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INTESTINAL METAPLASIA AND GASTRIC CARCINOMA

A THESIS SUBMITTED TO

THE UNIVERSITY OF GLASGOW

FOR THE DEGREE OF

DOCTOR OF MEDICINE

BY

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MARCH 1990

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I wish to acknowledge the use of diagrams of the staging, T classification and N classification of gastric cancer (Figures 3, 4 and 5) from Dr K Maruyama's monograph on the subject.

DECLARATION

I declare that the work of this thesis has not been previously presented for consideration of any degree. The work of the thesis and the analysis and presentation of the data was performed personally by myself with one exception, this being the classification of the gastric tumours using the Lauren system which was performed by myself and Dr I L Brown (MRC Path).

SUMMARY

Previous studies have suggested that gastric carcinogenesis is a multistage process involving first gastritis, then intestinal metaplasia, dysplasia of increasing severity and finally carcinoma. The exact role of intestinal metaplasia in the histogenesis of gastric carcinoma has been the subject of much debate. Recent work has identified an intestinal metaplasia variant (Type IIb) which appears to be significantly associated with a particular histological type of gastric carcinoma.

The aim of the studies carried out in the preparation of this thesis was to investigate the histogenesis of gastric carcinoma with particular reference to the role of intestinal metaplasia in this process.

The material studied was a series of gastrectomy specimens resected for both benign and malignant disease. In addition fetal stomachs, endoscopic biopsy material from a group of patients who had undergone gastric surgery for peptic ulcer disease, and an animal experimental model of gastric carcinogensis were studied to examine specific aspects of the carcinogenetic sequence. The methods utilised in the studies involved histological assessment, mucin and immunocytochemical techniques and cell kinetic analysis.

The results of the histological investigation indicate that the presence and amount of intestinal metaplasia in the gastrectomy material is related to variables such as age and inflammatory change rather than the presence of a tumour. The results also demonstrate that the presence of the type IIb variant is related to

age, inflammation and ulceration within the gastrectomy rather than the presence of any particular tumour sub-type.

The results of the mucin and immunocytochemical studies identified a series of phenotypic changes in the stages of gastric carcinogenesis which did not lend support to the role of intestinal metaplasia in the histogenesis of gastric carcinoma.

The cell kinetic study documented the changes in cellular proliferation that occur in the stages of gastric carcinogenesis. A series of cell kinetic abnormalities in the gastric mucosa of patients who had undergone gastric surgery for benign disease were identified. These abnormalities were shown to be related to alkaline reflux gastritis.

It is suggested that intestinal metaplasia and its variants do not represent a premalignant stage in the histogenesis of gastric carcinoma.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Intestinal metaplasia is defined as the appearance of intestinal epithelium in the stomach. The first description of this phenomenon was by Kupffer in 1883. Earlier this century the presence of intestinal epithelium in the stomach was regarded as a congenital heterotopia (Taylor 1927), caused by some "slight error of development" and plays "no part in the production of malignant disease". The embryological theory of the origin of intestinal metaplasia was challenged by Magnus in 1937 with his observation "the presence of intestinal epithelium in the stomach is the result of the faulty regeneration of surface epithelium in a mucosa repeatedly damaged by gastritis and is an example of metaplasia resulting from chronic irritation". In his study of 110 gastrectomy specimens, Magnus illustrated many of the features of intestinal metaplasia which have since been repeatedly demonstrated by successive studies:

1. Intestinal metaplasia involves the replacement of the columnar gastric epithelial cells and specialised gastric cells by goblet cells interspersed with columnar cells with a well developed brush border and Paneth cells at the base of the glands, and numerous argentaffin cells.
2. Intestinal metaplasia occurs only in association with gastritis.
3. Intestinal metaplasia is a common finding and occurs in two-thirds of gastrectomy specimens removed for both benign and malignant disease.

4. Emphasised the focal nature of the metaplastic process and the need to examine large amounts of the gastrectomy specimen to determine the true incidence of intestinal metaplasia.

The initial interpretation of intestinal metaplasia as an interesting congenital abnormality of the stomach thus shifted to a concept of an abnormal regenerative response in a mucosa damaged by chronic inflammation. By 1944 Warren and Meissner recognised intestinal metaplasia as the main epithelial feature of chronic gastritis and proposed the idea that chronic gastritis with the associated intestinal metaplasia might be a precursor of carcinoma of the stomach.

Three developments of major importance in defining the relationship of intestinal metaplasia to gastric carcinoma have emerged in the intervening 40 years since Warren and Meissner's work.

1. Jarvi and Lauren in 1951 recognised the histological heterogeneity of gastric carcinoma but identified a sub-set of carcinomas which displayed features characteristic of intestinal mucosa. On the basis of this finding they proposed a role for intestinal metaplasia in the histogenesis of this type of gastric carcinoma.
2. The second significant development appeared in consecutive papers by Morson in 1955 in the British Journal of Cancer. In his first paper, Morson repeated the earlier work of Magnus and examined large areas of gastric mucosa from 119 gastrectomy specimens from patients with duodenal ulcer,

gastric ulcer and carcinoma. He identified a higher incidence and extent of intestinal metaplasia in the stomachs removed for malignant disease.

This higher incidence remained even when allowance was made for age. In his second paper he illustrated five examples of gastric carcinoma arising from areas of intestinal metaplasia. This paper by Morson is the most frequently quoted reference in the literature on intestinal metaplasia and warrants close scrutiny.

The first example described by Morson is of a carcinoma with early invasion of the submucosa in a stomach in which the gastric epithelium is completely replaced by intestinal metaplasia. The second and third examples show transition from the metaplastic mucosa to carcinoma involving a gradual loss of differentiation of the metaplastic mucosa. The fourth and fifth examples show carcinoma in situ change in areas of intestinal metaplasia in gastrectomy specimens at a distance from the main tumour.

Morson's work is frequently misquoted as evidence that intestinal metaplasia is a premalignant state. His histological description is not of carcinoma arising directly from intestinal metaplasia but of a metaplastic epithelium undergoing dysplastic change and transforming into carcinoma with intestinal features.

Following Morson's work, Lauren 1965, extended his original observations on the histological appearances of

gastric carcinoma. He identified two major histological types of tumour; an "intestinal type" with features of intestinal epithelium as opposed to a "diffuse type" with the histological appearance of gastric epithelium. The implication of his study was that the two types of tumour have different origins, the intestinal type from intestinal metaplasia and the diffuse type from gastric epithelium.

From the work of Lauren and Morson intestinal metaplasia appeared to have some place in the histogenesis of gastric carcinoma. The clinical problem which became more apparent with the advent of endoscopic biopsy material was that intestinal metaplasia was a common finding in both benign and malignant disease. Twenty five years after his initial work Morson et al., 1980, defined intestinal metaplasia as a premalignant condition. A premalignant condition is regarded as a clinical state associated with a significantly increased risk of cancer, a premalignant lesion is a histopathological abnormality in which cancer is more likely to occur than in its apparently normal counterpart. Intestinal metaplasia represents the premalignant condition, dysplasia the premalignant lesion which may occur in both gastric epithelium and intestinal metaplasia.

3. The third most recent work was by Jass in 1980 who identified a variant of intestinal metaplasia - type 11b which was significantly associated with "intestinal type" cancers but not with "diffuse type" or benign lesions. Jass suggested

that this variant might be a premalignant form of intestinal metaplasia.

The hypothetical histogenetic sequence outlined in Figure 1 is a summation of the work originated by Magnus and culminating in Jass's description of the type IIb variant. This thesis is concerned with the investigation of aspects of this hypothesis.

The sequence outlined in figure 1 represents a simplified hypothesis for the histogenesis of gastric carcinoma based on this brief introduction. Chapter 1 is a review of the literature which examines the evidence for the various stages of the sequence.

The epidemiology, aetiological factors and current concepts of therapy and prognosis of gastric carcinoma are examined in the first section. In the second part the classification systems for gastric carcinoma are detailed and discussed. The third part involves a review of the premalignant epithelial alterations that occur within the stomach and that are outlined in figure 1. The fourth area to be reviewed is the phenotypic changes in the gastric epithelium that can be identified by mucin histochemistry and immunocytochemistry at the various steps of the sequence. The fifth section examines the changes in cell kinetics that accompany gastric mucosal abnormalities and animal experimental models of gastric carcinogenesis. The sixth part reviews the relationship of the cell mediated immune

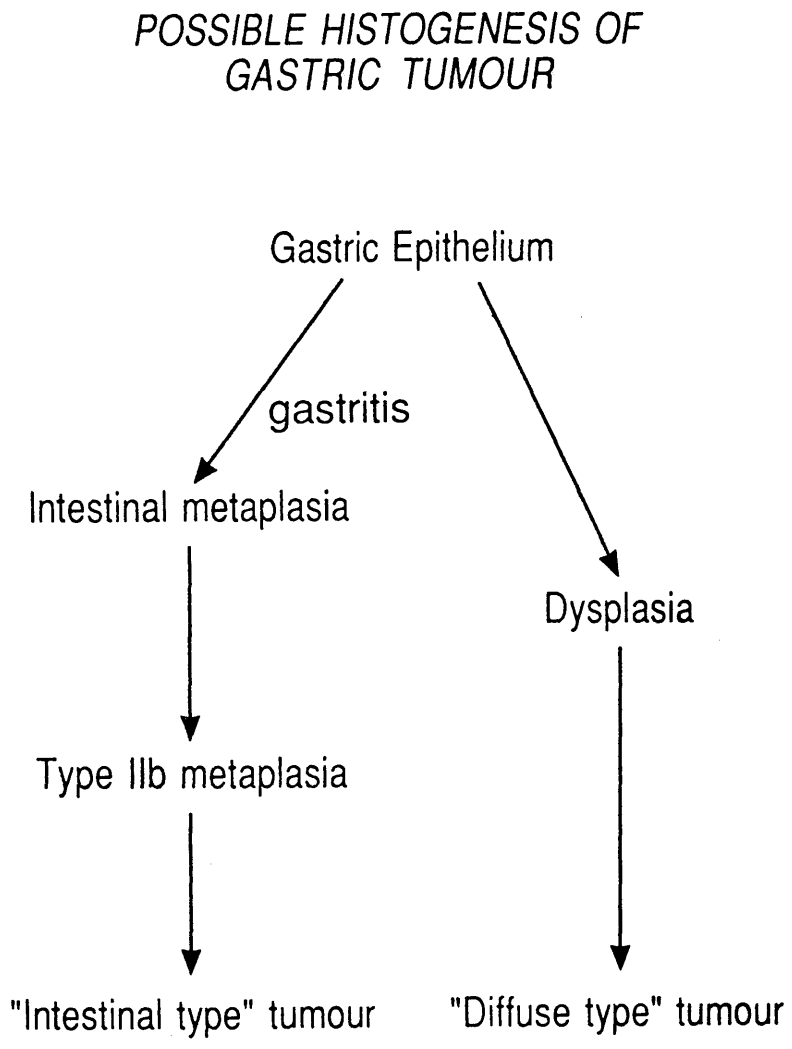


Figure 1. **Hypothetical sequence for the histogenesis of gastric carcinoma.**

response in the lamina propria to the gastric epithelial changes in the sequence. The final section briefly reviews the current knowledge regarding the embryonic development of the stomach and the relevance of this to gastric carcinogenesis.

Following the literature review a more complex hypothetical sequence for gastric carcinogenesis can be drawn. The specific aims and areas of interest of this thesis are discussed in relation to this sequence at the end of Chapter 1.

PART 1

GASTRIC CARCINOMA: EPIDEMIOLOGY, AETIOLOGY, PROGNOSIS AND TREATMENT

EPIDEMIOLOGY

Gastric carcinoma accounts for 24.8 male and 16.6 female deaths per hundred thousand population in England and Wales per year (OCPS, 1982). This places stomach cancer as the fourth commonest cause of death from malignant disease. As with most other cancers the mortality rate rises almost exponentially with age.

A constant worldwide feature is the decline in the incidence of gastric carcinoma. In terms of mortality rates (world standardised) there has been a decline in incidence from 1950-1978 (Waterhouse, et al., 1983). Over this period the mortality rate in USA and England and Wales has shown an almost linear decline to approximately one-half of the initial rate. It has been suggested that this worldwide decline may be due to the refrigeration of food replacing spices, smoking and pickling in the preservation of foodstuffs (Waterhouse et al., 1983).

