. 3 quite well formed, but 'square' villi were in the field of view. Many of the cells lining the mucosa appear to be 'pushed' out into the lumen, giving the surface a most irregular appearance. The majority of these appeared to be of a metaplastic type, but in various stages of differentiation. Many of the cells were darkly staining and had a necrotic appearance, being filled with vacuoles. Others were pale staining, though often contained Tol. Blue positive inclusions. No brush border was seen over any part of the specimen. Goblet cells, although sparse, were present in some regions.

B & W PRINTS NO: .....

ELECTRON MICROSCOPY

BLOCKS CUT: ... ... 3 ... ...

GRIDS MADE: ... ... 4 ... ...

STAIN: ... ... PbC + UA ... ...

REPORT: The mucosa was lined with metaplastic gastric surface mucous secreting cells in various phases of differentiation. There are numerous GMC containing large aggregates of mucus and many are seen 'explosively' secreting muco-substance into the duodenal lumen. There were some cells which contained mucus droplets, within which were osmiophylic inclusions, similar in many respects to those seen in Brunners glands. These droplets, however, appeared to contain rudimentary helical inclusions, similar to those that characterise the mucus in GMC.Intermediate phase metaplastic cells, had reduced quantities of more osmiophylic mucosubstance. Microvilli were quite numerous, but generally short. A thin glycocalyx projected from them. No goblet cells were seen. Occasional spiral bacilli were observed near to the luminal cell surface.

NEG NOS: ... ... 12911 - 12917 ... ...

NO. OF PRINTS: ... ... 7 ... ...

ANALYSIS OF ELECTRON MICROGRAPHS

Metaplastic cells contain a) large quantities of mucus, with extremely dilated smooth and rough endoplasmic reticulum and Golgi apparatus and b) cells with apically situated small aggregates of particularily electron dense mucogranules.

Type a) cells are typical of the surface gastric mucosa. Type b) are more similar to the surface pit cells. These cells do not exhibit the morphological features of excessive protein synthesis. The cytoplasm contains whorls of rough endoplasmic reticulum. The nuclei and nucleolemmae are normal with not nearly as much peripheral chromatin as in 'ischaemic' or more secretory orientated cells. Mitochondria were normal. Microvilli were 0,6um long and there were approx. 4:6MV/um. The glycocalyx was 86nm thick and attached to individual microvilli. There were some metaplastic cells which appeared to be in a phase between cell types a) and b). In addition some cells loosely resembled an admixture between surface and mucus neck cells and Brunners gland cells.

ANALYSIS OF CASE

		POINTS (MI1/2)	MI3
G.A.M: MF No	1	~~4~~ ~~3~~ 2 1 0	4 3 2 ~~1~~ ~~0~~
G.C.M: "	2	~~4~~ 3 2 1 0	- - - - -
M.C: "	3	- ~~3~~ ~~2~~ 1 0	- - - - -
N.M.C: "	4	- ~~3~~ 2 1 0	3 2 1 ~~0~~

M.F. No	MI 1 1+2+3+4	MI 2 1+3	MI 3 1+4
MAX	14	7	7
SCORE	13	6	0,5
PERCENTAGE	93	86	7

COMMENTS

A moderate DU presented for therapy. After treatment a 6mm, healing DU remained. Endo. score G, our score 1

APPENDIX D

KEY FOR MORPHOLOGICAL INDEX

S1: General Appearance of the Mucosa

Score	Morphology
4	An undulating mucosa exclusively populated with actively secreting PAS+/Toluidine Blue+ metaplastic gastric mucus secreting cells (MSC). No finger shaped villi are in evidence. No goblet cells present. Helicobacter pylori are often found in these specimens. **Exclusively populated with cell type a)++++**
3	Atrophic, undulating mucosa usually populated with actively secreting MSC together with MSC in various phases of differentiation. Occasional abnormal goblet cells may be interspersed with the MSC. Occasional groups of non-metaplastic, non-degenerative absorptive cells and normal goblet cells may be present, however, metaplastic cells are in the majority. Helicobacter pylori are sometimes found over secretory patches of well differentiated MSC. **Populated with cell types b)+++; a)++; c)++; dii)+; e)+ and f)+.**
2	An undulating mucosa which by light microscopy appears to be predominantly populated with non-metaplastic cells. Ultrastructurally, however, many of these cells are of a poorly differentiated metaplastic type. They are characterised by numerous intracytoplasmic electron-dense vesicles and quite long, densely packed microvilli surmounted by a discontinuous glycocalyx. These may be interspersed with occasional goblet cells containing abnormal mucodroplets. There may be quite extensive "normal" regions with morphologically normal absorptive cells being interspersed with normal goblet cells. Helicobacter pylori were rarely seen in these specimens. **Populated with cell types b)+++; dii)++ a)+; e)+; f)+.**

S1: General Appearance of the Mucosa

Score	Morphology
1	Finger like villi and a mucosa, which by light microscopy, appears normal. Helicobacter pylori were never found in these specimens. There are two fine-structural patterns of mucosal morphology:

a) Metaplastic - The majority of cells are absorptive, contain occasional osmiophilic inclusions and have tightly packed microvilli of normal length. Projecting from the microvilli is a well developed, continuous glycocalyx. There are occasional poorly differentiated MSC. The non-secretory cells are interspersed with a normal complement of goblet cells.

