MUCIN EXPRESSION IN NORMAL AND DISEASED STATES OF THE STOMACH - A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY.

BY

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To my family, Izak, Diana and Sean

for their support, encouragement and patience.

DECLARATION

I, Kathryn Taylor, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, or is being, or is to be submitted for a degree in this or any other University.

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ABBREVIATIONS

N	Normal stomachs
FS	Foetal stomachs
G	Gastritis
D	Low grade dysplasia
IM	Intestinal metaplasia
IAC	Early intestinal type adenocarcinoma
AIAC	Advanced intestinal type adenocarcinoma
DAC	Diffuse adenocarcinoma
PAS	Periodic Acid Schiff
APES	3-aminopropyltriethoxysilane
PBS	Phosphate buffered saline
HRP	Horse-Radish Peroxidase
LIMA	Large intestinal mucin antigen
SIMA	Small intestinal mucin antigen

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ABSTRACT

Nine human mucin genes have been described, which express glycoproteins *MUC1,2,3,4,5AC,5B,6,7*,and *8* in various tissues. It has been shown that different mucins are expressed in various gastric disease states as compared to the normal.

In this study histochemical and immunohistochemical methods were used to determine the type of mucin and the pattern of staining in 54 patients with a variety of gastric conditions [i.e. normal controls, foetal stomachs, chronic active gastritis, low grade dysplasia, intestinal metaplasia (associated with gastritis, benign ulcers, dysplasia and cancer), early and advanced intestinal type adenocarcinoma, and diffuse adenocarcinoma]. *MUC1-7* antibodies were used in the study, this being the first study to all assess seven *MUC* antibodies in the various conditions. It is also the first study to assess the pattern of mucin staining in foetal stomachs.

Normal controls were immunoreactive for *MUC4, 5* and *6* , and gastritis specimens showed similar results, although the latter showed more *MUC1* immunoreactivity. Whereas early foetal stomach showed no *MUC* immunoreactivity, *MUC4, 5* and *6* were present from the early second trimester onwards. There was no significant difference between dysplasia and intestinal metaplasia, both categories showing expression of *MUC2* and *3* predominantly.

Early intestinal type adenocarcinomas did not show any mucins in the majority of cases. Advanced intestinal type adenocarcinomas showed immunoreactivity predominantly for *MUC1, 5* and *6*, as well as *MUC2* in some cases. Diffuse adenocarcinomas showed strong positive *MUC2* and *MUC6* staining, and in some cases *MUC5* and *7*.

In conclusion this study has shown different patterns of mucin immunoreactivity in various gastric disease states. Specimens with dysplasia, intestinal metaplasia, late intestinal type

adenocarcinoma and diffuse gastric cancer were characterized by increased diversity of mucin gene expression, whereas early intestinal cancer showed loss of mucin immunoreactivity.

Key words: Gastric mucin, immunohistochemistry, dysplasia, intestinal metaplasia, gastric carcinoma, *Helicobacter pylori*.

GASTRIC MUCINS

Structure and function of mucins

Mucins are a heterogeneous group of highly glycosylated glycoproteins, which form the major component of mucus. They are composed of a polypeptide backbone (apomucin), which is extensively glycosylated by O-linked oligosaccharide chains. The O-linked chains are initiated by N-acetylgalactosamine, which links with threonine and serine residues in the protein core [1-6]. The oligosaccharides are arranged in a 'bottle-brush" fashion around the protein core, which allows the mucin to bind large amounts of water. The binding of water results in a mucous gel that expands rapidly after excretion into the gastric lumen [6].

Mucins are synthesized by many different secretory epithelial cells as membrane-bound or secreted products [7,8] They are a major component of mucous viscous gels and have cytoprotective properties. They play a role in mechanical protection, the maintenance of viscosity of secretions and in cellular recognition [1]. Mucus also acts as a free radical scavenger, partly because of its ability to bind lipids [6]. Gastric mucins, in particular, are needed to protect the stomach from damage by low pH and proteolytic enzymes [1, 8]. Bicarbonate ions, secreted by the gastric mucosa, are trapped within the mucous gel lining the stomach. It has been shown that pH is an important determinant of mucus function, and that movement of HCl through mucus is pH dependent .

Mucin genes

Mucins are derived from a heterogeneous family of genes [8]. Nine human mucin genes with different mucin protein cores have been identified to date (*MUC1, 2, 3, 4, 5AC, 5B, 6, 7*, and *8*)

[9-11]. *MUC 1-7* have been cloned and sequenced with the use of a variety of human organ specific cDNA libraries [12-17]. Nucleotide sequencing indicates that the mucin core proteins have tandemly repeated peptides with a high percentage of threonine and/or serine and proline, and a high content of O-glycosylation sites [1,12,13]. Each of the identified mucins has distinct characteristics, being expressed in an organ and cell specific manner [1,3,9]. These mucin genes have been labelled *MUC 1 - 7* (See table 1), and more recently *MUC8* has been identified, but no details of its sequencing are available.

The mucin genes are localized in 5 different chromosomes, but there is a clustering of some of the mucin genes at 11p15 [18,19].

Sites of mucin expression

MUC 1 is the best characterized mucin gene, and is localized to chromosome 1q21-24 [19]. It is a membrane associated mucin, also termed polymorphic epithelial mucin [6,15] which is expressed in many epithelia, including bronchus, breast, salivary gland, pancreas, prostate and uterus. It is sparsely expressed in gastric epithelium [3,5-7,20] and is highly expressed in many breast cancers [21]. The *MUC1* gene product shows a high degree of length polymorphism due to the presence of different numbers of tandem repeats [20]. In the stomach *MUC1* has been observed in the cytoplasm and apical membrane of the foveolar cells and of a few mucous gland cells. In the body of the stomach parietal cells have been shown to stain with *MUC1* [7]. *MUC1* has, in addition, more recently been found to be expressed in normal and neoplastic haemopoietic tissues [22].

MUC 2 is expressed in the duodenum, jejunum, ileum, right colon, and weakly in the tracheobronchial tree and gallbladder [1, 3, 6].

MUC 3 is expressed mainly in the small intestine, in both goblet cells and absorptive cells [9], but also in colon and gallbladder [6]. It has been localised to chromosome 7q22 [19].

MUC 4 has been localized to chromosome 3q29 [19], and is present in normal bronchial and colonic mucosa. Most major studies do not show any expression in gastric mucosa [6,12].

MUC 5 is a major gastric mucin, being expressed on the surface and in mucous cells of normal stomach. It is also expressed in the tracheobronchial tree, gallbladder and endocervix [1,6,8,12,17,23-25]. *MUC 5* was initially divided into three distinct groups, *MUC 5A, 5B* and *5C*. *MUC5A* and *MUC5C* have since been shown to belong to the same unique gene, and *MUC5B* to a distinct one, and *MUC5* has, therefore, been divided into 2 groups *MUC5AC* and *MUC5B* [19]. There are high levels of *MUC5AC* in gastric epithelium, mainly confined to surface foveolar cells, and mucous neck cells, but it is not seen in deep gastric glands [10].

MUC5, together with *MUC2* and *MUC6* are localized to chromosome 11p15.3-15.5. It appears that the 11p15 region contains several distinct gene loci for mucins [18,19].

MUC 6 is expressed in mucous neck cells of gastric glands and pyloric gland cells, as well as in the gallbladder, Brunner's glands, pancreatic ductules and seminal vesicles [1,6,8,12,17,24,25].

MUC 7 is a low molecular weight mucin, which has been detected in salivary gland [12,13], and is localised to chromosome 4 [19].

MUC 8 has recently been identified, and is briefly mentioned in recent papers [10,19], but there are no details as to where it is expressed.

MUC1, MUC5 and *MUC6* are expressed in gastric mucosa in most studies. Many studies have shown weak expression of *MUC1*, while others found *MUC1* to be strongly expressed in surface mucous cells and mucous neck cells [3,5-7,12,20,25,26]. Parietal cells have also shown weak

positive staining with the *MUC1* antibody [26]. Normal stomach lacks *MUC2*, *MUC3*, and *MUC4*, according to most studies [12], but *MUC2* and *MUC3* are both expressed in intestinal metaplasia and have been reported in gastric carcinomas [6]. *MUC4* expression has also been reported in carcinomas. *MUC7* has not been studied in gastric mucosa.

Table 1. Summary of sites of expression of mucin genes.

MUCIN GENE	SITES OF EXPRESSION	REF.
MUC 1	Membrane associated mucin with widespread expression throughout the body. Sparse expression in gastric epithelium. Highly expressed in breast carcinoma.	3,5-7,20,21
MUC 2	Jejunum, ileum, right colon, weakly in tracheobronchial tree .	1,3,6
MUC 3	Small intestine predominantly. Also colon and gallbladder.	6,9
MUC 4	Bronchus and colon.	6,12
MUC 5	Stomach surface and mucous cells. Tracheobronchial tree.	1,6,8,12,17,23-25
MUC 6	Antral glands and gallbladder.	1,6,8,12,17,24,25
MUC 7	Salivary gland.	12,13

Mucins in disease

It has been shown that different mucins are expressed in diseased states as compared to normal [3,12,27]. Abnormalities of gastric mucin have been associated with a number of diseases such as atrophic gastritis, peptic ulcer disease, and chronic active gastritis associated with H pylori [8,9]. Mucin gene expression is relatively organ specific, and loss of normal mucin gene regulation may occur with metaplasia, dysplasia and particularly with malignancy. In intestinal metaplasia it has been shown that there is down regulation of the *MUC5* and *6* expression in the gastric mucosa, with increased expression of *MUC2* and *MUC3* (intestinal type mucins) [12,11]. It has also been shown that intestinal type carcinomas express *MUC2* and *3* [12]. Changes in mucin expression have been noted in association with malignant transformation, with some carcinoma cells expressing aberrant mucin expression, with either an increase in expression or a loss of mucin expression [12,17]. These changes imply that mucin may play a significant role in the biological behaviour of cancer cells [20]. Mucins synthesized by cancer cells are thought to contribute to cancer invasion and metastasis , via altered cell adhesion and immune recognition [4,5,12,28]. Although alterations in mucin are thought to contribute to the biological behaviour of cancer it is also possible that alterations in mucin expression may merely be a reflection of the biological changes that have occurred.

The expression of mucin epitopes is modified in carcinomas, mostly due to alterations in glycosylation, [7,20] but alterations in the apomucin core have also been cited as contributing to the tumour mucin phenotype [26]. Abnormal glycosylation results in hypoglycosylated mucins that have lost their luminal polarity [3,12,29]. This loss of carbohydrate side chains results in exposure of the tandemly repeated protein core which, combined with the loss of luminal

polarity, exposes antigenically active epitopes on the surface of these tumours [21]. Altered cell adhesion and/ or immune recognition may follow.

Mucin gene expression has also been shown to play a role in prognosis in some instances. High levels of *MUC1* have been associated with poor survival in breast cancer [21] carcinoma of the ampulla of Vater [30] and gastric carcinoma [31,32]. It has been suggested that increased *MUC1* expression in gastric carcinoma is also associated with aggressive features and a poor prognosis, [31-33]. In one study [32] this increased aggressiveness was associated only with the diffuse type carcinomas, and not with the intestinal type carcinomas. As the diffuse type of cancer is associated with a poorer prognosis anyway *MUC1* expression is unlikely to have independent prognostic value. There are conflicting and inconclusive reports from other studies [7] and it appears that *MUC1* expression cannot reliably be used as a prognostic factor in gastric carcinoma [7]. Positive *MUC 2* expression in gastric carcinomas has been associated with an improved prognosis [32,33].

Histochemical classification

Human gastrointestinal mucins have conventionally been classified histochemically as neutral mucins, sulphomucins and sialomucins according to their reaction with PAS, High iron diamine and alcian blue respectively [34]. Normal gastric mucin is entirely neutral in type, except for a small amount of sialomucin production by the mucous neck cells. No sulphomucin is present in normal stomachs [35].

GASTRIC CANCER AND PRECURSOR LESIONS

Aetiologic factors

Gastric carcinoma is one of the most prevalent cancers in the world [36], and despite a declining incidence in the USA and Western Europe, as well as in Asian and White populations locally, it remains a major killer worldwide [37,38]. There is a particularly high incidence in the Western Cape population of South Africa, especially amongst the so-called coloured (mixed race) population [39]. This population has the fourth highest incidence in the world with an estimated rate of 50-69,9/100 000 [40-42].