International comparisons

Crude mortality rates are not suitable parameters to make comparisons between different countries because of the differences in age structure that exist particularly between industrialised and Third World Countries. A comparison of age adjusted mortality rates from the Segi Institute of Cancer Epidemiology (1984) compares the rates between countries (see figure 2). There is a striking variation in the frequency throughout the world. Japan

Age adjusted male death rates for cancer of the stomach per 100,000 population.

Country	*Rank*	*Death rate*
Japan	1	50.40
Chile	2	46.06
Portugal	6	30.88
Uruguay	12	24.04
Germany	15	22.58
Scotland	20	18.77
England/Wales	22	18.06
Norway	31	14.81
France	35	13.08
USA	42	6.4
Nicaragua	46	0.11

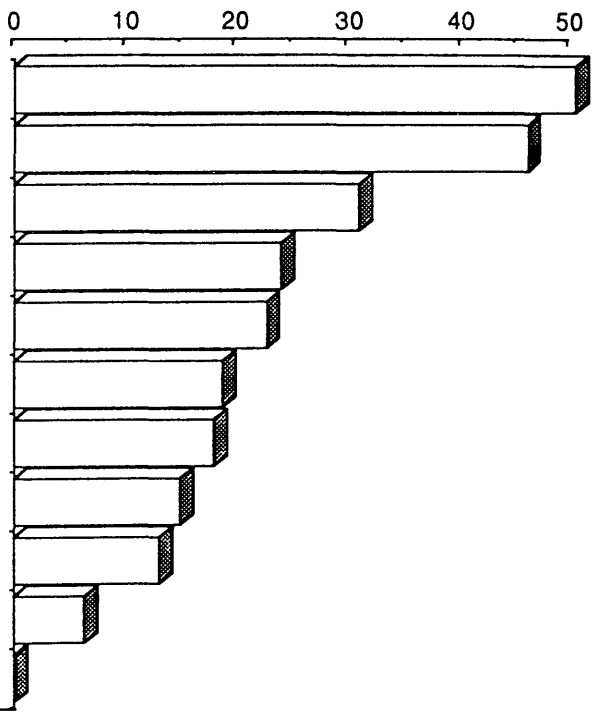


Figure 2. Age adjusted male death rates for cancer of the stomach per 100,000 population in various countries. Data derived from Segi Institute of Cancer Epidemiology (1984).

and the United States, both highly industrialised countries, are at the top and near the bottom respectively; and the same disparity is true of less developed countries such as Chile and Nicaragua. The countries with a high risk of gastric carcinoma have been shown to have a high incidence of the "intestinal type" carcinoma (Munoz et al., 1968; Correa et al., 1973). Further epidemiological studies with age specific comparisons have shown no differences in the type, site or morphology of gastric carcinoma between high and low risk countries (Kubo, 1974). The controversy regarding this point will be further discussed in the classification of gastric carcinomas.

AETIOLOGY

Diet

The results of studies into the effect of diet on gastric carcinoma have been confusing with uncertain conclusions. Diet is difficult to study accurately. In gastric cancer dietary factors which may be most important are those of decades before. Eating habits are inextricably linked to culture and socio-economic status.

The factor in diet considered to be most related to gastric carcinoma is nitrate. The nitrate ion itself is relatively innocuous, however certain species of bacteria found in the mouth and sometimes the stomach can enzymatically reduce nitrate to nitrite (Reed et al., 1981). Nitrite represents a potential hazard as its participation in nitrosation reactions may give rise to N-nitroso compounds which are carcinogenic. Following this argument

there might be expected to be a relationship between the level of nitrate ingestion and gastric carcinoma. In the most well controlled and detailed study in the UK no direct relationship between environmental nitrate levels and gastric carcinoma risk has been demonstrated (Forman et al., 1985). This apparent lack of correlation between environmnental nitrate levels and gastric carcinoma may have several explanations. Firstly, nitrates are not related to gastric carcinoma or secondly, that exposure to nitrates per se does not increase the risk of gastric carcinoma but that enzymatic reduction of the nitrate to nitrite is required. The experimental evidence to support this second explanation comes from studies in the hypochlorhydric stomach . These studies show that in conditions of low acidity N-nitroso compounds are generated by the presence of nitrate reductase from gastric flora (Reed et al., 1981). This rise in gastric pH favouring the production of N-nitroso compounds fits well into the hypothesis of atrophic gastritis and intestinal metaplasia acting as precursor states which predispose to gastric carcinogenesis.

Polycyclic hydrocarbons are potential carcinogens widely distributed in food particularly if smoked or grilled. Experimental evidence has shown that they produce tumours in rats (Dungal, 1961) and may be responsible for the high incidence of gastric carcinomas in communities with a high proportion of smoked food in the diet.

Two other dietary factors which have been shown to be associated with hypochlorhydria and a degree of gastric atrophy are

the ingestion of salt and protein malnutrition (Thomason et al., 1980; Joossen and Geboers, 1983). These factors are not thought to be carcinogenic but may result in damage to the gastric mucosa, cause achlorhydria and create the environment for the formation of N-nitroso compounds.

Genetic factors

Apart from sporadic reports in the literature of a "gastric cancer family syndrome" in which there is a high incidence of cases within a kindred family (Woolf and Isaacson, 1961), there are three lines of evidence for a role for genetic factors in the aetiology of gastric cancer; blood group associations, predisposing diseases, and risk of the disease in relatives of gastric cancer patients.

In 1953 Aird et al., noted that "the frequency of blood group A is greater and the frequency of blood group O is less in patients suffering from cancer of the stomach than in the general population .....". These findings have been confirmed by other workers (Roberts, 1959; Hoskins et al., 1965). The increased risk is modest, relative risk of gastric cancer in patients with blood group A compared to blood group O is 1.2. The blood group association is linked to the "diffuse" type of tumour, but not the "intestinal type" (Correa et al., 1973).

Pernicious anaemia and associated atrophic gastritis are thought to constitute a predisposing disease to gastric cancer. Pernicious anaemia is genetically determined and is itself associated with blood group A (Carter 1969; Callender et al., 1971). There is debate in the literature concerning the reality

of the risk associated with pernicious anaemia. The main point of dispute is the selection of a normal population to compare with pernicious anaemia sufferers. The risk of gastric cancer in pernicious anaemia patients has been estimated to be 3-6 times that of a "normal population" (Caygill et al., 1984; Svendsen et al., 1986).

Relatives of patients with gastric carcinomas appear to be at risk of developing gastric cancer depending on the histopathology of the tumour. Lehtola (1978), in an extensive review showed that "intestinal" type carcinoma conferred no excess risk, but relatives of patients with the "diffuse type" had a sevenfold risk.

Iatrogenic causes of gastric carcinoma

The possibility that surgical treatment for peptic ulceration may be an iatrogenic risk factor in the development of gastric carcinoma is a controversial subject. There is a large body of published evidence which would appear to support the claim that previous gastric surgery for peptic ulceration leads to an increased incidence of gastric carcinoma.

The published data suggests that there is a latent period of 15-20 years from gastric surgery after which the risk of dying from gastric cancer is increased by three to six times in these patients (Stalsberg and Taksdal, 1971; Watt et al., 1984C; Caygill et al., 1986; Viste et al., 1986). The risk appears to be greater in patients who have undergone surgery for the treatment of gastric rather than duodenal ulcer (Caygill et al., 1987) and a Billroth II resection confers greater risk than a Billroth I gastrectomy. The

increased risk of gastric carcinoma is not restricted to patients who have had gastric resection but is also reported following truncal vagotomy and drainage procedures (Stalsberg and Taksdal, 1971; Totten et al., 1983; Caygill et al., 1986). There is some evidence that the latency period between surgery and the development of carcinoma is shorter in the vagotomy and drainage group than in the gastrectomy patients (Totten et al., 1983; Watt et al., 1984C).

The evidence from some studies relying solely on necropsy material must be interpreted carefully and not all studies have confirmed the increased incidence of gastric carcinoma (Ross et al., 1982), following surgery for benign disease.

If one accepts the observed increased risk of gastric carcinoma following surgery for benign disease then it is important to examine this finding in the context of other causes of death in these patients. Most studies demonstrate that the excess mortality from smoking related disease was greater by a factor of three or more than the total mortality from gastric carcinoma (Ross et al., 1982; Caygill et al., 1987). These findings have led some authorities to suggest that persuading patients to stop smoking after peptic ulcer surgery would be of greater benefit than any form of post-operative endoscopic screening programme (Logan and Langman, 1986).

The aetiology of this reported higher incidence of gastric carcinoma in the post-operative stomach requires to be fully answered. Achlorhydria or hypochorhydria resulting in bacterial

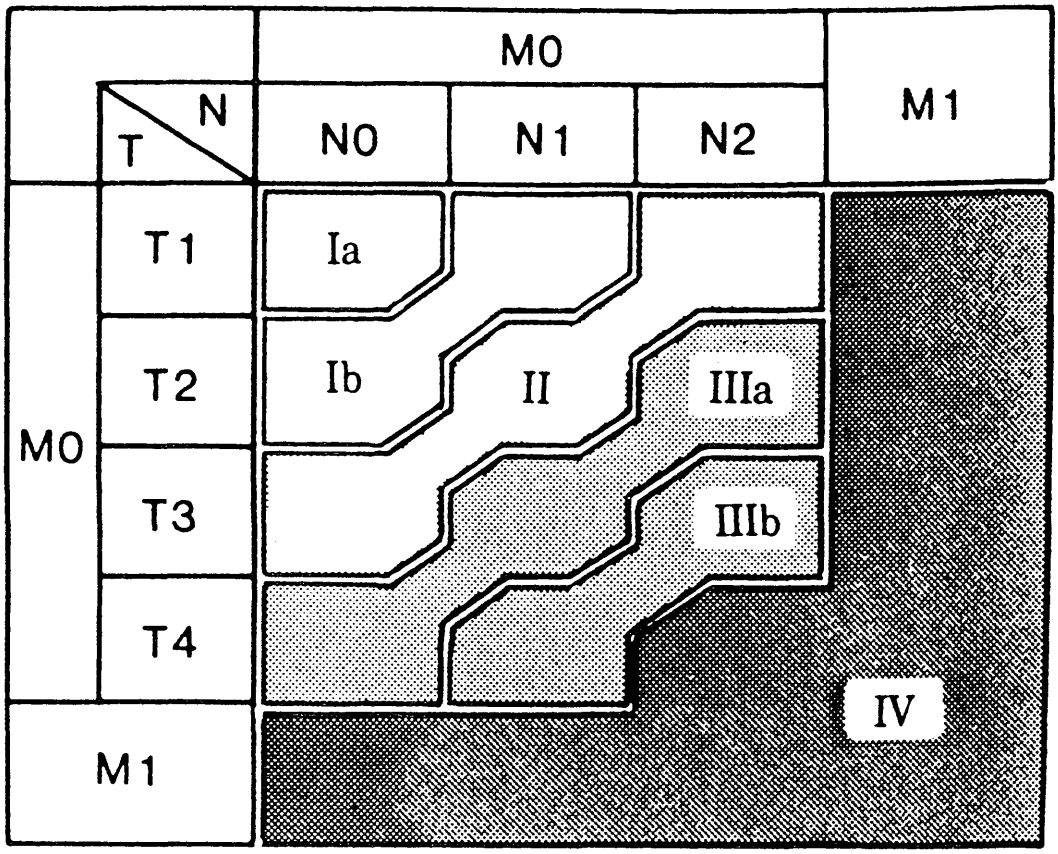


Figure 3. **TNM staging for gastric carcinoma UICC, AJC and JRSSC (1985)**

From the monograph "Surgical Treatment and End Results of Gastric Cancer" by Dr K Maruyama.