Populated with cell types dii)+++; e)++; f)++; b)+.

b) Non-metaplastic - The majority of cells are absorptive. Most appear normal, but some may have swollen cytoplasmic organelles, long, densely packed microvilli from which projects a fragmented glycocalyx. There are often glycocalyceal bodies associated with the glycocalyx of these cells. Normal goblet cells may be increased in number. Occasional poorly differentiated MSC may be present, although their occurrence is rare.

Populated with cell types e)+++; f)++; di)++; b)±

0	Normal or atrophic villi predominantly populated with abnormal epithelial cells. Such a mucosa may look normal by light microscopy, however, by electron microscopy, the majority of absorptive cells are electron dense and have swollen organelles and vacuoles. In the most abnormal of cases, the microvilli may have been eroded from the cell surface leaving only sub-plasmalemmal rootlets. In less severe cases, most microvilli are long and densely packed over the cell surface but have no glycocalyx. Goblet cell numbers are generally increased. No MSC in any stage of differention are present in these specimens. Helicobacter pylori were never found in these specimens.

Populated with cell types di)+++; f)++; e)+.

S2: Goblet Cell Morphology

Score	Morphology
4	No goblet cells in a mucosa exclusively populated with well differentiated MSC (as in **S1:4** above).
3	Substantially reduced number of goblet cells, most of which contain abnormal mucodroplets within which are osmiophilic inclusions. These cells are present in variable numbers in metaplastic mucosa (as in **S1:3** above).
2	Goblet cell numbers may be normal in a moderately metaplastic mucosa. The number of goblet cells with normal mucodroplets increases as the overall degree of mucosal metaplasia decreases (as in **S1:3 & S1:2** above).
1	A normal complement of goblet cells containing normal mucodroplets (as in **S1:1a** above).
0	An increased complement of morphologically normal goblet cells (as in **S1:1b & S1:0** above).

S3: The General Ultrastructural Appearance of the Majority of Metaplastic Cells in the Mucosa.

Score	Morphology
3	Fully differentiated, actively secreting MSC (as in **S1:4** above). Helicobacter pylori were often associated with these cells.
2	Well differentiated MSC. Such cells contain quite large aggregates of mucodroplets in the cytosol. Droplet numbers are less than in **S3:3** above and mucosecretion is not seen. There may be crinophagic vacuoles and occasional osmiophilic vesicles in some cells. Microvilli were short and sparse and the glycocalyx was thin and sometimes discontinuous. Helicobacter pylori were sometimes seen in association with the more well differentiated MSC. These cells predominated in **S1:3** above.

Microvilli increase in length and number as the quantity of cytoplasmic mucosubstance decreases. The thickness of the glycocalyx increases with increased microvillous length. Score 2.5 points for very well differentiated and 1.5 points for moderately well differentiated MSC. Depending on the degree of differentiation, these cells will predominate from **S1:3 to S1:2** above.

1	Poorly differentiated MSC. Such cells contain small secretory droplets and numerous osmiophilic vesicles in the sub-plasmalemmal cytosol. Microvilli have increased to near normal length and numbers and are surmounted by a thin or normal thickness glycocalyx. The glycocalyx is generally continuous, but may fragment in places. These cells predominate in **S1:1a** above.
0	Non-metaplastic cells. Only score 0 when no MSC occur in the specimen. These cells predominate in **S1:1b** and **S1:0** above.

S4: General Ultrastructural Appearance of the Majority of Non-Metaplastic Cells in the Mucosa.

Score	Morphology
0	Degenerative absorptive cells have a swollen nucleolemma, mitochondria, rough and smooth endoplasmic reticulum and generally have a particularly osmiophilic cytoplasm. Mitochondria are usually pale and rounded and enveloped by whorls of rough endoplasmic reticulum. Microvilli may have been eroded from the cell surface. In other instances, microvilli may be of varying lengths and densely packed over the surface of the cell. Some microvilli may be vesiculating into large membrane bound, cytoplasm filled spheres. In no instance does a glycocalyx project from the microvillous surface. These cells usually predominate in **S1:0** above.
1	The cytoplasm is less osmiophilic than in **S4:0** above. The organelles are less swollen with mitochondria often appearing normal. The microvilli are usually long and densely packed over the cell surface. In some instances, occasional microvilli may be degenerating into membrane bound, cytoplasmic spheres or swollen to form "blebs" that can project up to 5um into the lumen. The glycocalyx may be discontinuous, thin or absent. There are often glycocalyceal bodies associated with the abnormal glycocalyx. These cells predominate in **S1:1b** above.
2	Absorptive cells whose morphology resembles that described as normal (Long, densely packed microvilli from which projects a thick, continuous glycocalyx. These cells may be found in **S1:3; S1:2; S1:1a & 1b and S1:0** above.
3	Mucosa exclusively populated with well differentiated MSC. Only score 3 when no non-metaplastic cells occur in the specimen.

Notes: It is rare for specimen morphology to conform exactly to the descriptions in the key. Where the degree of metaplastic or degenerative non-metaplastic transformations vary substantially between and within villi in a single specimen, it is necessary for the investigator to use discretion when applying the key to morphology. 0,5 points ratings are useful to describe more or less "severe" transformations.

Exclusive well differentiated metaplasia scores 14 points (100%), severe degenerative non-metaplasia scores 0 points (0%).