Aetiologic factors and the pathogenesis of gastric carcinoma are not fully elucidated, but there is little doubt that *Helicobacter pylori* has a very important role to play in gastric carcinogenesis (discussed later in the text), and that well defined precancerous entities relating to *Helicobacter pylori* infection exist. Intestinal metaplasia, chronic atrophic gastritis and dysplasia are considered to be precancerous lesions and to precede gastric carcinoma in many instances, especially the intestinal type adenocarcinomas [43-60]. Factors such as diet, climatic, environmental and genetic influences may partly explain the different geographical incidence of gastric carcinoma [35].

Precancerous lesions

Intestinal metaplasia is characterized by a change in morphology from a gastric to an intestinal phenotype, associated with a change from neutral to acid mucin, and as the process becomes more advanced sulphomucins make their appearance [61,62]. Intestinal metaplasia is divided into complete IM (type I), which resembles small intestinal epithelium, and incomplete, which

resembles colonic epithelium. Incomplete IM is divided into types IIa and IIb (or type II and III), depending on the presence or absence of sulphomucins [54,56-58,63-65]. Intestinal metaplasia is a common association with benign as well as malignant conditions, and this limits its use as a selective marker for increased cancer risk [27,46-48,54,56-58,66]. It is far too common to have any predictive value in an individual patient [61]. Sulphomucin secreting IM (type IIb), however, is considered to have a higher correlation with subsequent gastric cancer (particularly intestinal type adenocarcinoma), than non-sulphated types (types I and IIa) [45-47,56-58,62,64-67], and is possibly a marker for malignant change . It is not known for certain whether intestinal metaplasia is, in fact, truly precancerous, or merely a parallel response to a carcinogenic environment [34,48,50,54,64]. Not only is the histochemical type of mucin in intestinal metaplasia different from normal, but mucin gene expression is also altered. There is a decrease in expression of the normal stomach mucins *MUC5* and *6* in intestinal metaplasia, and an upregulation of *MUC2* and *3* [11].

Atrophy of the gastric mucosa is defined as loss of glandular tissue, and it occurs with all pathological processes that cause severe mucosal damage. Extensive chronic atrophic gastritis, usually associated with intestinal metaplasia carries an increased risk of malignancy [67, 68].

Dysplasia is characterized by architectural disorganization, cellular atypia and abnormal differentiation, and is classified as high or low grade [69-71]. Low grade dysplasia (Fig. 1) is composed of simple tubules with little branching. Neoplastic cells are pseudostratified, tall columnar cells with dense, spindled hyperchromatic nuclei and abundant cytoplasm. Mitoses, if present, occupy the superficial half of the mucosa. High grade dysplasia shows a greater degree

of architectural complexity of the neoplastic glands, and the cells show pleomorphism, loss of polarity and frequent mitoses [72]. High grade dysplasia is generally accepted as a marker of risk of malignant change [43,50,64,69-73], being common in stomachs with established carcinomas, whereas low grade dysplasia in the majority of cases regresses. [69, 73]. Although most low grade dysplasias regress, it has been shown that a small percentage do progress to invasive carcinoma [73].

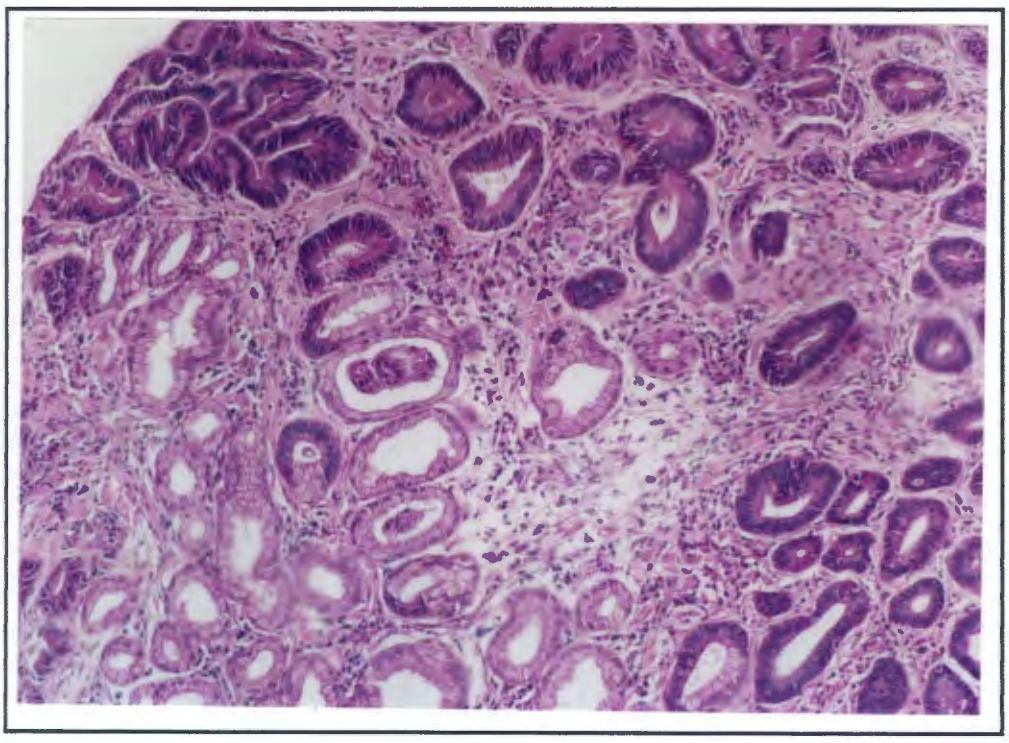

Figure 1. Low grade dysplasia of gastric glands with adjacent non dysplastic glands (Haematoxylin and eosin stain).

The Lauren Classification

There are a number of classifications for gastric carcinoma, but the most widely used classifications in the Western world are the Lauren [74] and WHO classifications [43]. For the purpose of this study the Lauren classification was used, and is the only one that will be mentioned from here onwards.

The **"Lauren Classification"** divides adenocarcinomas of the stomach into two major types, the intestinal type and the diffuse type. The **intestinal type** is composed of well-formed glands, lined by mucous secreting epithelium, resembling colonic carcinoma. These tumours are usually well demarcated. The **diffuse type** does not usually demonstrate recognisable glands and is composed of poorly cohesive single cells and small groups of cells, which infiltrate widely through the stomach, and often have a signet-ring pattern of mucin secretion [35].

The **intestinal type** (Fig. 2) of carcinoma is the most frequent type in countries with a high gastric cancer risk, and occurs in an older age group. It is preceded by well defined pre-cancerous lesions, such as intestinal metaplasia and atrophic gastritis. A putative sequential precancerous process has been described with intestinal type adenocarcinoma. It is postulated that chronic active gastritis, usually the result of H pylori infection, may progress to chronic atrophic gastritis, to intestinal metaplasia, dysplasia and finally to carcinoma. [60,75-79]. This is a long process in general requiring 20-40 years to complete.

The **diffuse type** (Fig. 3) occurs in relatively low risk populations and is not preceded by intestinal metaplasia or other defined precancerous lesions [12,38,43,46,62]. It is the type of carcinoma most commonly seen in young persons [35]. Although environmental factors are

important in this type of cancer it seems that genetic influences have a more important role to play [79].

CLASSIFICATION OF GASTRIC CARCINOMA

World Health Organisation 1990

Papillary

Tubular

Mucinous

Signet ring

Lauren classification 1965

Intestinal type

Diffuse type

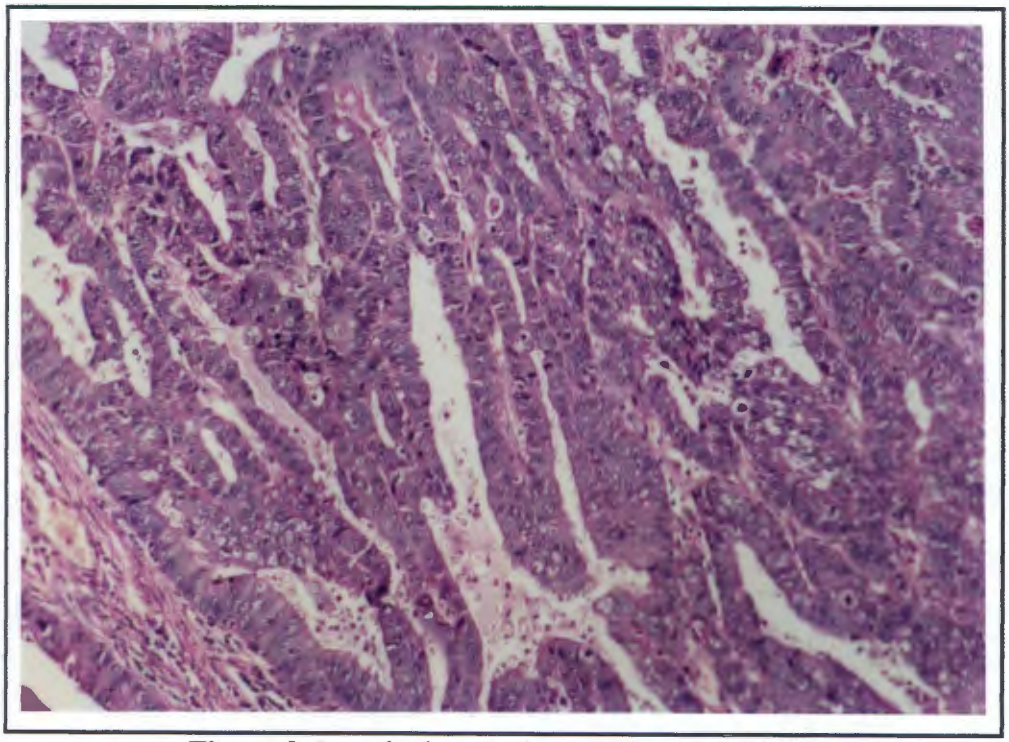

Figure 2. Intestinal type adenocarcinoma (H & E stain).

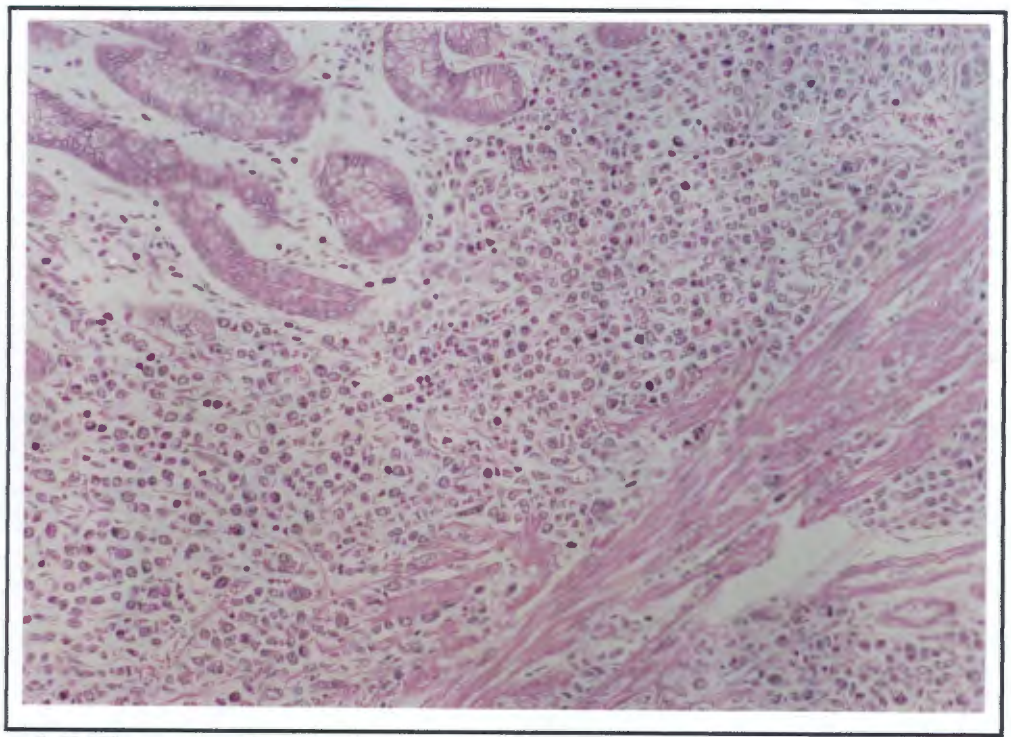

Figure 3. Diffuse type adenocarcinoma infiltrating between the layers of the muscularis propria.