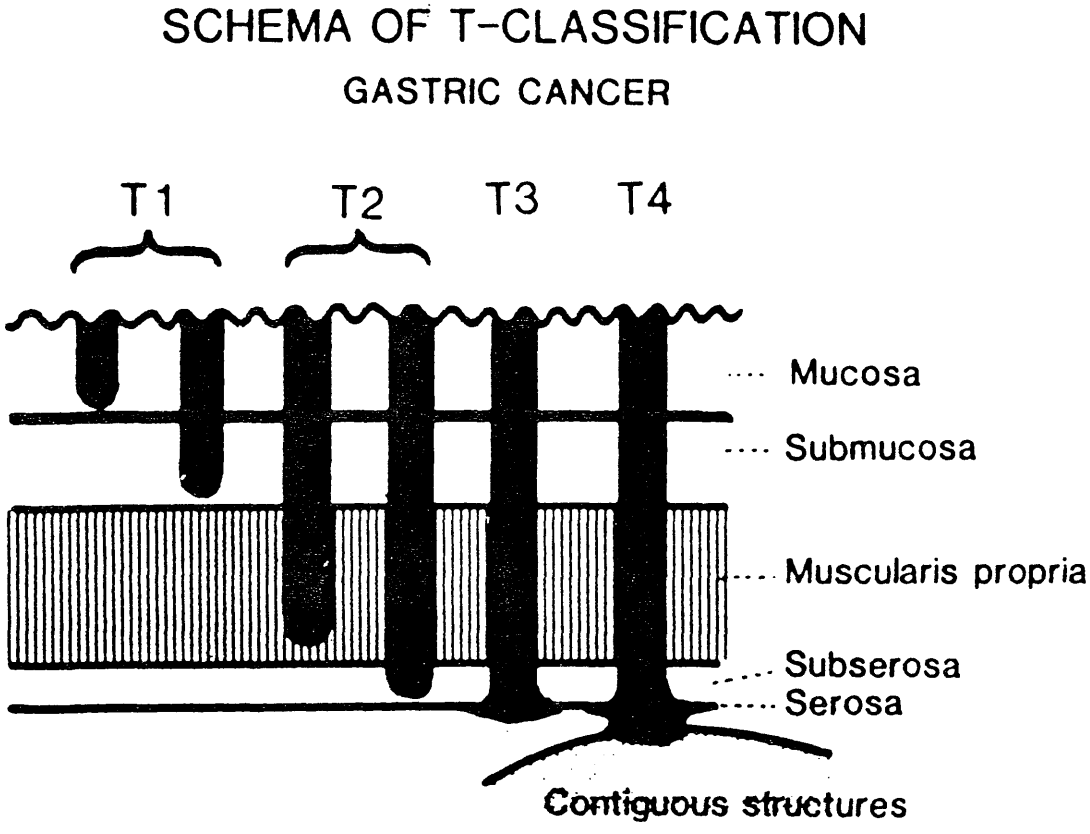


Figure 4. Diagrammatic representation of T staging (depth of penetration) for gastric cancer.

From the monograph "Surgical Treatment and End Results of Gastric Cancer" by Dr K Maruyama.

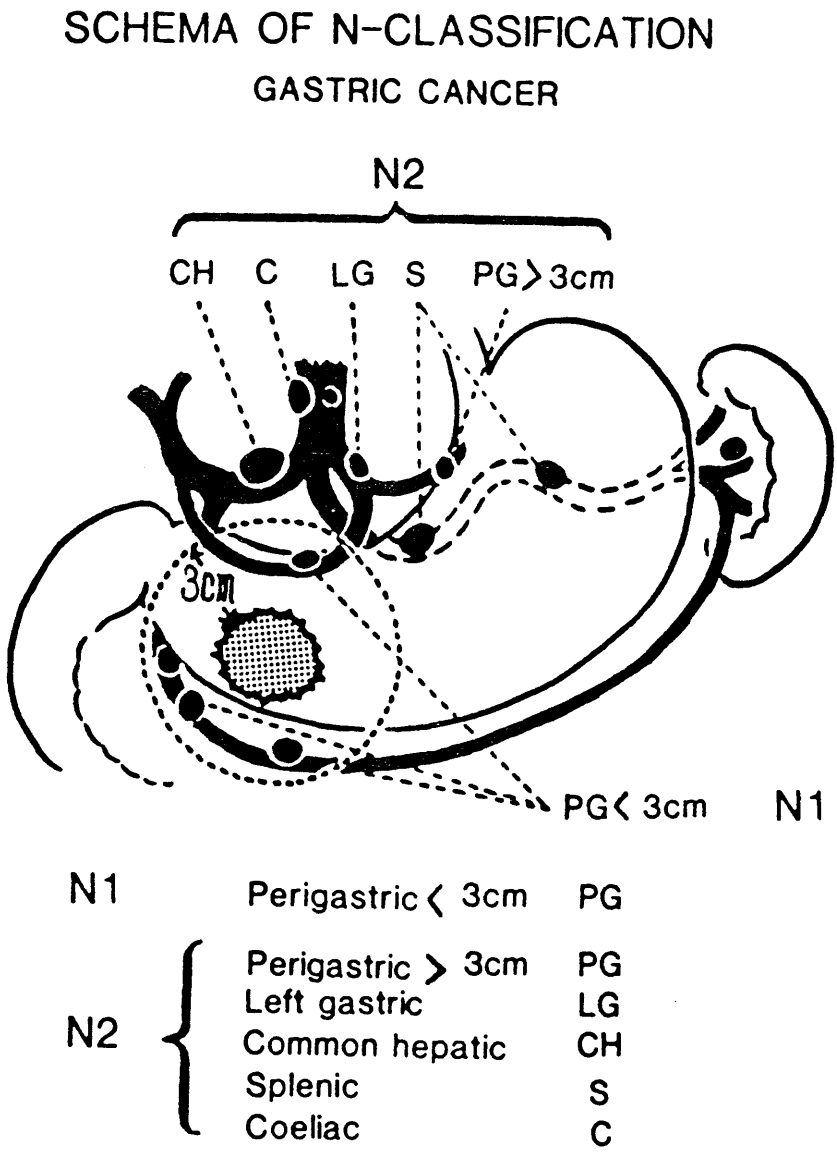


Figure 5. Diagrammatic representation of N staging (Nodal) for gastric cancer.

From the monograph "Surgical Treatment and End Results of Gastric Cancer" by Dr K Maruyama.

growth and N-nitrosamine formation (Reed et al., 1981) and bile reflux (Domellof, 1979) are two of the factors which may be implicated in carcinogenesis. The exact mechanism and the importance of these post-operative changes require further study.

PROGNOSIS AND STAGING

The staging of gastric carcinoma is based on the T. tumour, N. Node, and M. Metastasis system. The most recent staging system was agreed by the UICC, Japanese Research Society for Gastric Cancer (JRSSC) and the American Joint Committee (AJC) in 1985 and takes into account that survival is most strongly related to both the depth of invasion and the lymph node metastasis. This system adheres to the basic principle of TNM staging that "each stage group is more or less homogenous in respect of survival and the survival rates in the four stage groups are distinctive". This new TNM classification was based on statistical analysis of 15,589 cases from Japan and 4,785 cases from the USA of primary resectable gastric cancer (figure 3).

The depth of penetration of the tumour is shown clearly in figure 4 and is self-explanatory. The nodal status employed by the Japanese is based on the concept of defined rings of nodes (figure 5). N_1 nodes are perigastric nodes in the greater or lesser omenta within 3 cm of the tumour. Spread then continues via the arterial supply, left gastric, right gastric and right and left gastro-epiploic arteries to involve the coelic, suprapyloric, infrapyloric and splenic nodes respectively and these constitute the second ring of N_2 nodes. The M stage involves metastases to

different organs ie. liver or nodes outwith the N_2 ring. The third ring of nodes involves the nodes around the abdominal aorta - retrapancreaticoduodenal, middle colic, and hepato-duodenal arteries.

Table 1 outlines the major series in the American and European literature compared with the figures from the Japanese Research Society (1973) published by Maruyama in 1982.

The first (Oschner et al., 1958) and last (Dupont et al., 1978) series originate from the same American centre. The 5 year survival has not increased (and shows a slight decrease) over the past 20 years. The Japanese figures show a stark contrast with the American and European experience. Although there are minor differences in the survival rates in the Western literature there is nothing to compare with the difference between the West and Japan. The Oriental and Occidental cultures are not only different but so is the philosopy to gastric carcinoma; the Japanese are reporting increasing resectability rates coupled with improving five year survival (whereas in the West an attitude approaching therapeutic nihilism prevails). Why is there such a difference? Many Western clinicians write off the Japanese success by claiming that the Orientals are dealing with a different disease, treating at an earlier stage and that the biological behaviour of the tumours are different.

What evidence is there for stating that the disease is different? Table 2 compares a European series classified according to the Japanese histological criteria.

Series	No of Patients	Resectability %	5 yr survival	Hospital mortality
Oschner et al. 1958	193	33	7.5%	9.3%
Remine et al. 1966	11,817	63	20.0%	8.9%
Nadler et al. 1968	680	20	13.0%	-
Hawley et al. 1970	205	-	19.4%	25.0%
Hoerr et al. 1973	478	63	15.0%	10.0%
Desmond et al. 1976	1,363	53	8.0%	25.0%
Costello et al.1977	226	66	8.5%	-
Cady et al. 1977		58	10.6%	11.0%
Buckholtz et al.1978	201	70	11.5%	-
*Japanese 1973	12,535	80	53.25%	1.6%
Dupont et al. 1978	1,497	48	7.4%	26.5%

*Japanese Research Society for Gastric Cancer (Maruyama, K. 1982)

Table 1: Major published series of gastric cancer with resectability rates, 5 year survival for surgical treatment and hospital mortality.

	Histological Type				
Series	Tubular	Signet ring cell	Papillary	Mucinous	Undifferentiated
Paginini & Rugge 1982	62%	10.5%	6.5%	10.5%	10.5%
Maruyama et al., 1982	52%	25.0%	5.0%	4.0%	23.0%*

*calculated

Table 2: Comparison between histological types of gastric cancer in Europe (Paginini and Rugge, 1982) and Japan (Maruyama, 1982)

The published standard photographs of the Japanese classification correspond to those of the WHO system. From the table (table 2) the broad proportions of the tumour types are similar, with difference in numbers between the Western and Eastern series it would not be appropriate to draw any more detailed comparison.

A more detailed comparison between the European and Japanese experience has been undertaken in the specific area of "early gastric cancer" (Evans et al., 1978). Morphologically and histologically there appeared to be no difference between the two groups and importantly, this study involved discussion between Western and Japanese pathologists.

The Japanese have the highest incidence of gastric carcinoma in the world. The combination of screening programmes and high public awareness results in 14% of tumours (Miwa, 1979) being identified at the T_1 stage, compared with 1-2% in most Western series (Kennedy, 1970). The Japanese have rigid protocols for pathological staging and therapy but unfortunately this is not so in the West. Large multicentre reports from European centres have a complete absence of TNM staging (Lundh, 1974). A higher incidence of early lesions in Japanese series does undoubtedly improve survival overall. Comparison of stage and survival between East and West is difficult because of the aforementioned lack of standardisation of criteria. Hoerr's (1973) personal series of 498 is the only Western study with adequate pathological data that can be compared with the Japanese. The overall 5 year survival is 15%: comparable to most Western series. However the

5 year survival rate for patients undergoing a "curative" resection was 42%. The staging used by Hoerr involved stage AI which was defined as superficial cancers with negative nodes and was equivalent to Stage I, tumours extending on to the serosa or spreading to regional nodes are Stage AII, BI and BII in Hoerr's classification and Stage II and III in the Japanese classification.

A comparison of the five year survival rates between the series published by Hoerr (1973) and the Japanese data from Maruyama (1982) is shown in table 3.

This data suggests that the progress in surgically treated early lesions is not greatly different between East and West but that the Japanese are better at treating more advanced lesions. To support this the Japanese have noted that the prognosis of early lesions has not changed significantly over the past 20 years, the improvement in survival has come from the treatment of advanced lesions (Nagata et al., 1983).

Is there a difference in the biological nature of factors affecting prognosis between the West and Japan? The two factors identified by the Japanese which most affect prognosis are the depth of penetration of the primary tumour and the nodal status. These two factors have also been shown to be the main prognostic determinants in several series from the West (Monafo et al., 1962; Hawley et al., 1970).

In summary, the Japanese gastric cancers appear histologically similar to those in the West. Although the Japanese see a greater proportion of early lesions their survival

	Stage I	Stage II + III
Japan (Maruyama)	96% (34)	53 (21.2)
USA (Hoerr)	91% (12)	38 (57)

Stage I = Stage Al (Hoerr) = Superficial tumours, node negative

Stage II + III = Stage AII, BI, BII (Hoerr) = Extension to serosa ± node positive

(Figures in parentheses % of total number of cases)

Curative Gastrectomy = Resection extends to a greater degree of node clearance than the tumour

Table 3: Comparison of five year survival of "curative" gastrectomy between USA (Hoerr, 1973) and Japan Maruyama, 1982).

figures are better for more advanced lesions and the prognostic variables appear similar to those in the West.

TREATMENT

The mainstay of current therapy for gastric carcinoma is surgical excision. Surgical treatment may be broadly divided into curative and palliative. As the depth of penetration and spread to nodes are the two most important prognostic variables, a curative resection must be directed towards these factors.

To treat effectively and compare methods of treatment an exact definition of therapy is required. The Japanese have introduced this concept.

In addition to the TNM staging of gastric carcinoma, the Japanese have also introduced the concept of standardisation in the type of resections performed for carcinoma of the stomach and by such standardisation the type of surgical treatment can be assessed ie "curative" or non-curative. Four types of gastrectomy are described:

R_0 Gastric resection including the incomplete removal of lymph nodes of Group 1.

R_1 Gastric resection including the complete removal of Group 1 lymph nodes (N_1).

R_2 Gastric resection including the complete removal of Group 1 and Group 2 lymph nodes.

R_3 Gastric resection including the complete removal of Group 1, 2, 3 lymph nodes.

In practical terms the R_1 resection involves clearance of the

	No of cases	Survival %
Curative resection		
Absolute	2,706	78
Relative	823	39
Non-curative resection		
Relative	281	16
Absolute	923	1.4

Table 4: Cumulative 5 year survival based on radicality of surgical resection as determined by histology (Maruyama et al., 1982)

N_1 group of nodes in the lesser and greater omenta. The R_2 resection involves clearance of the N_2 and involves splenectomy and hemi pancreatectomy. An R_3 resection may require partial colectomy, hepatectomy, sub total pancreatectomy or pancreatico-duodenectomy.

For each case the resections are classified into:

(a) Curative resection

A. Absolute curative resection

B. Relative curative resection

(b) Non-curative resection

A. Relative non-curative resection

B. Absolute non-curative resection

An absolute curative resection is defined as R >N ie. the resection has extended to a greater degree of lymph node clearance than the tumour, a relative curative resection R = N. In both these categories M = 0 and resection margins (proximal and distal) are greater than 5 cm. In a relative non-curative resection M = 0, R = N, but resection margins are not clear. In an absolute non-curative resection there is clear residual cancer.

This system has the advantage of standardisation and combines surgical and pathological data. The prognostic implications of the types of resections are shown in table 4.

The Japanese are at a considerable advantage over those in the West, being able to perform a large number of curative resections. Unfortunately there is no comparable data from Western sources as the definition of curative is usually based

solely on operative staging without pathological input (Lundh et al., 1974).

Two-thirds of patients in the West will have such advanced disease (Hoerr, 1973) that palliation is all that can be offered. The type of palliation required depends on the clinical context and the predominant symptoms. The common distressing symptoms are obstruction, pain or haemorrhage.

Failure of therapy

Table 4 shows that approximately 40% of patients who had a curative resection failed to survive five years. The pattern of recurrence in patients who have had a gastric resection is predominantly local and/or regional to the area of the gastric bed, or to the regional lymph nodes (Maruyama, 1982).

Adjuvant therapy

To try to overcome the local and regional node recurrence attention has been directed to the use of chemotherapy and radiotherapy as an adjunct to resection. No randomised controlled trial has shown any benefit from adjuvant therapy. However trials with multi-agent cytotoxics in combination with radiotherapy are being undertaken but no results are yet available.

PART 2

PATHOLOGY AND HISTOLOGICAL CLASSIFICATION

PATHOLOGY

Neoplasms of the stomach are predominantly adenocarcinomas. In the largest unselected series reported with histological data the Third National Cancer Survey 1969-1971 (Cutler and Young, 1975) from the USA reviewed 5041 gastric neoplasms, of which 7.2% were not adenocarcinoma and comprised lymphomas, squamous carcinomas and soft tissue tumours. This thesis deals with adenocarcinomas, any reference to gastric cancer or carcinoma may be taken to be adenocarcinoma unless otherwise specified.

HISTOLOGICAL CLASSIFICATION SYSTEMS

"All tumours are different. Some tumours are more different than others".

The histological classification of gastric carcinoma is a complex problem. Any classification system is a compromise; artificially grouping tumours into loosely defined categories. The biological nature of gastric carcinoma with widely varying structure within a single tumour makes attempts at classification especially difficult.

There are four main classifications for gastric carcinoma in the recent literature: WHO 1977, Mulligan and Rember (1972), Lauren (1965), Ming (1977). The simplest method of classification is on purely descriptive histological grounds and this is the basis of the WHO system. The three remaining systems have examined

gastric carcinoma on a more conceptual level. Mulligan (1972) identifies three morphological types of gastric carcinoma and relates this to the origin of the tumour from different gastric cell types in a histogenetic classification.

Lauren (1965) divides gastric carcinomas into two main types on the basis of a combination of morphological variables. The two types have differing epidemiological and clinical behaviour and Lauren himself describes this classification as "histo-clinical".

Ming (1977) divides gastric cancer into two types, infiltrative and expanding using the mode of growth of the tumour as his main criteria. Like Lauren (1965) this patho-biological classification results in two groups of tumours with differing clinical and epidemiological features.

These attempts at classification almost reach a complexity which mirrors that of gastric carcinoma itself. The fact that so many classifications exist serves to illustrate the problem which confronts the histopathologist in categorising gastric tumours.

None of the systems are free from criticism. Each attempt at defining sub-groups has illustrated some facet of the histology of gastric carcinoma which was previously not appreciated. The classifications, which are now discussed in detail, should not be regarded as such, but as contributions to the understanding of the histological diversity of gastric carcinoma.

Mulligan classification

The initial classification proposed by Mulligan and Rember (1972) was updated by Mulligan (1975) and is based on a

histological analysis of 297 gastric carcinomas. Mulligan (1975) argues that the gastric mucosa contains a variety of cell types; the mucous cells of the crypt surface, the mucous neck cells, the pyloric and cardiac gland cells deep in the mucosa of the antrum and cardia, parietal and chief cells, metaplastic intestinal epithelial cells and Paneth cells. All of these cell types theoretically may be progenitors of gastric carcinoma. Using this histogenetic concept Mulligan (1975) classifies the tumours into three main groups, (1) mucous cell, (2) pyloro-cardiac, (3) intestinal cell.

Mucous cell

The hallmark of this type is the signet ring cell. These cells are arranged in cords, widely invading and eliciting a stomal response in the form of a dense fibrosis with a marked lymphocytic infiltrate. Microscopic satellite foci often appear in the mucosa adjacent to the main carcinoma. The cell of origin is presumed to be from the mucous cells and the mucous neck cells.

Pyloro-cardiac

These tumours tend to be located in the antrum or the cardia and are presumed to arise from the pyloric and cardiac gland epithelial cells deep in the gastric mucosa. Macroscopically the tumours are well demarcated and tend to fungate into the lumen. Microscopically the tumours form glands of varying size lined by cells which have stratified or in a single layer. The cells vary from low to tall but have central to basal nuclei, clear cytoplasm and distinct cell borders. The cells are strongly PAS positive.

These tumours tend to spread both into the lumen and into the deeper layers of the stomach. Deep invasion is not associated with inflammatory or fibrotic response unless there is marked ulceration of the tumour.

Intestinal Cell

This is subdivided into two types:

Intestinal Cell 1: The tumour is composed of glands formed from stratification of cells with indistinct cell borders and irregular nuclei. Polymorphonuclear leucocytes are often present in the centre of the glands and lymphoid tissue is associated.

Intestinal Cell 2: The tumour is formed by loose sheets of tumour cells with a few small glandular areas with intermingled lymphoid tissue.

Ming Classification

Ming (1977) bases his classification on a study of 171 gastric carcinomas. Ming argues that the classification systems previously described are based on purely descriptive histological features and do not represent the biological behaviour of the tumour. His classification divides gastric carcinomas into two groups, expanding and infiltrative, using the mode of growth of the tumour. This system incorporates the biological behaviour and the resultant appearance of the tumour.

Expanding type: The characteristic features of this type is the presence of aggregates of cells maintaining a

coherent relationship. The periphery of the cell aggregates is sharply delineated and the surrounding tissue compressed. The aggregates of cells in well differentiated tumours contained well formed glands.

Infiltrative type: In contrast to the first type the tumour cells are isolated individual cells. Occasional aggregates of cells are present. The tumours were composed of cells of differing degrees of differentiation, the well differentiated cells appearing as signet ring cells.

Lauren Classification

Lauren drew attention to the intestinal features displayed by many gastric carcinomas before Mulligan's publication of his classification (Jarvi and Lauren, 1951). Lauren (1965) subsequently reviewed 1344 cases of gastric carcinoma in an effort to establish whether a distinct group of carcinomas with intestinal features could be identified, and if by approaching the classification of gastric carcinomas in this manner other groups of gastric carcinomas with specific histological properties could be determined. On the basis of four structural characteristics Lauren divided the tumours into two main groups, intestinal and diffuse.

(1) General structure: Intestinal type carcinoma are composed of distinct glandular lumina. Diffuse carcinoma exhibited single or small clusters of cells with little cohesion.

(2) Cell structure: Intestinal type carcinoma are composed of large

cells of variable shape with irregular hyperchromatic nuclei.

The cells lining the glandular lumina possessed a well developed brush border. The diffuse tumour cells tend to be smaller and more regular in shape with more uniform nuclei. Enterochromaffin cells stained by the argyophil and argentaffin technique are more frequent in diffuse type carcinoma.

(3) Secretion: Mucous secretion products are present in scattered cells in 80% of the intestinal type tumours. In diffuse type carcinomas all tumours display secreting cells in almost all of the tumour cells. In intestinal type tumours the secretion forms a distinct theca in the cytoplasm: in diffuse carcinomas it is evenly distributed in the cytoplasm.

(4) Mode of Growth: Intestinal type carcinoma spread in a clearly defined area, but with variation in structure between the centre of the tumour and the periphery. Large glandular lumina are present in the central area whilst at the margin of spread small solid cords of cells.

Diffuse carcinoma forms poorly defined tumours and this pattern of growth was consistent throughout the tumour.

The mode of growth differ in three other aspects:

mucosal infiltration, connective tissue proliferation and inflammatory response. Intestinal tumours infiltrate the mucosa directly above the tumour resulting in ulceration. Diffuse tumours spread widely within the mucosa and do not result in ulceration as the surface mucosa remains intact. Diffuse tumours provoke a marked stromal proliferation compared with the intestinal type. Intestinal type tumours provoked a largely polymorphonuclear response whilst diffuse tumours provoked a scanty mononuclear infiltrate.

COMPARISON OF HISTOLOGICAL FEATURES

The histological criteria which determines these three classifications contain common features. The Lauren (1965) and Ming (1977) classification recognise two main categories of tumour. Lauren's (1965) description of the mode of growth of the intestinal and diffuse type carcinoma is comparable to Ming's (1977) expanding and infiltrative type respectively. The mucous cell and intestinal cell group of Mulligan coincide with the diffuse and intestinal type of Lauren. The Lauren (1965) and Mulligan (1975) classifications differ in the recognition by Mulligan (1975)of the pyloro-cardiac gland carcinoma as a separate entity, whereas Lauren (1965) includes the intestinal cell and the pyloro-cardiac gland cell carcinoma in his intestinal type.

The three classifications have independently divided gastric carcinoma into two broad types as shown below (assuming intestinal cell and pylorocardiac are grouped together). Figure 6 illustrates graphically the sub-division of gastric cancer by Lauren, Mulligan and Ming.

The morphological criteria embodied in any classification must be easy to apply and be reproducible in order to permit comparisons between different research groups. Paginini and Rugge (1982) examined the diagnostic agreement between two pathologists in 75 cases of gastric carcinoma using the WHO, Lauren, Ming and Mulligan classifications, the results of this study are shown in table 5.

The authors ascribe the high figure for the WHO classification as being due to the pathologists familiarity with the architectural features which form the basis of this classification. The diagnostic disagreement in Lauren 's classification was in cases with a high content of mucus which the authors found obscured the morphological features.

The diagnostic disagreement in the Ming classification occurred in cases where the main mass of the tumour exhibited an expanding pattern with peripheral areas showing infiltrative features. It is important to note that Lauren (1965) draws attention to this pattern in his description of the mode of growth of intestinal type tumours. The Ming (1977) classification although superficially simpler by concentrating solely on the mode of growth according to Paginini & Rugge (1982) interpretation has resulted in a greater diagnostic confusion than Lauren (1965).