THE ROLE OF *HELICOBACTER PYLORI* IN GASTRIC DISEASE, AND ITS EFFECT ON GASTRIC MUCIN.

Disease processes associated with *Helicobacter pylori*

The presence of curved and spiral organisms in the stomach has been reported in the literature for many years, but they were not initially considered to be pathogenic. In 1982 Marshall [80] isolated these organisms, and named them *Campylobacter pylori*, the name later being changed to *Helicobacter pylori*. *Helicobacter pylori* is a gram negative, microaerophilic, curved bacillus, [80,81] which is found in gastric mucus in close apposition to the gastric mucosa [82] (Fig. 5).

Infection with *Helicobacter pylori* is the most important cause of chronic active gastritis (Fig. 4) worldwide, and is associated with evolution to chronic atrophic gastritis and intestinal metaplasia at a later stage. It is also associated with gastric and duodenal ulcers, and there is general agreement that *Helicobacter pylori* increases gastric cancer risk. There is a link between *Helicobacter pylori* infection and gastric cancer of both intestinal and diffuse histological types. [37,59,60,76,83]. *Helicobacter pylori* associated gastritis is more frequently located in the antrum, [25,59,67,75,81,84,85], but atrophy, intestinal metaplasia and carcinomas occur in both the antrum and the body. *Helicobacter pylori* associated carcinomas are rarely present in the cardia. [37]. A large study from Italy [59] showed an eighty five percent incidence of *Helicobacter pylori* colonization in patients with early gastric carcinomas. This percentage underscores a highly significant association between *Helicobacter pylori* and gastric cancer [86]. *Helicobacter pylori* infection is common worldwide, but is particularly prevalent in underdeveloped countries, where most adults are infected [86]. The majority of these infections are asymptomatic. The present incidence in Cape Town is known to be high, and there are

ongoing studies to establish the incidence, and the relation with gastric carcinoma in the local population (Louw et al, personal communication, 1999). In South Africa there have been studies from Natal which showed an incidence of *Helicobacter pylori* in seventy nine percent of patients undergoing routine endoscopy, with up to ninety five percent of the patients with gastritis having *Helicobacter pylori*, and ninety percent of patients with duodenal ulcers, and fifty percent of patients with gastric ulcers demonstrating the organism [87,88]. Previous observations from an outpatient population in Cape Town showed an overall incidence of seventy three percent [89]. The incidence of *Helicobacter pylori* infection has been shown to increase with age [81].

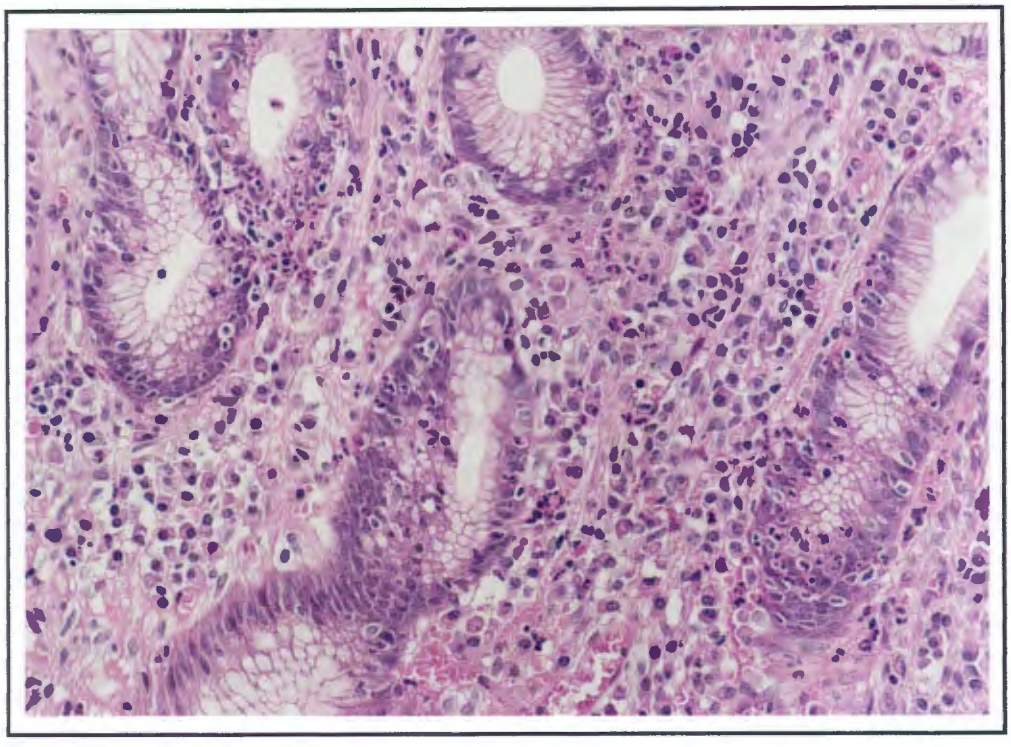

Figure 4. Chronic active gastritis. There are numerous plasma cells in the lamina propria, and neutrophils are seen infiltrating the glandular epithelium. (H & E stain).

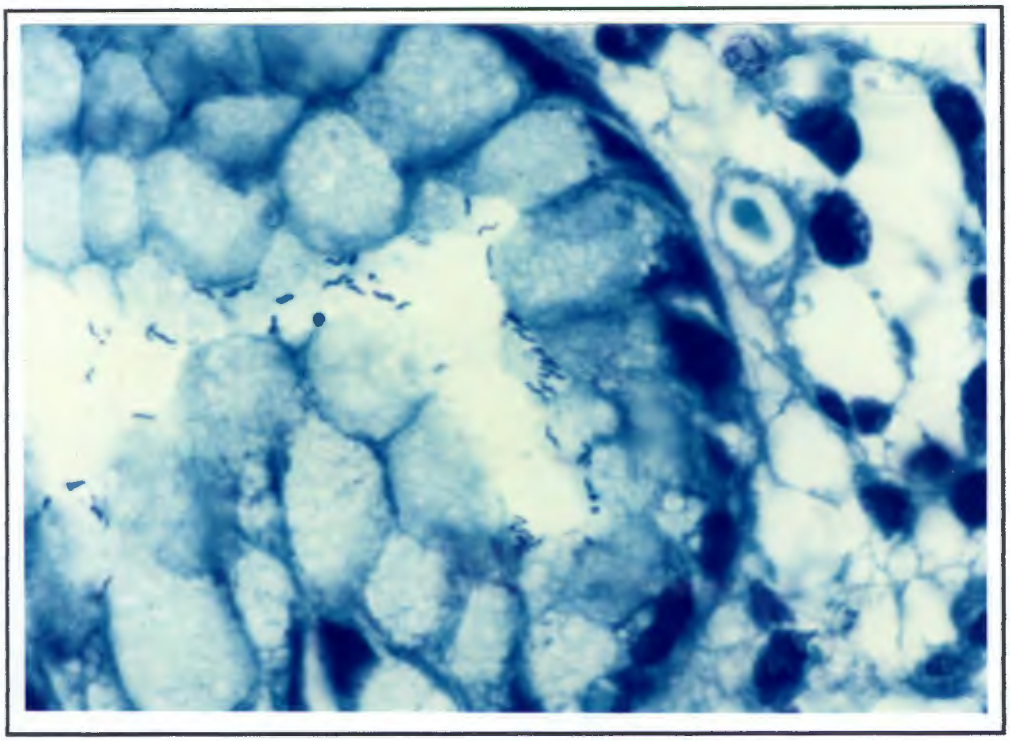

Figure 5. H*elicobacter pylori* adherent to the gastric mucosa (Giemsa stain).

Adherence to the mucosa

Helicobacter pylori is located primarily within the adherent mucus barrier of the gastric mucosa, (Fig. 5) but the bacteria may also adhere to the gastric surface mucous cells [25,90]. Colonisation usually takes place on normal gastric epithelial surfaces, and *Helicobacter pylori* does not in general adhere to areas of intestinal metaplasia or advanced atrophy in the stomach. In specimens showing gastritis with advanced intestinal metaplasia, or with gastric atrophy *Helicobacter pylori* are often undetectable, even if there is evidence of serologic evidence of infection [85,91].

The reason for the preferential colonisation is uncertain, but it may be that the acid mucins produced by intestinal metaplasia are toxic to *Helicobacter pylori,* and that the evolution of intestinal metaplasia is a defensive mechanism by which the gastric mucosa eliminates *Helicobacter pylori* [75]. There have been some studies, however, which have shown adherence of *Helicobacter pylori* to metaplastic cells in type III (incomplete, sulphomucin-producing) intestinal metaplasia [84,91,92], but not to areas of intestinal metaplasia which don't produce sulphomucins. This study would suggest that sulphomucins may not be as toxic as sialomucins to *Helicobacter pylori,* and perhaps may in some way promote the growth of *Helicobacter pylori.* This is of interest because it is this type of intestinal metaplasia that is linked to the development of gastric carcinoma. This finding suggests that adherence of *Helicobacter pylori* plays a role in the progression of metaplasia to dysplasia and carcinoma. Other studies are, however, at variance with this finding [75], with fewer *Helicobacter pylori* being detected in association with type III intestinal metaplasia.

Once invasive carcinoma has developed it seems that the neoplastic environment becomes inhospitable to *Helicobacter pylori* [75], and the organisms are hardly ever seen in association with the mucosa in patients with advanced carcinomas.

Pathogenic mechanisms in *Helicobacter pylori* associated gastric disease

Helicobacter pylori infection leads to gastric disease as a result of epithelial damage caused by active ongoing inflammation. A multistep sequence of events leading to the development of intestinal type gastric carcinoma has been postulated, in which chronic active gastritis ,if

untreated, leads to chronic atrophy, intestinal metaplasia, dysplasia and then finally to carcinoma. [75-77,81]. The relationship between *Helicobacter pylori* and diffuse type gastric carcinoma does not seem to follow the same route as with intestinal type carcinomas. Studies have shown that DNA damage due to oxygen radicals induced by persistent inflammation resulting for *Helicobacter pylori* infection may play a role in the development of diffuse type gastric cancer [76].

The exact mechanism for the pathogenesis of *Helicobacter pylori* induced gastric disease is not well understood, but it appears that the organism may injure the gastric epithelium by impairing the effectiveness of the protective function of mucins [90]. *Helicobacter pylori* adversely affects the chemical and physical properties of the mucus layer, with alteration of the general quantity and quality of mucin, resulting in diminished gel forming properties of the mucin[8], and decreased viscosity [93,94]. It appears that *Helicobacter* pylori per se causes decreased polymerization of the mucus gel [95], but according to one study does not cause a decrease in the actual thickness of the mucus layer, except where there is significant atrophic gastritis or ulceration.[95]. The ability of *Helicobacter* pylori to degrade mucin is controversial with conflicting results from various studies [25,95]. Alterations in the glycosylation process of the mucin have been shown to have an effect on the structure and function of mucins and might affect their ability to maintain an effective barrier in the stomach [90]. It has been postulated that *Helicobacter pylori* can induce alterations in the gastric mucus by abnormal glycosylation of the mucin. The changes in glycosylation would bring about altered expression of carbohydrate antigens on the gastric surface. The pattern of glycosylation has been shown to return to normal after treatment of the *Helicobacter pylori* [90]. Urease produced by *Helicobacter pylori* has been

shown to be an aetiologic factor in mucus breakdown. [86,96] *Helicobacter pylori* secrete urease and other proteolytic enzymes, which may have important pathogenic effects. Alterations in the mucus layer by whatever mechanism may interfere with the pH gradient in the mucus layer, exposing the surface epithelium to excessive hydrogen ions, and therefore leading to further damage [6]. In one study by Markesich et al [97] the authors argue against degradation and decreased viscosity of mucus in *Helicobacter pylori* infection, but their results differ from most other studies.

In addition to causing alterations in the mucus layer *Helicobacter pylori* has also been shown to induce gastric epithelial proliferation, either directly or indirectly, which is suggestive evidence that it may be an initiating step in the development of gastric carcinogenesis [77]. *Helicobacter* pylori infection alone is insufficient to account for gastric carcinoma, as numerous people having *H* pylori infection never develop gastric carcinoma. Dietary factors probably also play a role. [37] Age at infection probably also is important, earlier acquisition of the infection being associated with an increased incidence of carcinoma [37,78].