Comparison of Classification Systems

Classification system	Number of cases	Histological types % total
Laurén	1,344	Intestinal 53
		Diffuse 33
		Unclassified 14
Ming	171	Expanding 67
		Infiltrative 33
Mulligan	297	Intestinal cell 24 Pylorocardiac 28
		Mucous cell 45
		Unclassified 3

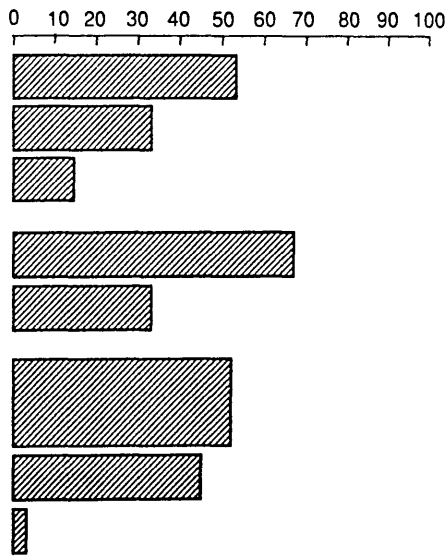


Figure 6. Comparison of sub-divisions of Lauren (1965), Ming (1977) and Mulligan (1972) Classification Systems.

	WHO	Lauren	Ming	Mulligan
Diagnostic agreement	96%	92%	88%	82.7%

(Paginini and Rugge, 1982)

Table 5: Diagnostic agreement of classification systems based on study with two observers (Paginini and Rugge, 1982).

The poor reproducibility of the Mulligan (1977) classification has been noted by other authors (Day and Morson, 1978). The area of diagnostic confusion is in the recognition of the pyloro-caridiac cell carcinoma as distinct from the intestinal cell type unless obvious clear cells are present.

The heterogenous appearance of gastric carcinoma has been described previously. There is not only variation between cases but marked differences in histological appearance within the same tumour. The larger the area of tumour sampled the greater the variation (Day & Morson 1978). Extensive sampling of tumour frequently results in more than one histological type (in all classification systems) being identified (Paginini and Rugge 1982). The application of a classification system is then based on the histological appearance of the largest area within the tumour (Paginini and Rugge 1982). Therefore the observer in classifying the tumours is using the subjective impression of the most frequent feature.

CLINICAL CORRELATION OF CLASSIFICATION SYSTEMS

The three classifications also examined the clinical features of the sub-types. Lauren (1965) discovered marked differences between the intestinal and diffuse types. The intestinal group showed a 2:1 male:female ratio with a mean age of 55.4 years. The diffuse group had a male:female ratio of approximately one and a lower mean age of 47.7 years. Ming (1977) describes a similar sex ratio; expanding carcinoma being twice as common in the male than the female and infiltrative carcinoma being equally distributed between the sexes. The age distribution also corresponded to that found by Lauren (1965).

Mulligan's (1975) analysis of the variables of age and sex show approximately comparable data if the intestinal cell and pyloro-cardiac cell carcinomas are considered together. A striking feature is a 4.13 male to female ratio for the pyloro-cardiac group alone compared with 2.81 for all types.

Lauren (1965) assessed the prognosis in 153 cases in which curative treatment had been given. Intestinal type had a more favourable prognosis with 43% survival at three years compared with 35% for diffuse carcinoma. In an age and stage adjusted comparison (Stemmerman and Brown 1974) this prognostic difference between Lauren's sub-types was confirmed with a 27.4% five year survival for intestinal type and a 9.9% five year survival for diffuse type. No difference in 5 year survival between the intestinal and diffuse types was seen in one series (Hawley et al., 1970), however, in this series the age and stage of tumour were not taken into account.

Mulligan (1975) in evaluating the prognostic significance of his classification describes the stage of the sub-types of tumour in terms of extension outwith the stomach: 92.4% of mucous cell carcinomas, 77.8% of pyloro-cardiac gland tumours and 58.7% of intestinal cell tumours extended outwith the stomach. These differences in stage are reflected in corresponding differences in prognosis. No data is available for stage adjusted data using the Mulligan system so differences in biological behaviour between the sub-types is impossible to assess.

Ming (1977) although claiming that there is a survival difference between his two types of tumour, produces no data to support this and

merely quotes the survival data for intestinal and diffuse type.

The largest series of survival data based on the WHO classification is from a Japanese series (Maruyama 1982) which gives five year survival rates of 74% for well differentiated tubular adenocarcinoma, 61% for moderately differentiated tubular adenocarcinoma, 54% for signet ring cell, 46% for poorly differentiated carcinoma, 46% for papillary carcinoma and 39% for mucous carcinoma.

EPIDEMIOLOGICAL APPLICATION OF CLASSIFICATION SYSTEMS

Extensive epidemiological data is available on the Lauren classification. The marked worldwide variation in the incidence of gastric carcinoma has been previously described. The incidence of diffuse carcinoma is similar between high risk and low risk areas, the excess of stomach cancer in high risk areas is predominantly of intestinal type (Munoz et al., 1968). This data is supported by migrant studies (Correa et al., 1973). Migration of Japanese from high risk Japan to low risk Hawaii results in a decrease in intestinal tumour incidence whilst the diffuse type incidence is not significantly altered. The two studies quoted (Correa et al., 1973; Munoz et al., 1968) which support the concept of two distinct types of tumour with differing epidemiology are both by protagonists of the Lauren classification. The differing incidence of intestinal and diffuse type tumours has not been supported by other studies (Kubo 1974) and in this study the age distribution of the populations was taken into account.

IMPLICATIONS FOR HISTOGENESIS

Lauren (1965) describes four structural characteristics of the tumour as the basis for his classification system. It is important to

note that his original description does not include any allusion to metaplasia in the mucosa adjacent to the tumour as a criteria used in the classification. What Lauren (1965) does describe is that once classified on structural characteristics the intestinal type tumours are surrounded by mucosa with a higher incidence and severity of intestinal metaplasia than the diffuse type. Although this may seem a subtle point it is important. The Lauren (1965) classification has been "modified" by several authors (Jass 1980) so that the presence of intestinal metaplasia adjacent to a tumour is used as one criteria for classifying that tumour into the intestinal group.

On the basis of the histological features, the differing age and sex characteristics, prognosis and intestinal metaplasia in the adjacent mucosa Lauren put forward the hypothesis that there are two biologically different types of gastric carcinoma with differing aetiology and pathogenesis. Although there is some conflict in the epidemiological data, the majority of work supports this hypothesis. Although the Lauren classification identifies and the epidemiological data appears to confirm two biologically distinct tyepes of gastric carcinoma, the histogenesis of the two types remains hypothetical. The inference is often made that the intestinal type arises from intestinal metaplasia and the diffuse type from "true" gastric epithelium. The evidence for this histogenetic sequence is discussed in the next chapter.

PART 3

PRECANCEROUS STATES OF THE GASTRIC MUCOSA

The term precancerous states in the chapter title is used in the broadest sense and includes both precancerous conditions and precancerous lesions as defined by Morson et al., 1980. The precancerous states generally recognised in the stomach are gastritis, intestinal metaplasia, gastric ulcer, gastric polyps and Menetrier's disease.

GASTRITIS

Histological appearance

The term "gastritis" means "many things to many men" (Warren and Meissner 1944). To the clinician it means a change in colour of the gastric mucosa visible endoscopically, to the pathologist it means a wide range of histological appearances. Generally the term gastritis implies inflammation of the gastric mucosa. Histologically gastritis is characterised by two distinct changes; an inflammatory component and alteration in the epithelial structure. These two components display a broad range of severity. At the extremes of severity different histological features are clearly seen but as the various stages probably represent different steps in one dynamic process, the distinction between successive grades are not so clear cut.

The inflammatory component in mild forms consists predominantly of polymorphs in the superficial layer of the epithelium. More severe inflammation is typified by the presence of chronic inflammatory cells with plasma cells in the upper layers

and lymphocytes in the lower layers of the mucosa often forming follicular lymphoid aggregates.

The epithelial component ranges from loss of one or two tubules to almost complete loss of all tubules. This atrophy is normally associated with severe inflammation in the deeper layers. The atrophy of gastric tubules coincides with the development of metaplastic glands and an end stage is recognisable with severe atrophy of gastric tubules, widespread intestinal metaplasia with lymphoid aggregates and a negligible inflammatory infiltrate.

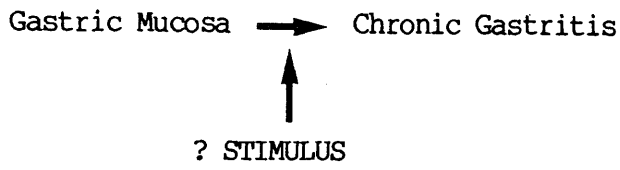
Histological classification

A variety of classifications of chronic gastritis have been proposed (Motteram 1951; Whitehead et al., 1972). Two major subdivisions are advocated; superficial and atrophic gastritis. Superficial gastritis involves only the superficial epithelium above the level of the gastric pit. Atrophic gastritis is defined as deep extension of the inflammatory infiltrate around the tubules associated with atrophy of the tubules which may be classified as mild, moderate or severe. The end stage lesion is often termed "gastric mucosal atrophy" or "gastric atrophy" to emphasise the severe loss of gastric epithelium and quiescent inflammatory component.

A scoring system has been devised to grade chronic gastritis (Watt et al., 1983). Two separate scores ranging from 0 - 6 are given depending on the severity of the inflammatory component and the degree of atrophy. This numerical scoring system allows for statistical comparisons between different groups to be made but has

simply replaced a descriptive histological term with a number.

Aetiology of Gastritis



The stimulus which induces the inflammatory change leading to chronic gastritis is of vital importance.

The first step in unravelling the aetiology of chronic gastritis was the recognition that chronic gastritis may result from differing stimuli. Strickland and McKay (1973) recognised two distinct types of atrophic gastritis, type A and type B on the basis of gastric morphology, function and pathogenesis. Type A gastritis involves a diffuse atrophy predominantly in the fundus and is associated with parietal cell and intrinsic factor antibodies and decreased acid secretion. Type B gastritis which was the most prevalent type was focal in distribution predominantly in the antrum and was associated with an absence of antibodies to parietal cells and only slightly impaired acid secretion. The aetiology of type A gastritis on the basis of this evidence is related to pernicious anaemia. The aetiology of the numerically larger group type B remained speculative with ingestion of alcohol, salt and salycylates or the reflux of bile being promoted as possible factors.

The commonest cause of acute inflammation is bacterial infection in any body system. The secretion of acid by the stomach constitutes a major non-specific defence mechanism. Until

recently the normal stomach has been regarded as virtually sterile with bacterial counts less than 10^5 organisms/ml. Reduction in acid secretion predisposes to infection with a variety of organisms - tubercle, salmonellae, brucella and dysentery bacilli. Infection with these organisms are rare clinically. The description of campylobacter-like organisms (CLO) in a high percentage of gastric mucosal biopsies by Warren and Marshall in 1984 identified a possible infective agent responsible for gastritis, and aetiological factor for peptic ulceration.

The campylobacter-like organisms have been identified as gram negative, flagellate and micro-aerophillic and have recently been named as Helicobacter pylori. The organism appears to lie close to the mucosal surface deep to the mucus layer (Steer 1985). Numerous studies have shown a significant association between the presence of the Helicobacter pylori and histological gastritis (Rollason et al., 1984; Jones et al., 1984). The relationship between the organism and peptic ulcer disease is still disputed with some authors finding no significant association (Forrest et al., 1984; Langenberg et al., 1984) whilst others have demonstrated a significant association (Warren and Marshall 1984).

The exact role of Helicobacter pylori in gastric pathology requires further clarification and the current hypotheses advanced regarding the organism and peptic ulceration are outwith the scope of this thesis. The original observation of Magnus 1937, that gastritis results from a mucosa repeatedly damaged by chronic irritation suggests that Helicobacter pylori may at least in some

cases represent the cause of the chronic irritation. Correa (1984) in his hypothetical aetiological model of gastric carcinogenesis present chronic superficial gastritis as the initial step in the carcinogenetic process. The cause for the chronic superficial gastritis has been presumed to be ingested dietary contents such as alcohol, salt and salicylates. What evidence is there to suggest that Helicobacter pylori may play a role in this process and how is this related to the development of intestinal metaplasia and carcinoma?

There is now considerable evidence that the presence of Helicobacter pylori in the gastric mucosa is significantly associated with chronic active gastritis. Does the organism cause the gastritis or is it simply an opportunist capable of colonising an altered environment caused by the gastritis? Marshall himself answered this question by ingesting a culture of Helicobacter pyloridis and proving Koch's third postulate that the organism reproduces the gastritis when inoculated into a susceptible host (Marshall et al., 1985). Serological studies have also shown a strong correlation between the presence of the organism, detectable circulating antibody and gastritis implying a host response to the organism (Jones et al., 1984). Immunoperoxidase studies on gastric biopsies demonstrate IgA, IgM, and IgG coating of Helicobacter pylori again implying a host response to the organism (Wyatt et al., 1986).