The effect of *Helicobacter pylori* on mucin gene expression

There have not been many studies of the effect that *Helicobacter pylori* has on expression of individual mucin genes in gastric mucosa, but one study has shown that *Helicobacter* pylori infection does cause abnormalities of mucin gene expression [25]. In this study it was shown that *Helicobacter pylori* caused aberrant expression of *MUC6* in surface mucous cells , where it is not normally expressed, and that there was significantly less than normal *MUC5* expression in the

surface mucous cells. Aberrant expression of mucin could facilitate the adherence of *Helicobacter pylori* to the epithelial surface, and promote dissolution of the protective mucous barrier. The study also showed that eradication of *Helicobacter pylori* in these cases caused a reversion of the mucin expression to normal [25].

AIMS OF THIS STUDY

1. The main aim of this study is to examine the different types of mucin expressed in normal stomachs, and in various gastric disease states.
2. Gastric carcinoma is often detected very late in our community and another aim of the study is to attempt to show if there is any use for mucin immunohistochemistry in detecting precancerous states and early cancer, and therefore aiding in earlier diagnosis.
3. The third aim is to assess the association of *Helicobacter pylori* with the various non-neoplastic and neoplastic disease states in the stomach.

In this study histochemical and immunohistochemical methods were used to determine the type of mucins present, and the pattern of mucin immunoreactivity in the stomach in a variety of conditions. These included normal gastric controls, foetal stomachs, stomachs with chronic active gastritis, low grade dysplasia, intestinal metaplasia (associated with benign ulcers, dysplasia and cancers), early and advanced intestinal type adenocarcinoma, and diffuse adenocarcinoma.

Selection of cases.

Gastric tissue from a total of 54 cases was specifically selected from recent laboratory material. These included five normal controls, six foetal stomachs (two were under 12 weeks' gestation and four were over 12 weeks' gestation), nine cases of chronic active gastritis (three with associated intestinal metaplasia), seven mild dysplasias (all of which had associated intestinal metaplasia), nine benign ulcers (six with associated intestinal metaplasia), six early intestinal type adenocarcinomas (five with associated intestinal metaplasia), five advanced intestinal type adenocarcinomas (two with associated intestinal metaplasia) and seven diffuse adenocarcinomas. In total twenty three cases had intestinal metaplasia as one of the abnormalities. Two of the normal controls were biopsies, and three were from cadaveric organ donors who had no known underlying pathology in other organs. The foetal material was derived from autopsies. The chronic active gastritis cases were all biopsies, as were all the dysplasias except for one which came from a gastrectomy specimen. The ulcer specimens were all obtained from partial gastrectomies, and all the carcinoma samples were derived from gastrectomy specimens.

Mild gastric epithelial dysplasia was characterized by low grade cellular atypia, slight nuclear stratification, and an increased nuclear/cytoplasmic ratio, as well as mild architectural

derangement [38]. Early carcinomas were defined as those that had not penetrated the muscularis propria [43] (Fig. 6).

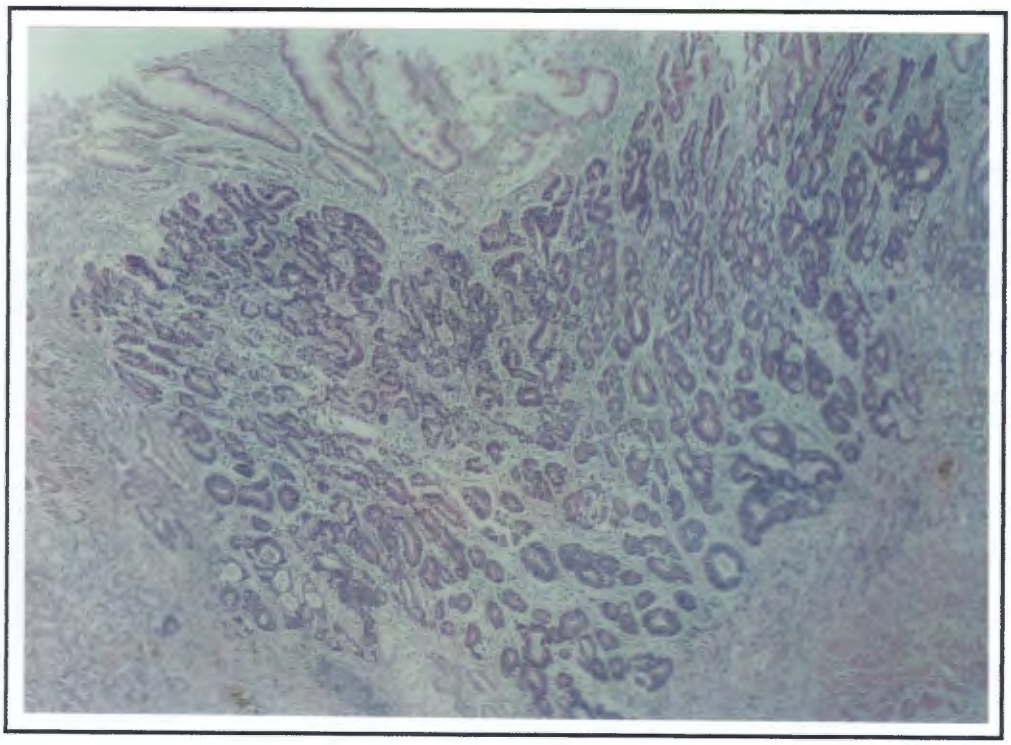

Figure 6. Early intestinal type adenocarcinoma confined to the mucosa (H & E stain).

Histochemistry

Representative paraffin-embedded tissue blocks were recut to provide 2um thick sections. These were stained with routine haemotoxylin and eosin [98], Giemsa to detect *Helicobacter pylori*, and special stains for the determination of mucin type i.e. Periodic acid -Schiff (PAS), for the detection of neutral mucins; Alcian Blue (pH 2.5), which stains acid (sialo) and most sulphated mucins blue; Mucicarmine, for the detection of acidic (sialo) mucins, and High iron

Diamine [98]. The latter stain was used to differentiate sulphated mucins (which stain black) from non - sulphated mucins (which stain blue) [44,63,99].

Immunohistochemistry.

Antibodies for this study (*MUC1-7*) were kindly provided by Professor Sam Ho (University of Minnesota and Veterans Administration Medical Center, Minneapolis) [see appendix]. For immunohistochemical staining 2um sections were cut and placed on glass slides coated with 3-aminopropyltriethoxysilane (APES). Sections were incubated at 37 degrees overnight, and then dewaxed and rehydrated.

Endogenous peroxidase activity was quenched with a 1% hydrogen peroxide methanol solution for 15 minutes at room temperature. After washing 3 times in phosphate buffered saline (PBS) those tissues requiring antigen retrieval were microwaved in citrate buffer (pH 6.0) for 10 minutes on full power (600 watts) [98].

Each section was stained immuno-enzymatically in a humidity chamber using a modified 3 step peroxidase conjugated avidin-biotin method [98]. Non-specific binding was blocked with non-immune serum (swine, rabbit or chicken) at a 1:20 dilution for 10 minutes. Optimal dilutions of the Primary antibodies were determined using the "checkerboard titration" method [98,100]. Sections were washed thoroughly in PBS after each antibody incubation. The sections were subsequently incubated with a 1:250 biotinylated rabbit-anti-mouse, swine anti-rabbit (Dako, Copenhagen, Denmark), or rabbit-anti-chicken 1:500 (Biogenesis, Poole, U.K.) antibody for 30 minutes at room temperature and then with a strep-avidin HRP (Dako, Denmark) for 30 minutes.

After washing, peroxidase activity was detected using 3.3' diaminobenzidine (Sigma, St Louis, U.S.A.) as a chromagen with 1% hydrogen peroxide solution as the substrate. Sections were washed to arrest the reaction after 10 minutes, counterstained with haematoxylin, blued in Scott's tap water substitute and then dehydrated through graded ethanols to xylol and mounted in a synthetic resinous medium.

The above method [98] was used for *MUC 1-7* and for *FHF*, which is a deglycosylated mucin for *MUC 5* and *6* [12]. Positive immunohistochemical staining is considered to be cytoplasmic staining of more than 50% of the cells in the various conditions examined. In cases where staining was less than 50% or was very weak this is reported in the text.

Positive controls were from normal tissues known to express the relevant antibody. Normal breast was used for *MUC1*, colon for *MUC2* and *MUC4*, small intestine for *MUC3*, stomach for *MUC5* and *MUC6*, and salivary gland for *MUC7*. Negative controls, using the same tissues as for the positive controls were incubated with no primary antibody, and then they were incubated with primary antibody, but no link antibody. In a third step the controls were incubated with strep-avidin to ensure that there was no non-specific linking of primary antibody to the tissues.

All cases studied had evidence of *Helicobacter pylori* infection, except normal controls (n=5), foetal stomachs (n=6), diffuse adenocarcinomas (n=7) and 3 of 5 advanced intestinal adenocarcinomas. The organism was found in the mucus on the surface of the gastric epithelium, and attached to the gastric epithelium. It was seen in biopsies with intestinal metaplasia, but not attached to the portion of the epithelium showing metaplastic change. Although *Helicobacter pylori* were not seen in the advanced carcinomas, it is unlikely, considering the high prevalence of *Helicobacter pylori* in our population that the advanced carcinomas never had *H. pylori* infection. It has been shown previously [25] that the environment associated with advanced adenocarcinomas is unsuitable for the continued presence of these organisms.

Histochemical stains

(The results are summarised in table 2).

Normal stomachs all stained with neutral mucin as expected, and there was no significant staining for acidic mucin.

Foetal stomachs in the first trimester showed no mucin staining, but neutral mucin was positive from the second trimester onwards.

Chronic active gastritis specimens all showed neutral mucin staining. Acidic mucin was only demonstrated in foci of associated intestinal metaplasia. No sulphomucin was detected in the intestinal metaplasia associated with gastritis.

The low grade dysplasias stained positively for neutral and acidic mucins, and half of them showed the presence of sulphomucin.

In the intestinal metaplasia group there was positive staining for neutral and acidic mucins in all cases (Fig 8a & b, and 9). Sulphomucin was present in the goblet cells in 14 of 23 cases. (Fig. 7). Of the cases of intestinal metaplasia associated with sulphomucin, 4 were associated with early gastric intestinal type adenocarcinoma, 2 with advanced intestinal type adenocarcinoma, 2 with benign ulcers, and 6 were associated with dysplasia. Although the numbers are small there does seem to be a higher incidence of sulphomucin in association with gastric cancer and with dysplasia, compared to gastritis and benign ulcers. The majority of intestinal metaplasia cases associated with ulcers (n = 6) displayed no sulphomucin staining, and there was no sulphomucin in the intestinal metaplasia associated with gastritis (n = 3).

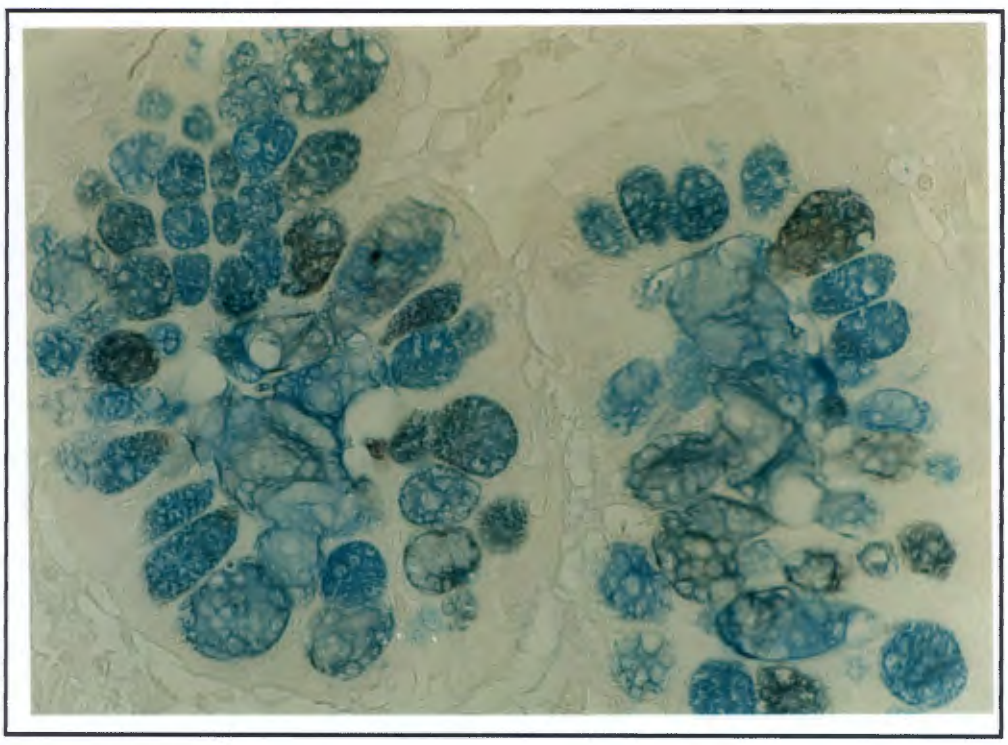

Figure 7. Intestinal metaplasia stained with the high iron diamine stain. Sulphomucins stain black.

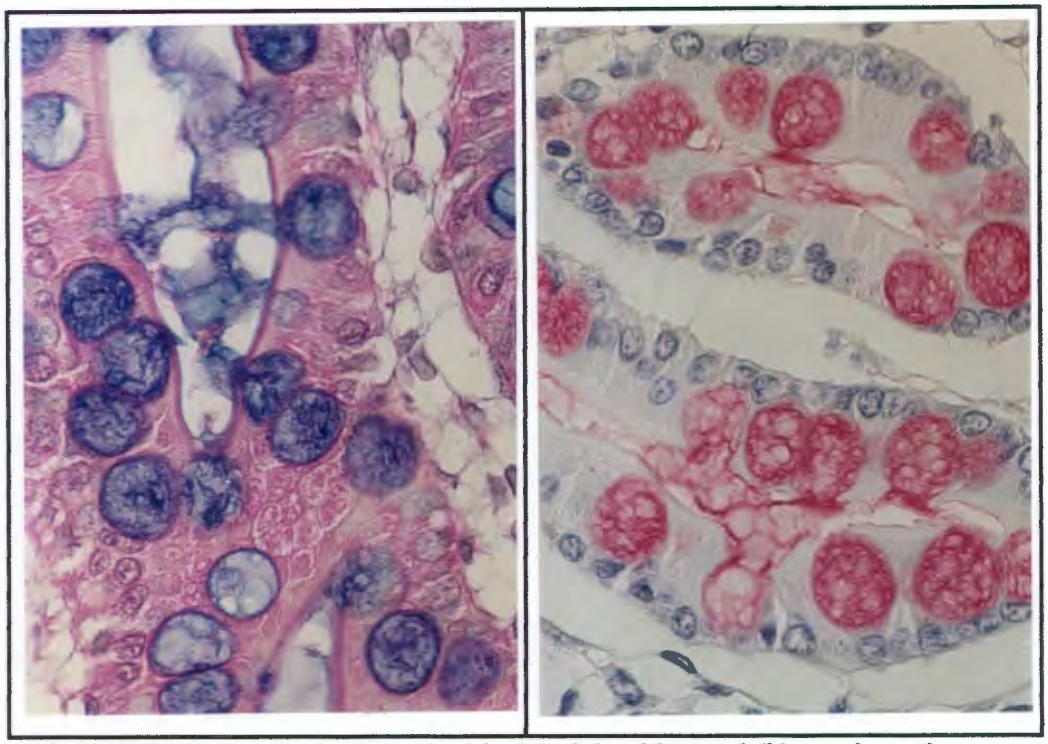

Figure 8. Intestinal metaplasia stained with (a) alcian blue and (b) mucicarmine.

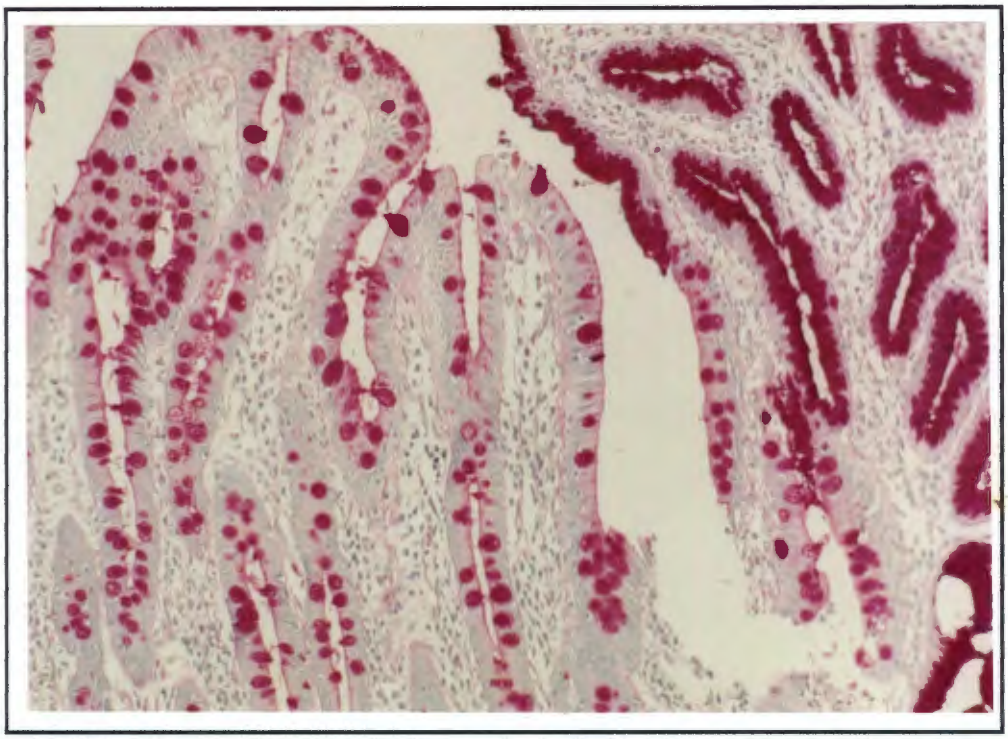

Figure 9. PAS stain showing intestinal metaplasia (left) adjacent to normal gastric mucosa.

Table 2. Results of Histochemical staining in normal and diseased states.

	N	FS*	G	D	IM	IAC	AIAC	DAC
No of cases	5	6(4/6>12 weeks)	9	7	23	6	5	7
Neutral mucin	100%	100%	100%	100%	100%	83%	80%	86%
Acidic/sulphated mucins	0%	0%	0%	100%	100%	50%	60%	50%
Acidic mucins	0%	0%	0%	100%	100%	33%	60%	50%
Sulphated	0%	0%	0%	57%	61%	0%	40%	16%

Abbreviations: N, normal stomachs; FS, foetal stomachs; G, gastritis: D, low grade dysplasia; IM, intestinal metaplasia; IAC, early intestinal type adenocarcinoma; AIAC, advanced intestinal type adenocarcinoma; DAC, diffuse adenocarcinoma

*Foetal stomachs in the first trimester showed no mucin expression at all. The figures in the table are for foetal stomachs after 12 weeks.

Early intestinal type adenocarcinomas showed staining for neutral mucin in 5 of 6 cases, and staining for acidic mucin in 2 of 6 cases with the Alcian blue stain, and in 3 of 6 cases with mucicarmine.

Advanced intestinal type adenocarcinomas showed neutral mucin in 4 of 5 cases, acidic mucin in 3 of 5 cases, and sulphomucin in 2 of 5 cases.

The diffuse adenocarcinomas showed neutral mucin in 6 of 7 cases, acidic mucin in 2 of 7 cases and sulphomucin in one case.

Immunohistochemistry Results

(The results are summarised in table 3.)

For origins of the antibody clones see appendix.

MUC 1 showed weak positive staining in only one of five normal stomachs. The staining was in the superficial mucous cells of the foveolae, in surface mucus and in the parietal cells. The majority of cases of gastritis (8 of 9) showed positive staining with *MUC1*. *MUC1* was present predominantly in surface mucus and in superficial glands, and in three cases there was staining in the foveolar mucous cells as well. There was positive staining in four of five advanced intestinal adenocarcinomas (see Fig. 18a). The remaining categories showed no staining.

MUC 2 showed patchy positive staining in 60% (3 of 5) of normal stomachs, predominantly in mucous neck cells; no staining in early foetal stomachs, and positive staining in foveolar mucous cells in 50% (2 of 4) of foetal stomachs after 12 weeks of gestation. There was patchy positive staining in foveolar mucous neck cells in 2 cases of gastritis, and in one case the parietal cells stained positively. Mucous cells in dysplastic glands showed positive staining in all the low grade dysplasias (Fig. 10), and there was also *MUC2* staining in all the intestinal metaplasias (Fig. 11), the staining being confined to the goblet cells. There was very focal staining in 33% (2 of 6) of early intestinal type adenocarcinomas, positive staining in 60% (3 of 5) of advanced intestinal adenocarcinomas (although in one case the staining was weak and focal) and strong staining in 85% (6 of 7) of diffuse adenocarcinomas (Fig. 17a).

MUC 3 showed no staining in normal stomachs, and focal staining in foveolar mucous cells in 25% (1 of 4) of the second trimester foetal stomachs. There was no significant staining in

gastritis. There was positive staining in 71% (5 of 7) of dysplasias and in 56% (13 of 23) of the intestinal metaplasias, within goblet cells, focal staining in 16% (1 of 5) of early intestinal adenocarcinomas, no staining in advanced intestinal adenocarcinomas and staining in 29% (2 of 7) of diffuse adenocarcinomas.

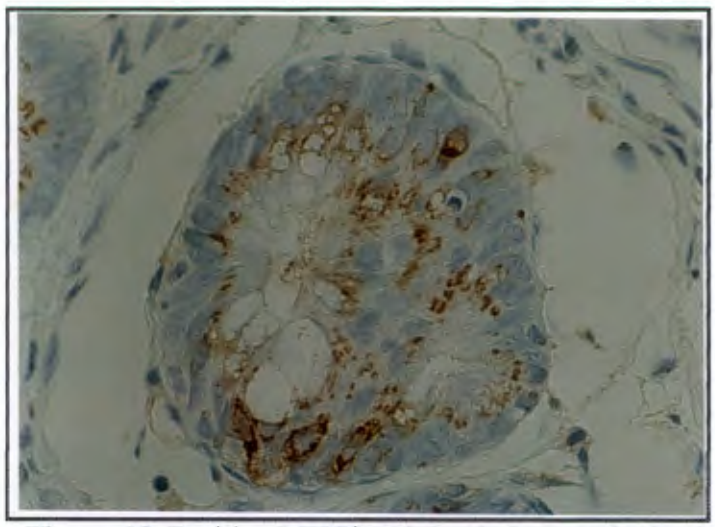

Figure 10. Positive *MUC2* staining in low grade dysplasia.

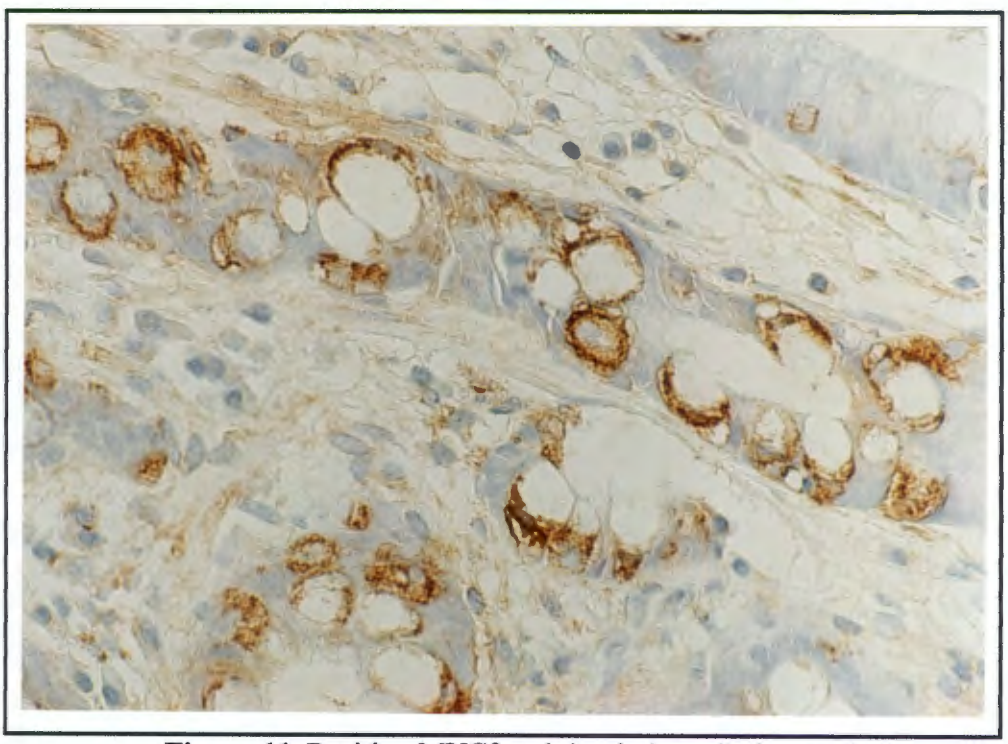

Figure 11. Positive *MUC2* staining in intestinal metaplasia.