Gastritis in the post-operative stomach

In addition to the genetic cause and possible infective cause for gastritis attention has been directed to iatrogenic causes - namely

gastric surgery for benign disease.

The development of gastritis has been reported after both Billroth I and Billroth II gastrectomy and after proximal gastric vagotomy alone and in combination with gastro-enterostomy (Pulimood et al., 1976; Roland et al., 1975; Watt et al., 1983). Gastric mucosal abnormalities have been reported to occur more frequently in patients after gastric surgery than in age and sex-matched controls (Savage and Jones 1979; Watt et al., 1983).

A significant increase in dysplasia, the frequency and severity of gastritis and intestinal metaplasia have been described in the post surgical group compared with medically treated peptic ulcer patients (Watt et al., 1983). Post-operative gastritis occurs even when there has been no surgical manoeuvre to physically alter drainage of the stomach ie. highly selective vagotomy (Dewar et al., 1983).

The two factors which may cause gastritis post-operatively are reflux of alkaline small bowel contents (often termed bile reflux) and an increase in the gastric pH with subsequent N-nitroso compound formation.

The exact role that the reduction in gastric pH or of bile reflux (that occurs after most forms of gastric surgery) play in the aetiology of the gastric mucosal abnormalities is difficult to differentiate. The changes in peak acid output following proximal gastric vagotomy alone are not sufficient to explain the subsequent gastritis. Highly selective vagotomy has been shown to result in less severe post-operative gastritis and bile reflux than other types of surgery (Dewar et al., 1983) but unfortunately the effect

of changes in pH were not determined in this study.

A significant correlation between high pH and the incidence of gastric mucosal abnormalities has been demonstrated in patients after vagotomy and gastroenterostomy. However the high pH correlated with nitrite concentration but not with total N-nitroso-compound concentration (Watt et al., 1984B).

Reflux Gastritis

The concept of gastritis caused by the reflux of alkaline bile containing duodenal or small bowel contents has been gaining acceptance (Dewar et al., 1983; Ritchie 1984). The term "bile reflux" has now been changed for the more accurate and descriptive terminology - alkaline reflux gastritis (Ritchie 1984). Alkaline reflux gastritis is a common clinical problem causing considerable morbidity in patients after gastric surgery. Alkaline reflux gastritis has recently been shown to have distinct histological features compared with other types of gastritis (Dixon et al., 1986). The main and most distinctive mucosal abnormality is an elongation, tortuosity and hypercellularity of the gastric pits leading to foveolar hyperplasia. This gives the mucosa a villous appearance (Dixon et al., 1986; Niemela et al., 1987). In addition to this foveolar hyperplasia there is vasodilatation and congestion of capillaries in the superficial lamina propria; oedema and increased numbers of smooth muscle fibres in the lamina propria. Although this may appear to be a contradiction in terms in the description of a gastritis there is a striking paucity of neutrophil polymorphs and chronic inflammatory cells in the lamina

propria (Dixon et al., 1986).

Dixon et al., 1986, combined these histological features to give a composite reflux score. The variables were graded from 0-3. The inflammatory cell infiltrate was graded in an inverse manner with 0 representing a severe increase to 3 representing normal or reduced numbers. The reflux score was shown to be correlated to the bile acid concentration and hypochlorhydria in the stomachs of post-operative patients who had undergone their surgery for peptic ulcer disease. The histological scoring system for the reflux score is shown in table 6.

The importance of Dixon's work is that it has drawn attention to changes in the mucosal architecture which appear to result from alkaline reflux gastritis. The foveolar hyperplasia has been noted by other authors (Niemela et al., 1987) but the radical concept that reflux gastritis is characterised by a decrease in inflammatory cell infiltrate has not yet gained acceptance. The foveolar hyperplasia found with alkaline reflux gastritis may be misinterpreted as dysplasia and this may account for the finding of this mucosal abnormality in endoscopic surveys of post-operative patients (Watt et al., 1983; Watt et al., 1984B).

The mechanism by which reflux of the alkaline bile containing small bowel contents cause gastric mucosal injury is not entirely explained. Bile acids act to remove membrane-associated material from cells and act as co-carcinogens in experimental models of gastric carcinogenesis (Domelloff 1979). Small bowel contents appear to have a trophic action on the progenitor cells in the

REFLUX GASTRITIS SCORING SYSTEM

Histological variables	Score
Foveolar hyperplasia	
Oedema and smooth muscle fibres	0-3 for each
Vasodilatation and congestion	variable
Polymorphonuclear infiltrate	
Chronic inflammatory infiltrate	
Total score possible	15

NB. Polymorphonuclear and chronic inflammatory infiltrate were graded in an inverse manner ie 0 = severe increase in cells,
3 = normal number of cells
after Dixon et al., 1986.

Table 6: Reflux gastritis scoring system described by Dixon et al., 1986.

gastrointestinal tract and increased cell proliferation (Weser et al., 1977). This may be the mechanism for the development of the foveolar hyperplasia.

Relationship of chronic gastritis to gastric cancer

The reported increased incidence of gastric carcinoma in patients with pernicious anaemia and type A gastritis has been discussed in Part 1. There have been several reports of a high incidence of gastric carcinoma in patients with type B gastritis. There appears to be an approximately 10% incidence of gastric carcinoma developing in patients with chronic gastritis after 15-23 years follow-up (Walker et al., 1971; Siurala et al., 1974). Epidemiological surveys indicate that chronic gastritis is more prevalent in high risk than low risk populations for gastric carcinoma, and that it occurs at a younger age group (Correa et al., 1976).

The increased incidence of gastric carcinoma in post-operative reflux gastritis has been discussed in Chapter 1, Part 1.

INTESTINAL METAPLASIA

Metaplasia is defined as the transformation of one type of differentiated tissue into another (Muir's Textbook of Pathology (1978)). Intestinal metaplasia has been regarded as the histological hallmark of chronic gastritis and intestinal metaplasia is thought to represent an adaptive change of the lining epithelium to a type likely to be more resistant to injury.

The classical description of intestinal metaplasia in the stomach is of an epithelium identical to that of the small

intestine. The metaplastic glands are formed by two main types of cell - the goblet cell and columnar absorptive cells with a well developed brush border. At the base of the glands Paneth cells are frequent and neuro-endocrine cells are present.

Mechanism of metaplastic transformation

The gastric crypts are formed by division of stem cells in the neck of the glands (Winawer and Lipkin 1969). Division of stem cells produces columnar mucus cells which migrate upwards and the specialised parietal and peptic cells which migrate downwards (Stevens and Leblond, 1953; Creamer et al., 1961; Winawer and Lipkin 1969). The transformation to metaplastic epithelium involves the proliferation of the stem cells into goblet and columnar absorptive cells (Mukawa et al., 1987). This process results in the gradual development of metaplastic glands. The stimulus for this change in differentiation of the stem cells has classically been thought to be chronic inflammation ie. chronic gastritis (Warren and Meissner, 1944). Recent histological studies have identified that minute foci of intestinal metaplasia are associated with areas of regenerative epithelium formed during healing of erosions. The aetiology of the erosions was not determined and chronic gastritic changes were apparent in the mucosa examined in this study (Mukawa et al., 1987).

The origin of intestinal metaplasia in the neck zone of gastric crypts affected by inflammation explains the focal distribution of metaplasia often seen in gastrectomy specimens (Stemmerman and Hayashi 1968). Some authors have questioned the

association of intestinal metaplasia with chronic gastritis because of the absence of lymphoid aggregates or lymphocytic infiltration in areas completely replaced by metaplasia. This absence of an inflammatory component may be explained by the concept of metaplasia and consequent gastric atrophy representing the end stage of the inflammatory process.

Inherent in the transformation involved in metaplasia is that some biological advantage is accrued. Histologically there is a reduction in inflammation in areas with metaplasia. The recent identification of Helicobacter pylori as a common cause for type B gastritis may explain the biological advantage (Warren and Marshall, 1984).

A consistent feature in the reports concerning Helicobacter pylori is the absence of the organism in foci of intestinal metaplasia (Rollason et al., 1984; Steer 1985) in stomachs in which the organism was present. This finding leads to the hypothesis that intestinal metaplasia may represent a host response which results in eradication of the organism. Isaacson (1982) in a study performed prior to the recognition of Helicobacter pylori illustrated the adaptation of gastric epithelial cells to inflammation by synthesising secretory component and transporting IgA. This ability of the gastric epithelium is strikingly enhanced in areas of metaplastic epithelium (Valnes et al., 1984). Does the enhanced secretory immunity demonstrated in intestinal metaplasia result in eradication of the organism and the resolution of the associated gastritis?

Relationship of intestinal metaplasia to gastric carcinoma

The finding of extensive intestinal metaplasia in gastrectomy specimens removed for gastric carcinoma was the initial stimulus for interest in intestinal metaplasia as a possible precancerous state (Morson 1955A). The incidence and the severity of intestinal metaplasia has consistently been shown to be higher in stomachs removed for malignant disease rather than for benign disease (Morson 1955A; Stemmerman and Hayashi 1968). Furthermore the distribution of intestinal metaplasia in the pyloric antrum corresponds to the commonest site for gastric carcinoma (Morson 1955A). These findings are not consistent, however, and some authors have described individual gastrectomy specimens removed for carcinoma with little or no evidence of metaplasia (Stemmerman and Hayashi, 1968).

The description of two types of gastric carcinoma, a diffuse type and intestinal type by Lauren (1965) may explain this absence of metaplasia adjacent to some tumours. The intestinal type of tumour appears to be associated with marked metaplasia whereas the diffuse is not (Lauren 1965; Ming 1967; Stemmerman et al., 1977). This histological evidence is supported by epidemiological studies in which the incidence of metaplasia runs parallel to that of the intestinal type tumour (Munoz and Matko 1972).

The evidence presented above supports an association with intestinal metaplasia and intestinal type cancer but the causal sequence is not defined. There are three possible explanations of the association.

1. Intestinal metaplasia transforms into intestinal type of cancer.

2. Intestinal metaplasia and gastric carcinoma arise from a common stimulus to the undifferentiated stem cells of the gastric mucosa.

3. Intestinal metaplasia within the stomach results in an environment ie. rise in pH, nitrate formation that increases the exposure of the gastric mucosa to carcinogens.

The first explanation has attracted the greatest interest. There are two arguments proposed to counter this. Firstly that small intestinal tumours are rare lesions and that malignant transformation of mature small intestinal epithelium in the stomach would seem unlikely.

Second that intestinal metaplasia is a common abnormality, occurring in 20- 37% endoscopic biopsies (Rothery and Day 1985; Filipe et al., 1985). This problem has vexed the histopathologist as the presence or absence of intestinal metaplasia has no easily definable prognostic value when detected in endoscopic biopsies.

The realisation that intestinal metaplasia was not a homogenous entity brought renewed interest in the structure of intestinal metaplasia. Several variants and classifications of intestinal metaplasia have been described in an attempt to identify a sub-group of intestinal metaplasia that might represent a possible "marker" for gastric cancer in biopsy material. Three main classifications of intestinal metaplasia have been proposed. The literature is confusing as different authors have used differing criteria for their systems. The three classifications and relationship to gastric carcinoma are discussed below.

Classification of Intestinal Metaplasia

Complete and incomplete metaplasia

Ming et al., (1967) in an ultrustructural study first identified columnar cells in the gastric mucosa affected by intestinal metaplasia that had characteristics of both intestinal and gastric foveolar epithelia. These cells contained small amounts of mucus granules and rudimentary microvilli and were thought to represent intermediate forms in the transition from gastric to metaplastic epithelium. Further studies identified complete glands of intestinal metaplasia containing these abnormal columnar cells, and these were regarded as being an intermediate type of metaplasia which eventually would develop into complete metaplasia and the term incomplete coined (Iida et al., 1978). The enzyme and mucin histochemistry of this variant have been characterised by other authors and the results summarised in the table 7 (Matsukara et al., 198ØA).

Studies on gastrectomy specimens have identified an association between the incomplete variant and the intestinal type of gastric carcinoma. The hypothesis that incomplete metaplasia is an immature type and that the intestinal type of gastric carcinoma originates from this variant is proposed on the basis of these findings (Matsukara et al., 198ØB).