MUC 4 showed positive staining in mucous neck cells and strong staining in basal mucous glands in 100% (5 of 5) of normal stomachs. (Figs. 12 and 13). There was no staining in first trimester foetuses, but positive staining was present in 75% (3 of 4) of second trimester foetal stomachs, the staining in the latter being weak surface and mucous gland staining. There was positive staining in all cases of gastritis, the staining pattern being the same as that of normal controls. In dysplasias there was weak, variable staining in 42% (3 of 7), weak patchy staining in 22% (5 of 23) of intestinal metaplasias, no staining at all in early intestinal type adenocarcinomas, weak staining in 20% (1 of 5) of advanced intestinal adenocarcinomas and no staining in diffuse adenocarcinomas. Where residual 'normal' basal glands were present in any of these conditions there was positive staining with *MUC 4*.

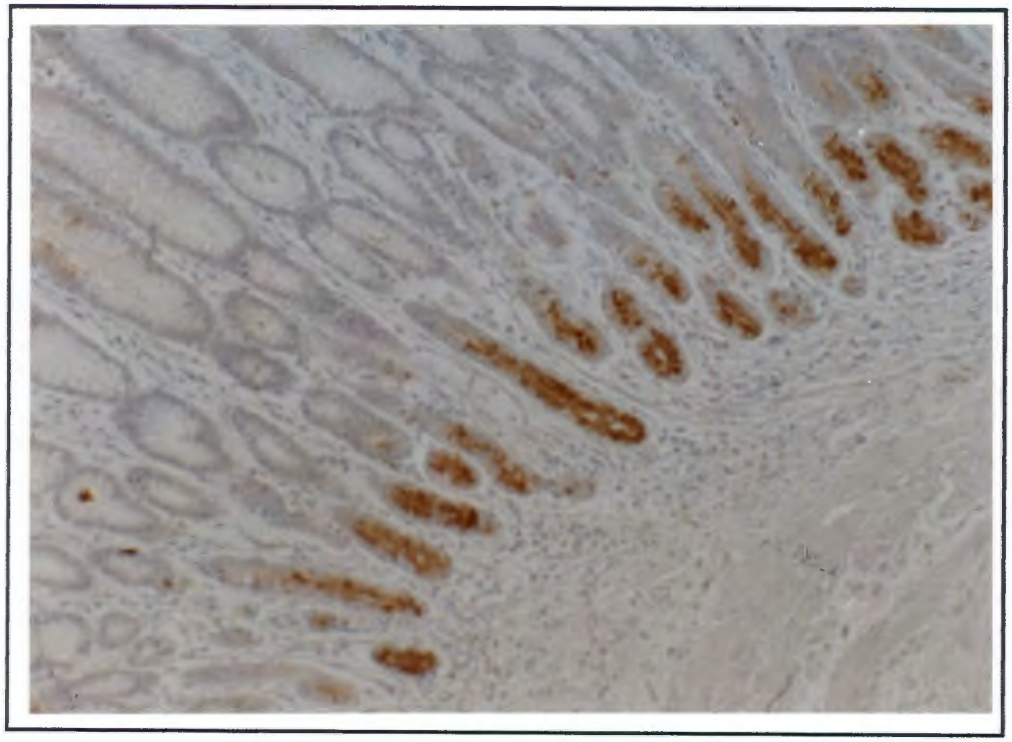

Figure12. Normal stomach showing positive staining with *MUC4* (100x magnification)

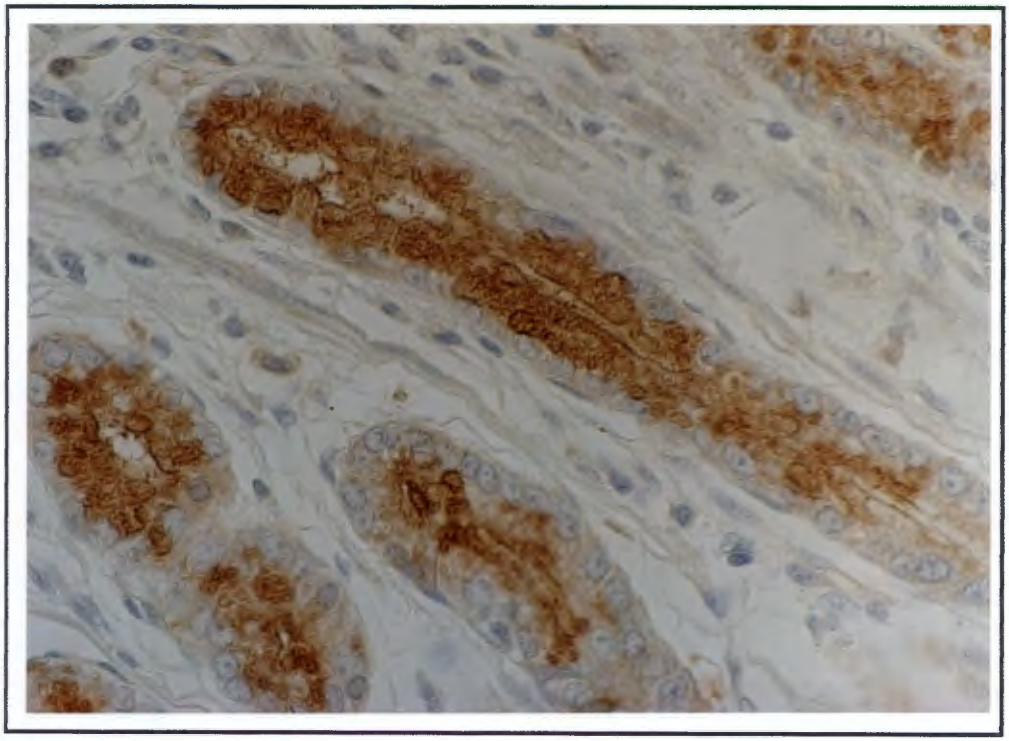

Figure 13. *MUC4* staining at 400x magnification. Same case as figure 12.

MUC 5 showed weak positive staining in 60% (3 of 5) of normal stomachs, in mucous neck cells and basal mucous glands; no staining in first trimester foetal stomachs and positive staining in 75% (3 of 4) of second trimester stomachs, mainly surface staining. All cases of gastritis showed positive staining with *MUC5*. The staining was found predominantly in the surface mucous cells and the foveolar neck cells, but in 6 cases also in the basal glands. There was positivity in 14% (1 of 7) of dysplasias, variable staining in 38% (7 of 18) of intestinal metaplasias, no staining in intestinal type adenocarcinomas, and positive staining in 60% (3 of 5) of advanced intestinal adenocarcinomas and in 57% (4 of 7) of diffuse adenocarcinomas. (Fig. 17b)

MUC 6 showed staining in 80% (4 of 5) of normal stomachs (Fig. 14), predominantly in gastric surface epithelial cells and gastric mucous neck cells. The presence of *MUC6* in the mucous neck cells corresponds with the findings in other reports [1, 8,25], but *MUC6* is usually not found in the surface mucous cells [8,25]. There was no *MUC 6* staining in first trimester foetal stomachs, but there was predominantly surface staining in second trimester stomachs (Fig. 15). 89% (8 of 9) of gastritis cases showed positive staining with *MUC6*, the staining being similar in distribution to that *MUC5*. Positive staining was seen in 29% (2 of 7) of dysplasias, in 17% (4 of 23) of intestinal metaplasias, in none of the early intestinal type adenocarcinomas (Fig. 16), in 80% (4 of 5) of advanced intestinal adenocarcinomas (Fig. 18b) and in 85 % (6 of 7) of diffuse adenocarcinomas (Fig. 17c), the latter showing strong positive staining.

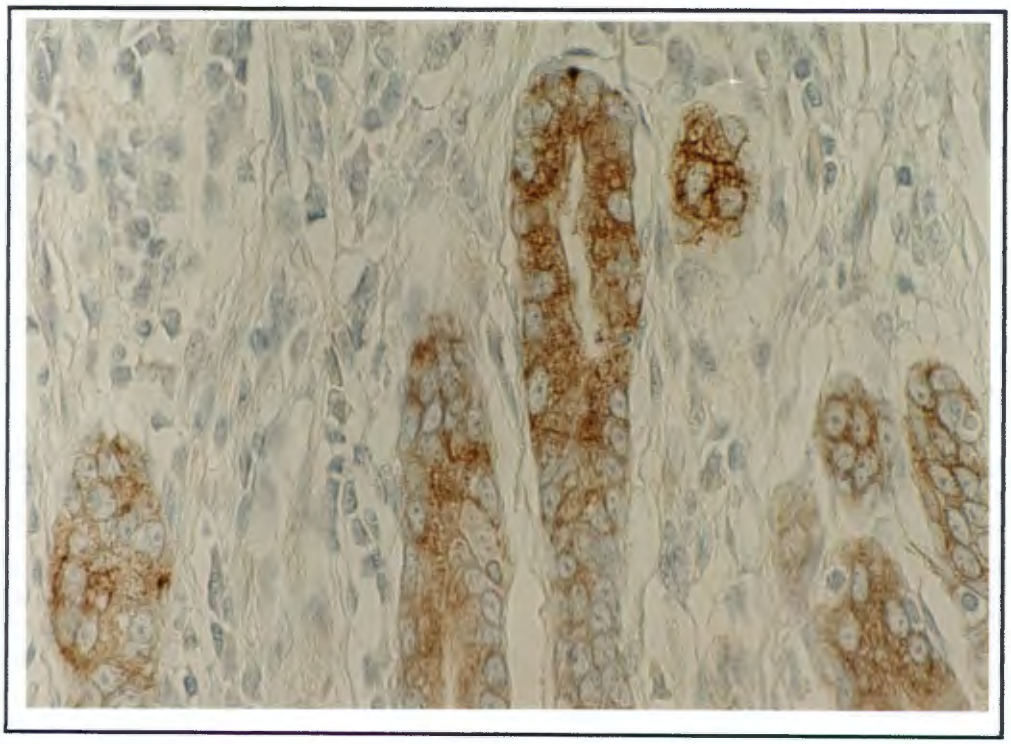

Figure 14. Normal stomach showing positive staining of gastric antral glands with *MUC6*.

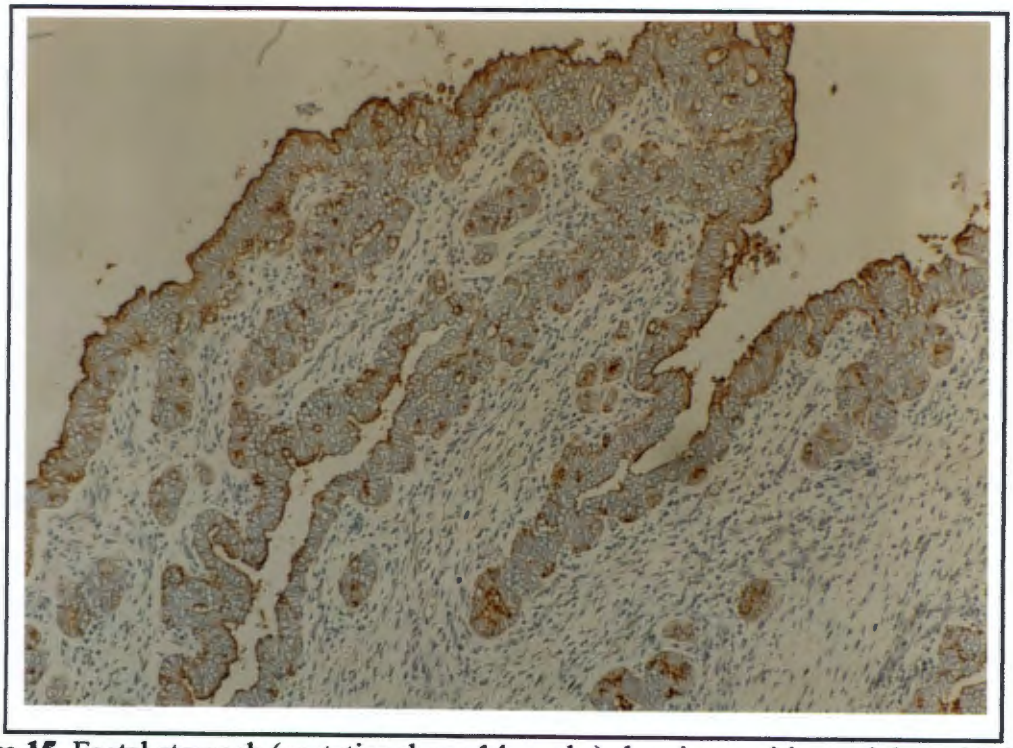

Figure 15. Foetal stomach (gestational age 14 weeks) showing positive staining with *MUC6*. The staining is seen on the surface of the stomach and in glands.

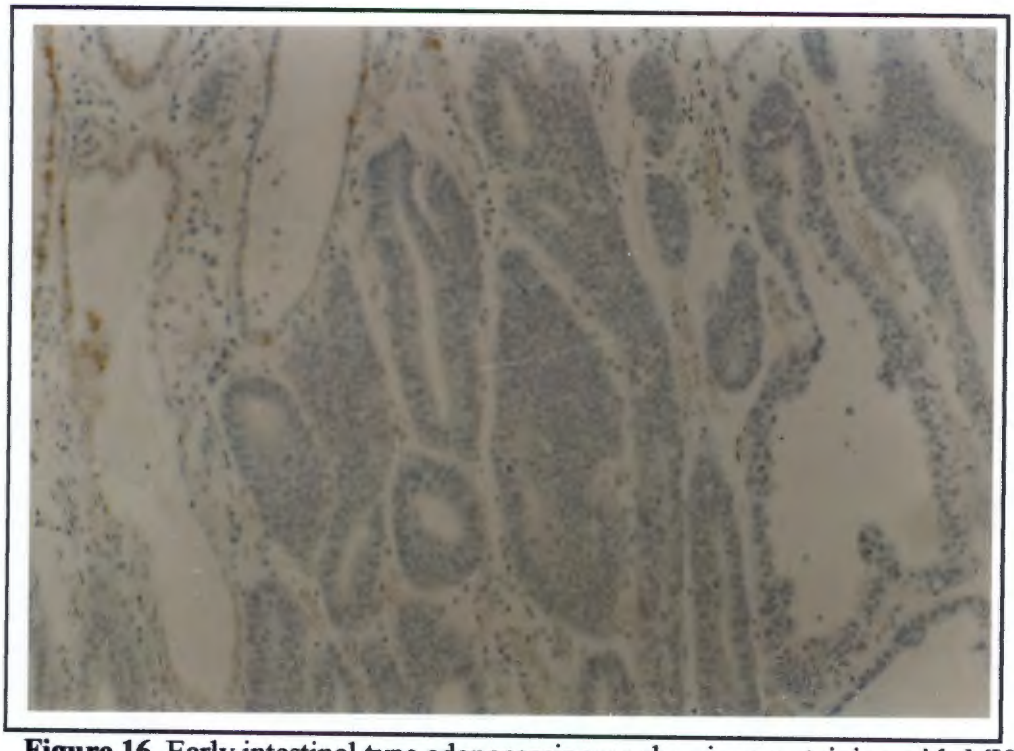

Figure 16. Early intestinal type adenocarcinoma showing no staining with *MUC6*.

MUC 7 showed staining in 40% (2 of 5) of normal stomachs. The staining was found mainly in the mucous neck cells, but also in parietal cells in one case, and in neuroendocrine cells in most cases. There was surface staining in 50% (2 of 4) of second trimester foetal stomachs, positive staining in 78% (7 of 9) of gastritis cases, 57% (4 of 7) of dysplasias, and in 35% (8 of 23) of intestinal metaplasias, no staining in early or advanced intestinal type adenocarcinomas, and positive staining in 57% (4 of 7) of diffuse adenocarcinomas (Fig. 17d).

FHF (rabbit polyclonal antibody), which is an antibody to deglycosylated gastric mucins (*MUC5* and *MUC6* core peptides) showed strong positive staining in all groups, except in early foetal stomachs, and is therefore considered to be unhelpful in this study.

It was noted that the distribution of *MUC 2* in the majority of cases correlated very closely with that of sialomucin. This was best assessed in intestinal metaplasia where there was striking *MUC2* positivity as well as abundant sialomucin. No definite correlation between *MUC2* and the presence of sulphomucin could be made . Very weak staining with *MUC4, 5,* and *6* was also present in some instances in areas where sialomucin was present, but the strength of *MUC2* staining was much greater than any of these and it would appear that *MUC2* therefore correlates with the presence of acidic (sialo)mucin.

These results have been published in Oncology Research 10: 465-473, 1998. [101]

Table 3. Results of *MUC* antibody expression in normal and diseased states.

	N	FS[a]	G	D	IM[b]	IAC	AIAC	DAC
No. of cases	5	6(4/6>12 weeks)	9	7	23	6	5	7
MUC1	20%	0%	89%	0%	0%	0%	80%	0%
MUC2	60%[1]	50%	22%	100%	100%	33%[2]	60%	85%
MUC3	0%	25%[2]	0%	71%	56%	16%[2]	0%	29%
MUC4	100%	75%	100%	42%[1]	22%[1]	0%	20%[1]	0%
MUC5	60%[1]	75%	100%	14%	30%	0%	60%	57%
MUC6	80%	75%	89%	29%	17%	0%	80%	85%
MUC7	40%	50%	78%	57%	35%	0%	0%	57%

[a]Foetal stomachs in the first trimester showed no *MUC* gene product expression at all. The figures listed in the table are for foetal stomachs after 12 weeks (second trimester).

[b]Of the 23 cases of IM 6 were associated with benign ulcers, 3 with gastritis, 7 with low-grade dysplasia, 5 with early intestinal adenocarcinoma, and 2 with advanced intestinal adenocarcinoma.

[1]The staining in these conditions was weak, and sometimes quite variable.

[2]These cases showed only focal staining.

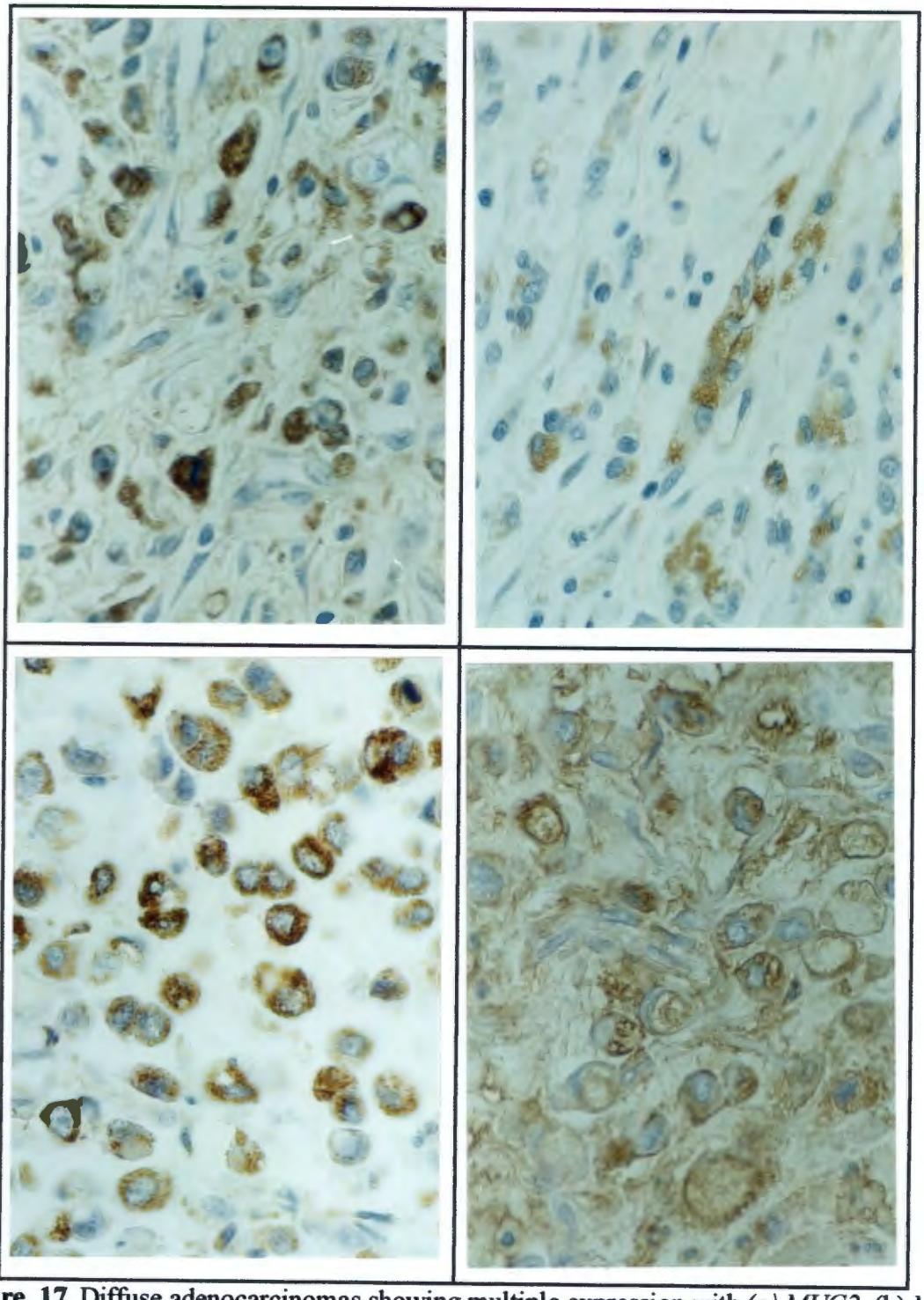

Figure 17. Diffuse adenocarcinomas showing multiple expression with (a) *MUC2*, (b) *MUC5*, (c) *MUC6*, and (d) *MUC7*.

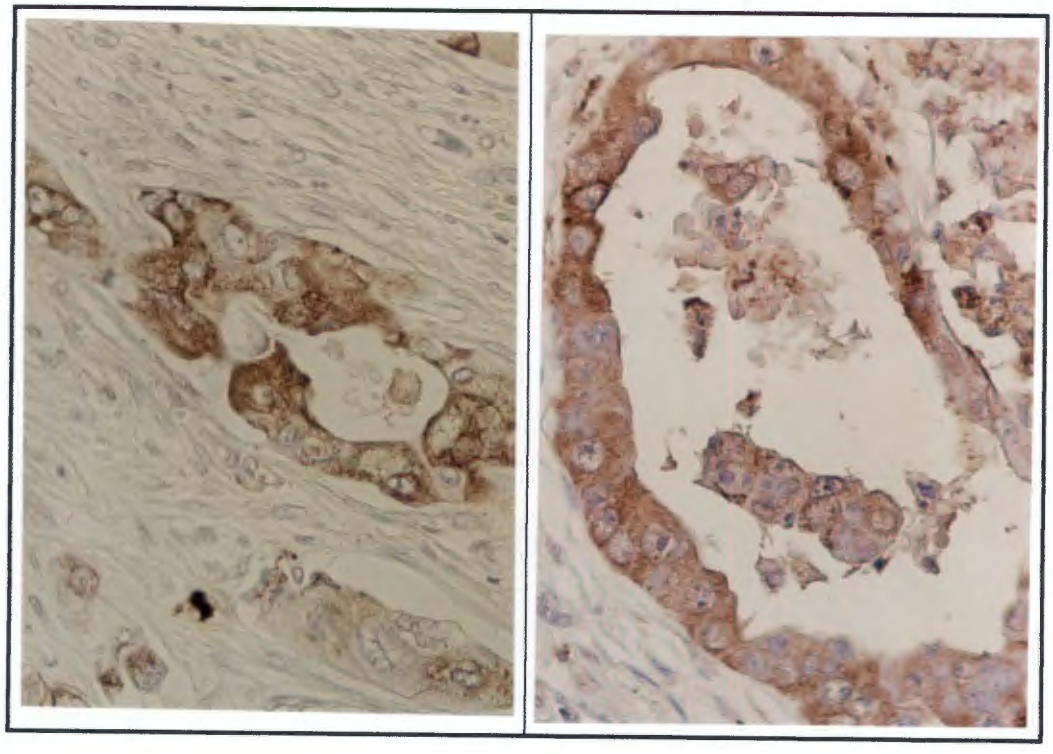

Figure 18. Advanced intestinal type adenocarcinomas showing expression of (a) *MUC1* and (b) *MUC6.*

Discussion of results and comparison to other studies

This is the first study to systematically examine all the *MUC* antibodies in human gastric mucosa, in normal, foetal and disease states by histochemical and immunohistochemical methods. Some of the immunohistochemical results in this study differ from those of previous studies. The following discussion, therefore, makes comparisons with other studies and sets out to show the validity of the findings in this study.