"Small Intestinal Type" and "Colonic Type"

Histochemical methods identified different type of intestinal metaplasia with differences analagous to those existing between normal colonic and small intestinal mucosa (Abe et al.., 1974;

	Complete	Incomplete
Goblet cells	Present, mainly sialomucins	Present mainly sulphomucins
Columnar cells	Well developed brush border	Absent brush border mucingranules with neutral or sulpho-mucins
Paneth cells	Present	Absent or reduced
Enzymes	Sucrase, leucine aminopeptidase trehalase, alkaline phosphatase	Sucrase, leucine aminopeptidase

Table 7: Characteristics of "complete" and "incomplete" intestinal metaplasia in terms of mucin and enzyme histochemistry and cell types.

Teglebjaerg and Nielsen 1978). On the basis of the type of mucus detected in the goblet cells a "small intestinal type" was found to be the predominant type with neutral mucins and sialomucins predominating in the goblet cells whereas the "colonic type" contained O-acetytl and sulphomucins (Teglebjaerg and Nielsen 1978). Unfortunately the mucus profile of the columnar cells was not discussed. The "colonic type" of tumour was found significantly more frequently in association with tumours of presumed intestinal histogenesis than in those with tumours of prescribed non-intestinal histogenesis. The incidence of the two sub types was not determined in non-malignant stomachs.

The classification into "small intestinal" and colonic type has certain drawbacks. Firstly the classification ignores the columnar cells and any mucus contained within them. Secondly the term "colonic" is a misnomer as the crypts do not resemble colonic epithelium and the presence of sulphomucin is not solely restricted to the colon (Filipe 1979).

The Jass Classification of Intestinal Metaplasia

Prior to the description of the incomplete sulphomucin positive type by Matsukara et al., (1980A), Jass and Filipe (1979) reported a similar variant with similar histological features. Jass (1980) followed this in the following year with a paper outlining a simple classification for intestinal metaplasia and the relationship of the sub types to gastric neoplasia. The crucial aspect of this classification was that it combined the mucin histochemistry of the goblet and columnar cells with the

morphological features of the metaplastic epithelium.

The intestinal metaplasia is divided into two types, I and II on the basis of the nature of the columnar cells. In type I metaplasia the columnar cells possessed a brush border, this type I with goblet cells and columnar absorptive cells is equivalent to the complete type of Ming (1967). The type II metaplasia is characterised by the loss of the brush border and the presence of mucus in the cells. The type II is further subdivided into type IIa in which the columnar cells secrete neutral mucins, in type IIb the columnar cells were distended with mucus and stained strongly for sulphomucin. In addition to the presence of mucin in the columnar cells the goblet cells showed differences in the nature of their mucin; in type I the goblet cells contained a mixture of 0 and N-acetyl sialomucin, in type II N-acetyl mucins predominated and 0-acetytl mucin was absent. The crypts of type IIa and b also varied in structure. The type IIb crypts were elongated and with an increase in cell height and branched cryps (see figure 7).

In his series of 48 gastric carcinomas and 25 benign specimens, Jass found that type IIb was significantly associated with gastric carcinomas of the "intestinal type". The advantages of the Jass classification are its simplicity and combination of easily recognisable morphological and histochemical variables. The histochemical techniques can be easily applied to formalin fixed paraffin embedded material which allows for greater application than the more sophisticated enzyme histochemical techniques. One of the main criticisms of Jass's work is the

DIAGRAM OF JASS CLASSIFICATION

Jass J.R., JClinPath., 1980

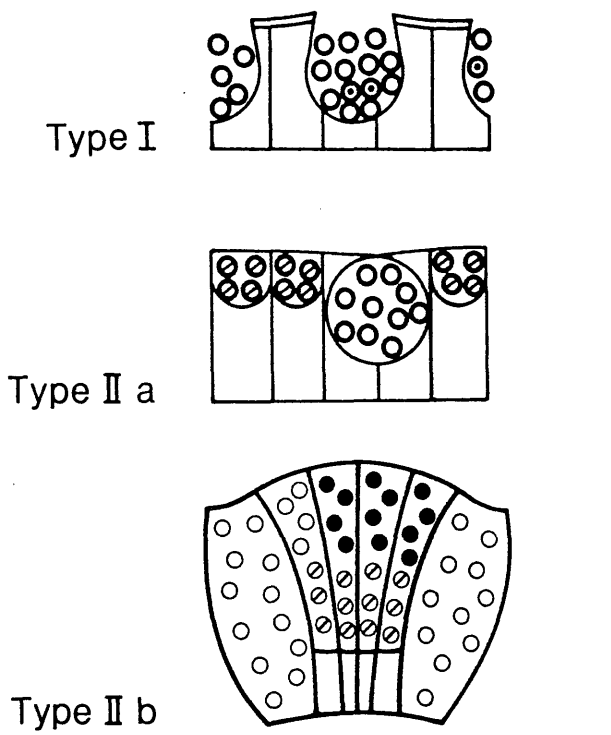


Figure 7. Diagrammatic representation of Jass Classification of intestinal metaplasia based on cell type and mucin histochemistry (after Jass, J R., 1980).

classification of tumours which did not adhere strictly to Lauren's (1965) definition. The use of a scoring system in which "extensive intestinal metaplasia round the tumour" is likely to result in an "intestinal" classification. This will inevitably result in tumours with extensive metaplasia and a higher chance of the type IIb sub type being classified as intestinal type and thus forming a circular argument.

Studying gastrectomy specimens is somewhat like shutting the gate after the horse has bolted. If type IIB metaplasia represents a variant of intestinal metaplasia associated with carcinoma then its detection in endoscopic biopsy specimens may be of value in the clinical situation.

A review of 1,465 endoscopic biopsies reviewed from a four year period showed that type IIb metaplasia was significantly associated with gastric carcinoma (Rothery and Day, 1985). In addition to this the frequency of type I, IIa and IIb was assessed (see table 8).

This study shows that type IIB metaplasia is not solely restricted to carcinoma, occurring in dysplasia and benign conditions. The presence of type IIB variant in benign conditions does not negate the association of this variant with gastric carcinoma as careful follow-up of these individuals would be required to determine if they subsequently develop carcinoma.

A retrospective study has been reported in which the question of the role of type IIb metaplasia as a marker of premalignant change has been partially answered (Ectors and Dixon 1986). After

Diagnosis	Types of Intestinal Metaplasia		
	I	IIa	IIb
Chronic gastritis	92%	31%	4%
Benign gastric ulcer	89%	42%	5%
Gastric dysplasia	100%	41%	7%
Gastric carcinoma	90%	19%	11%

Table 8: Frequency of Intestinal Metaplasia subtypes in Endoscopic Biopsies (Rothery and Day, 1985)

an eight year follow-up of 230 patients with chronic atrophic gastritis none of 28 patients with type IIb metaplasia identified developed gastric carcinoma. This indicates that the finding of type IIb on endoscopic biopsy material does not appear to confer a short term risk for the development of gastric carcinoma. This fact though of great clinical value does not fully answer the question of whether type IIb does have the potential to undergo malignant transformation.

A prospective multicentre study of gastric biopsies is currently under way (Filipe et al., 1985) but to date only the preliminary results are available that have shown an incidence and distribution of sub-types similar to those of Rothery and Day (1985). The results of this study should hopefully establish the clinical value of detecting the variants of intestinal metaplasia in gastric biopsies.

The Jass (1980) classification is simple, can be applied in paraffin embedded material using techniques available in most routine diagnostic laboratories, and allows comparison of results from different centres. Type IIb metaplasia and its relationship to intestinal type carcinoma as originally described by Jass (1980) appears superficially to be a strong candidate for a premalignant variant of intestinal metaplasia. However its finding in benign stomachs by subsequent studies (Rothery and Day 1985; Ectors and Dixon 1986; Filipe et al., 1985) and its lack of prognostic significant raises doubts. The finding that type IIb was present in a significantly older age group (Rothery and Day 1985; Ectors

and Dixon 1986) may indicate that the production of variant is simply a reflection of the duration and severity of gastric mucosal injury in older patients. As gastric carcinoma arises in this older age group the co-existence of type IIb with carcinoma may result by chance rather than being a premalignant change. Alternatively type IIb may represent a reactive phenomenon to malignant transformation.

Quantification of intestinal metaplasia

Intestinal metaplasia is a focal condition and the degree of intestinalisation of the gastric mucosa ranges from one or two crypts to almost complete replacement (Magnus 1937; Morson 1955A). The majority of published work has used semi-quantitative subjective assessments of the amount of intestinal metaplasia. Morson (1955) used a three part grading system; Grade I - scattered islands of intestinal epithelium, Grade II approximately half the mucosa affected by metaplasia, Grade III more than half the mucosa affected. Jass (1980) employed a similar semi-quantitative system but with different values for the grades viz:

Little or none	+/0	(<5%)
Moderate	++	(5-20%)
Extensive	+++	(20-100%)

These grading systems are extremely subjective and give only a broad indication as to the extent of intestinal metaplasia. They are, however, better than simply defining the presence or absence of metaplasia as is performed by some authors (Teglebjaerg and Nielson 1978).

An attempt at a more objective means of quantification has been produced (Rubio et al., 1985). An Intestinal Metaplasia Index was established by calculating the ratio between the length of mucosa affected by metaplasia and the length of gastric mucosa examined. The advantage of such a system is that statistical comparisons between benign and malignant specimens and intestinal and diffuse tumours can be made. Unfortunately the report using the quantitative data did not mention the statistical tests used. Although the report appeared to confirm the semi-quantitative findings of a higher degree of metaplasia in malignant stomachs than benign and in intestinal type rather than diffuse type, the lack of adequate statistical methods renders this finding valueless.

DYSPLASIA

Histological features

Epithelial dysplasia is recognised as the precancerous lesion in the stomach which may occur in gastric epithelium or in intestinal metaplasia (Morson et al., 1980). Dysplasia has been defined as having three histological features: (1) cellular atypia (2) abnormal differentiation (3) disorganised mucosal architecture. According to the severity of the changes dysplasia is classified into three grades, mild, moderate and severe. Although deceptively simple this classification has three major drawbacks (1) histological intrepretation (2) reproducibility and (3) prognostic significance.

Histological interpretation:

Where does dysplasia begin and end? The cytological abnormalities which accompany the disordered architecture of an inflamed or regenerative epithelium may be extremely difficult to separate from mild dysplasia. At the other extreme, severe dysplasia is difficult to differentiate from carcinoma in situ as it is difficult to be certain that the neoplastic cells have not breached the basement membrane of the crypts into the lamina propria.

Reproducibility

The use of highly subjective criteria in the interpretation of a rare lesion, often in epithelium with a significant inflammatory component, is bound to lead to some degree of observer error. An assessment of observer variation in the grading of dysplasia in the colon showed agreement between observers as low as 34% (Brown et al., 1985). No such study has been performed on gastric material but similar figures might be expected.

Prognostic significance:

The degree of risk conferred by epithelial dysplasia is not yet known. The finding of severe dysplasia is generally considered to indicate an inevitable progression to carcinoma but there is no firm evidence for this in the gastric epithelium. Several workers have examined the incidence and natural history of dysplasia in the post-operative stomach (Watt et al., 1983; Offerhaus et al., 1984) and shown patients with severe dysplasia in one biopsy subsequently having carcinoma identified in a subsequent biopsy. This, however, is not direct evidence that severe

dysplasia progressed to carcinoma. The interpretation of dysplasia in the post-operative stomach is also likely to be more liable to error in view of the characteristic foveolar hyperplasia identified in these patients (Dixon et al., 1986). In a study of 389 patients (Oehlert et al., 1979) severe dysplasia appeared to regress after 2-3 years in 23% of patients. Although the authors of such studies claim to have biopsied the same area the focal nature of the dysplastic change makes the interpretation of any study based on endoscopic biopsy material suspect because of the sampling error.

The prognostic significance of mild and moderate dysplasia is even less clear, and the reversibility of these lesions equally unknown.

Classification of Dysplasia

The classification of gastric dysplasia has been discussed by several authors (Cuello et al., 1979; Morson et al., 1980; Jass 1983; Ming et al., 1984). The majority of classification systems have concentrated on two aspects; the histological criteria to differentiate regenerative changes from true dysplasia and the grading of the severity of the dysplasia.

The classification systems all recognise an initial category of regenerative changes occurring in inflammation or ulceration where the cells are not proliferating in response to injury. There appears to be a general consensus in the middle ground that dysplasia has less severe ie. mild or moderate forms. At the furthest extreme there is some confusion of terminology - borderline lession, possible carcinoma, severe dysplasia etc. This

problem stems from the difficulty particularly in biopsy material of determining the presence of invasion of the lamina propria (Morson et al., 1980).