The histochemical findings are similar to those of other studies, which have shown that sialomucins are not found in appreciable amounts in normal gastric mucosa, gastric mucins being predominantly neutral [99], and that sialomucins are prominent in intestinal metaplasia [44,102].

Intestinal metaplasia, chronic active gastritis, and dysplasia have been shown to precede gastric carcinoma in many instances, particularly the intestinal type of adenocarcinoma [43-60].This has been discussed in more detail in the introduction. In this study a high association of sulphomucin secreting intestinal metaplasia with intestinal type adenocarcinoma was found (i.e 6 of 7 cases), which correlates with other studies, and a lower incidence was associated with gastritis (0 of 3) and benign ulcers (2 of 6). In addition all the cases of intestinal metaplasia associated with mild dysplasia showed the presence of sulphomucins. High grade dysplasia has been shown to have a higher risk of subsequent carcinoma, particularly intestinal type adenocarcinoma [38,43,50,64,71]. Mild dysplasia in itself is not thought to be an increased cancer risk [49], but

the fact that it has occurred together with type IIb intestinal metaplasia should perhaps alert one to follow these particular patients more carefully than if they had mild dysplasia alone. Further study is required in this area.

Sulphomucin positivity has been frequently observed in carcinomas [63,57]. Although the intestinal metaplasias in association with the cancers in this study showed sulphomucins only three of the cancers themselves showed a small amount of sulphomucin staining.

Human gastric mucosa has a specific mucin gene pattern that differs from that of other mucin-gene producing organs in the body [1,12]. In the following paragraphs the results of the immunohistochemical studies are discussed. In some respects these results correspond with other studies, but there are some differences.

Normal control stomachs were predominantly characterised by expression of *MUC 4, 5* and *6* predominantly. Previous reports [12] have shown *MUC 1* expression in normal gastric epithelium. Sakamoto et al [31] showed expression of *MUC1* predominantly in fundic glands and in parietal cells of normal stomachs. Weak *MUC 1* staining was only demonstrated in 20% of the normal controls in this study, which may be due to a technical problem. There was, however positive staining in 80% of the advanced intestinal adenocarcinomas, so that the lack of staining in the normal controls is difficult to explain, and needs further elucidation. The fact that most of the normal control material was obtained from cadaveric donors might be one explanation, as fixation may have been suboptimal. *MUC1* is thought to play an important role in tumour development [103], so that increased expression in the advanced adenocarcinomas in this study is not unexpected, and, in fact, supports its involvement in tumourigenesis. *MUC1* was also

positive in a large proportion of the gastritis cases. It is not possible to reach any definite conclusions as to the cause of this .

MUC 4, on the other hand, was expressed strongly and unequivocally in all normal stomachs, particularly in basally situated glands. This finding differs from some of the early studies in which *MUC4* was not found to be expressed in the stomach [8,12] , *MUC4* has, however, since been reported by a number of researchers. It was first reported by the author and colleagues in an abstract in 1996 (104). The presence of *MUC 4* in these cases appears genuine, as it was consistently and repeatedly positive in all normal stomachs. The whole *MUC4* gene has only recently been sequenced by Moniaux et al. (105), and these authors have now also reported finding *MUC4* in the stomach, in addition to the colon, cervix and lung. *MUC4* has been found in adult tissues, as well as in epithelial cells in embryos and foetuses. *MUC4* has been reported by Buisine et al (106), as being the first gene to be expressed in the foregut, where it can be detected as early as 6,5 weeks.

MUC5* and *MUC6 positivity in normal stomachs was similar to previous studies, except for the presence of *MUC6* in surface mucous cells, a finding that differs from other studies [8,25]. One study [25] has shown that *Helicobacter pylori* can induce surface mucin expression, but the normal controls in this study did not have histologically proven infection with *Helicobacter pylori*, and the reason for surface *MUC6* staining is uncertain.

MUC7 staining in neuroendocrine cells in normal control stomachs is unexplained. This has not been previously reported. It may be related to antigen retrieval methods, and is possibly a heat related artefact.

Foetal stomachs, in this study, were shown to have similar mucin gene expression to adult stomachs, but only from the second trimester onwards.

Disease processes in the stomach are associated with significant alteration in expression of gastric mucin genes. This was clearly shown in the study by Ho et al [12], who studied expression of *MUC 1 - 6* mucin genes in normal and diseased gastric epithelium. Other studies have shown increased expression of poorly defined antigens that occur in gastric cancer and intestinal metaplasia, but not in normal epithelium. Such antigens as SIMA and LIMA [27,52] are described, which may coincide with certain *MUC* genes, but thus far have not been identified as such. In a study by Filipe et al [27] it was shown that LIMA (large intestinal mucin antigen) was expressed in intestinal metaplasia and dysplasia in carcinoma bearing stomachs. Gastritis cases, in this study, showed similar immunoreactivity to normal stomachs, with *MUC4, 5*, and *6* staining, but there was also a greater amount of *MUC1* and *7* present. This study found that *MUC 2* and *3* expression is increased in intestinal metaplasia, corresponding with previous reports [12]. This was the case in all cases of IM whether it was associated with ulcers, dysplasia or malignancy. Mild dysplasias show a similar staining pattern to intestinal metaplasia in this study, and mucin gene product expression therefore does not assist in distinguishing one from the other. It was interesting to note that *MUC2* expression correlated with the presence of sialomucins. This was best assessed in cases of intestinal metaplasia, but could also be demonstrated in other categories.

Gastric cancers in this study showed striking differences in mucin gene expression as compared to the normal. Loss of mucin gene expression in early intestinal type adenocarcinomas was unexpected. There is a previous report of loss of mucin production in cancers, but these cancers were generally late cancers or less well differentiated cancers [52]. Very early cancers were not studied in other papers. Corresponding positive controls of intestinal metaplasia were present in

most of the early carcinoma specimens in this study, proving that the lack of staining does not have a technical basis.

Positive staining with multiple mucin antibodies was, however, found in the advanced intestinal type adenocarcinomas and in the diffuse adenocarcinoma group, and this corresponds with previous findings [12,27]. There is also considerable heterogeneity between different cancers in these two groups. *MUC1, 5* and *6* were identified most strongly in the advanced intestinal type adenocarcinomas, with variable expression of *MUC 2* in some cases. *MUC2, 5, 6* and *7* were expressed in various combinations in a large proportion of diffuse adenocarcinomas. Apart from one of the advanced intestinal type adenocarcinomas showing weak *MUC4* staining, there was a striking absence of *MUC4* staining in the latter two groups. Ho et al [12] showed that there was increased expression of mucin genes with advanced cancers as compared to early cancers, and that there was increased expression in well differentiated intestinal type adenocarcinomas as compared to the diffuse carcinomas. The results of this study are in agreement with Ho's finding of multiple expression in advanced cases. This multiple expression may reflect increasing "dedifferentiation" of the carcinomas. Alternatively the aberrant mucin genes may alter the biology of the cell so that it has greater potential for malignant transformation and metastasis.

Is there a role for mucins in the detection of precancerous/ early cancerous states?

As a result of the alterations that occur in mucin gene expression in disease states there has been an increasing interest in mucins as possible markers to detect pre-malignant states or early malignancy. Tumour markers useful for diagnosing gastric carcinoma at an early stage have not

been identified. Carcino-embryonic antigen and CA19-9 are markers that have been assessed in the past, but they are most often elevated in patients with incurable disease, and are, therefore, unsuitable for early detection of disease [107]. The association between mucin abnormalities and malignancy has been well described, and the identification of different mucin genes in humans has important applications for cancer research [19] in the detection and treatment of carcinomas in general, and also in their role as possible diagnostic and therapeutic agents [107].

This study was unable to show that using *MUC* antibodies on gastric biopsies has predictive value for detection of precancerous lesions. The mucins in gastritis did not differ significantly from those of normal controls. Intestinal metaplasia and dysplasia showed a similar staining pattern (increased *MUC2* and *MUC3*), and *MUC* immunohistochemistry is therefore of no use in separating the two conditions. It is possible that in the future screening tests for *MUC2* and *3* may alert the clinician to the presence of intestinal metaplasia, but even if this were the case intestinal metaplasia occurs in association with both benign and malignant conditions, and abnormal *MUC* expression is not a sensitive indicator of precancerous conditions. Advanced carcinomas in this study showed increased expression of different mucin genes, but by the time this amount of alteration in the mucin genes is present the carcinoma is already far advanced. At this point in time *MUC* antibodies are not freely available, and routine use of these antibodies, should they become available, is not a cost effective exercise.

The association of *Helicobacter pylori* with gastric disease

Of note is a very high prevalence of *Helicobacter pylori* in the population studied. As with other studies, this study found that the *Helicobacter* pylori did not adhere to the gastric epithelium

which showed changes of intestinal metaplasia, although the organisms could be found on adjacent "normal" epithelium. A relationship has been suggested between *Helicobacter pylori* infection and gastric carcinoma [51,82,108-112], particularly of the intestinal type. All the early intestinal type adenocarcinomas in this study, and 2 of 5 advanced intestinal adenocarcinomas had associated *Helicobacter pylori*, but none could be found in the diffuse adenocarcinomas. This may be because advanced malignancy, particularly diffuse adenocarcinoma presents an unfavourable environment for *Helicobacter pylori*, and this finding has been supported by other studies [25]. The organisms were most likely present earlier in the disease process in these stomachs. Metaplastic changes and advanced carcinoma are inhospitable to *Helicobacter pylori*, but it is uncertain if changes in mucin gene expression are implicated. Further investigations will be needed to assess the effect that *Helicobacter pylori* has on *MUC* gene expression, as well as the effect that changes in *MUC* gene expression have on *Helicobacter pylori*,and its ability to adhere to altered epithelium.

CONCLUSIONS

It can be concluded that specific human mucins are expressed in normal stomach, and that these become significantly altered in disease states. Foetal stomachs express the same mucin genes as adult stomach, but expression only begins from the second trimester. The mucins in gastritis do not differ significantly from the normal controls. Intestinal metaplasia and dysplasia show a similar staining pattern (increased *MUC 2* and *3*), and *MUC* expression is therefore of no use in separating the two conditions.

Mucin immunoreactivity appears to be switched off in early cancers, an observation which needs to be investigated further, while advanced intestinal type and diffuse adenocarcinomas show expression of multiple genes.

The clinical, pathological and biological significance of these patterns of mucin staining remains to be elucidated.

SUPPLIERS OF ANTIBODIES USED IN THE IMMUNOHISTOCHEMICAL STUDY

Biotinylated rabbit-anti-mouse antibody - Dako, Copenhagen, Denmark.

Biotinylated swine-anti-rabbit antibody - Dako, Copenhagen, Denmark.

Biotinylated rabbit-anti-chicken antibody - Biogenesis, Poole, U.K.

Strep-avidin HRP - Dako, Copenhagen, Denmark.

MUC1 - 7 antibodies were supplied by Professor Sam B. Ho, University of Minnesota and Veterans Administration Medical Center, Minneapolis (Table 4).

Mucin gene	Name	Type
MUC1	139H2	Mouse mAb[a]
MUC2	MRP	Rabbit pAb[b]
MUC3	M3P	Rabbit pAb
MUC4	M4P	Chicken pAb
MUC5	M5P	Chicken pAb
MUC6	M6P	Chicken pAb
MUC5 +MUC6	FHF	Rabbit pAb
MUC7	M7P	Chicken pAb

Table 4. Mucin core peptide antibodies

[a] mAb = monoclonal antibody
[b] pAb = polyclonal antibody

[Ref: 12 and personal correspondence]

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