Two authors (Cuello et al., 1979; Jass 1983) have noted heterogeneity within gastric dysplasia. Both authors describe two histological patterns with the cellular features of dysplasia. Jass (1983) in a detailed study of 53 carcinomas and eight adenomas classified dysplasia into two types I and II. Type I resembled dysplasia described in colonic adenomas with columnar cells, secreting sulphomucin, pseudostratified nuclei and occasional Paneth cells. Type II comprised of irregular glands with pseudo villi composed of both columnar cells and goblet cells. The columnar cells secreted acid or neutral mucins and had large ovoid nuclei with vesiculation and a prominent nucleus. Cuello et al., 1979, described essentially similar types but concentrated more on the glandular architecture naming his types adenomatoid and hyperplastic which correspond to type I and type II respectively. Jass also includes a category of "grade 0" dysplasia applied to incomplete intestinal metaplasia with architectural alteration but no cellular atypia which is synonymous with type IIb metaplasia.

Jass (1983) describes a statistically significant difference between type II dysplasia and poorly differentiated intestinal type of tumour, and an association between type I and well differentiated intestinal type. Jass (1983) advances the hypothesis that type I dysplasia represents maturation arrest at the stage of an undifferentiated cell whereas type II dysplasia (which consists of two populations of cells - goblet and columnar)

represents arrest at the level of an intermediate cell. His theory is that type I dysplasia may evolve gradually into well differentiated adenocarcinomas, type II dysplasia represents a more aggressive form which may transform into poorly differentiated intestinal tumours (see figure 8).

GASTRIC POLYPS

Polyps are considered uncommon in the stomach although no data exists as to their true incidence. Polyps derived from the mucosa are divided into three main groups (Morson and Dawson 1979)

(1) Hamartomatous polyps - such as juvenile polyps, polyps occurring in the Peutz-Jeghers syndrome and heterotopias commonly formed from aberrant pancreatic tissue. Such polyps are regarded as having no malignant potential and are not further discussed.

(2) Regenerative (hyperplastic) polyps.

(3) Neoplastic (adenomas) polyps.

The last two groups have premalignant potential but of different grade. The regenerative polyps are composed of mature hyperplastic foveolar cells and varying numbers of pyloric type glands. The neoplastic polyps are adenomas consisting basically of immature glands with pseudostratification and numerous mitoses and usually show areas of intestinal metaplasia.

Malignant transformation has been rarely reported in hyperplastic polyps, separate co-existing carcinomas are present in 22% of cases (Ming 1977B). Malignant change in neoplastic polyps is frequent and is in the area of 40% (Ming 1977B).

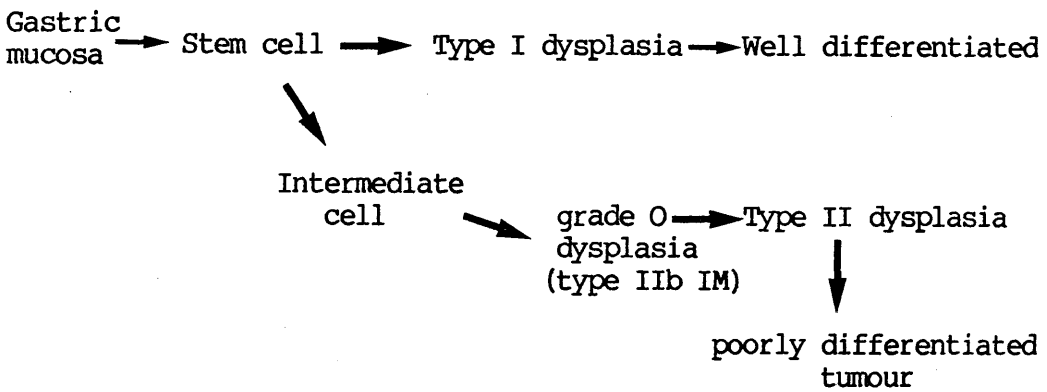


Figure 8. **Jass hypothesis of the histogenesis of gastric carcinoma (Jass, 1983).**

MENETRIERS DISEASE

In 1888 Menetrier described a diffuse mucosal hyperplasia (polyadenomes en nappe). He considered the lesion benign in itself but to have an affinity with a more malignant state and these lesions may be transformed into epitheliomas and gastric cancers. In the intervening 100 years there have been over 30 case reports of gastric carcinoma associated with Menetriers disease (Wood et al., 1983). However many of the case reports do not contain a clear histological diagnosis of Menetriers disease. There are only three reports in the literature of gastric carcinoma developing subsequent to a diagnosis of Menetriers disease (Von Loewenthal et al., 1960; Chusid et al., 1964; Wood et al., 1983). Menetriers disease although featuring prominently in most textbooks as a premalignant condition, numerically would appear to be of little importance.

GASTRIC ULCER

The role of chronic gastric ulcer as a precancerous state has been a controversial subject for several years. The controversy is due to the difficulty in distinguishing between a chronic gastric ulcer which has undergone malignant change and a cancer which has ulcerated. Histologically a chronic gastric ulcer will show complete interruption of the muscular coat. Although cases of ulcer - cancer do exist the incidence of gastric cancer in pre-existing ulcer has been estimated less than 1% (Mason 1974).

SUMMARY OF PRECANCEROUS LESIONS OF THE GASTRIC MUCOSA

"Chronic gastritis" results from several factors - genetic,

"infective" or chemical (alkaline reflux). Alkaline reflux gastritis has specific histological features. The inflammatory damage to the gastric mucosa is associated with transformation into metaplastic glands. This may represent an adaptive change to eliminate Helicobacter pylori. The transformation from gastric mucosa to classical intestinal metaplasia is associated with a variety of hybrid forms of intestinal metaplasia - these variants are associated with intestinal type cancer and may represent premalignant lesions. The role and assessment of dysplasia is unclear.

PART 4

ULTRASTRUCTURAL, MUCIN AND IMMUNOCYTOCHEMICAL STUDIES

There is considerable debate regarding the role of the precancerous states discussed in chapter in gastric carcinogenesis. This debate has led to much scientific endeavour being directed towards identifying changes in the phenotypic expression that accompany these histological abnormalities. Much of the published work is based on the premise that if factor X is identified in intestinal metaplasia and in the intestinal type of tumour then this is evidence of a histogenetic link. The logic of such arguments is discussed in the conclusion to this chapter.

The three areas which have received most attention are the ultrastructure, mucin histochemistry and immunocytochemistry of the precancerous states and of gastric carcinoma itself.

ULTRASTRUCTURAL STUDIES

The ultrastructure of normal gastric mucosa, intestinal metaplasia and gastric carcinoma have been examined in two major studies (Ming, 1967; Nevalainen and Jarvi, 1977). Foveolar and surface cells in normal gastric mucosa are morphologically similar with closely packed apical mucus granules and a few poorly developed microvilli. The microvilli on these cells possess no central core.

Metaplastic epithelium consists of two main cell types, the goblet cells and intervening columnar cells. Ultrastructurally these cells are indistinguishable from those of the small intestine. The most striking feature of the columnar cells is a well developed brush border on the apical surface formed by

microvilli. In contrast to the scarce rudimentary structures seen on the foveolar cells the microvilli on the metaplastic epithelium consist of an evenly distributed pattern with a central microfilament core. The mucus granules in the goblet cells were abundant throughout the cell with a pale staining granular inner texture compared with the small round homogenous darkly staining granules of the surface and foveolar cells of the gastric epithelium.

There is disagreement between the studies as to the presence of mucus granules in the columnar cells of intestinal metaplasia. No evidence of such granules was demonstrated by the first study, (Ming, 1967) whereas a variable number of granules in the apical zone of the columnar cells was seen by the second author (Nevalainen and Jarvi, 1977).

The cells of gastric carcinoma display a variety of features. Microvilli were seen particularly in cells lining tumour acini and the structure varied from well formed structures similar to those seen on the metaplastic columnar cells to the rudimentary structures of the foveolar cells. The mucus granules within the cells ranged from small dark oval bodies to pale structures often filling the entire cytoplasm to produce signet ring cells (Ming 1967; Nevalainen and Jarvi, 1977).

The two authors studied separate aspects of classification of gastric carcinomas. In the first study seven gastric carcinomas were examined (Ming 1967) and classified into undifferentiated and differentiated. The poorly differentiated tumour cells showed a variable number of microvilli and mucus granules. In the second

second study (Nevalainen and Jarvi, 1977) 47 gastric carcinomas were divided into diffuse and intestinal type using the Lauren 1965) classification. The intestinal tumour cells contained homogenous granules and well organised microvillar brush border. The diffuse type tumours contained cells with pale mucus granules and also areas of cuboidal cells with relatively well developed microvilli.

The interpretation of such ultrastructural studies is complex. As both intestinal and diffuse tumours possess cells with microvilli and such structures, however rudimentary, are present on gastric epithelial cells it is difficult to argue that the intestinal features of intestinal tumours result from an origin in intestinal metaplasia.

The disagreement between the two authors on the presence of mucus granules in the columnar cells of intestinal metaplasia may be explained by our present knowledge on the heterogeneity of intestinal metaplasia. The incomplete variants (see previous chapter) do possess columnar cells with mucus secretion detectable at the light microscopic level.

MUCIN HISTOCHEMISTRY

Introduction

Carbohydrate histochemistry has been extensively used in diagnosis and research in the gastrointestinal tract. The mucins (mucus glycoproteins) are the main carbohydrate-protein component of the secretory mucus and cell membranes that characterise the epithelial cells of the gut.

Three main categories of mucins are recognisable by

histochemical techniques: (a) Neutral mucins, (b) Sialomucins, (c) Sulphomucins. Histochemical studies have established the distribution and composition of mucins in the gastrointestinal tract and the alterations that occur in certain disease states. However the significance and physiological role that alterations in mucin histochemistry play remain to be fully elucidated. Experimental evidence suggests that mucins are of importance in immunological recognition (Deman et al., 1974), adhesion (Rios and Simmons, 1973) and the regulation of cell division (Weiss, 1973); processes fundamental to the development of metaplasia and neoplasia.

Current knowledge

Normal mucosa:

The mucous neck cells and the mucous cells of the surface epithelium in both the fundus, the antrum and the cardia, contain predominantly neutral mucins (Lev, 1965; Jass and Filipe, 1979). Traces of sulpho and sialomucins can be detected in the mucous neck cells and sialomucin in deeper foveolar cells in the body. In the antrum sialo and sulphomucins are found in small amounts in the lower part of the gland (Lev, 1965; Jass and Filipe, 1979). Near the oesophago-gastric junction marked staining for sulpho and sialomucin can be seen (Jass and Filipe, 1979).

Inflamed gastric mucosa:

There are few systematic studies on the histochemical appearances of gastritis and atrophy. Sialomucins and sulphomucins have been reported in mucous cells in stomachs affected by chronic gastritis (Gad, 1969; Jass and Filipe, 1979)

and a consistent feature is the reduction in mucus secretion.

Intestinal metaplasia

The mucin content of intestinal metaplasia and its relation to the various classification systems have been discussed in the previous chapter. The two important variants identified which appear to be linked to gastric carcinoma are the "colonic" type with O-acetyl and sulphomucins within the goblet cells (Abe et al., 1974; Teglebjaerg and Nielsen, 1978) and type IIb (Jass and Filipe, 1979) with sulphomucins in the apical mucus granules of the columnar cells. The relevance of this pattern of mucin secretion to that seen in gastric carcinoma is discussed below.

Gastric Carcinoma

Most authorities agree that gastric carcinomas contain a mixture of neutral, sialomucin and sulphomucin (Lev, 1965; Jass and Filipe, 1979; Lev, 1965; Filipe, 1979; Montero and Segura, 1980; Paginini and Rugge, 1983).

If Lauren's (1965) concept of two types of gastric carcinoma, intestinal and diffuse with a histogenesis from IM and gastric epithelium respectively is accepted, then differences in mucin histochemistry between the two types might be expected. Lauren himself (Lauren and Sorvar, 1969) identified a "higher proportion" of neutral mucin in diffuse carcinomas and sialo and sulphomucins in intestinal type tumours supporting the origin of diffuse tumours from the gastric epithelium. This fact has been used as a diagnostic aid in identifying secondary deposits of gastric carcinoma (Cook, 1982).

The difficulty with using this expression of mucin by tumours

as evidence of histogenesis is that most tumours produce all three major classes of mucin (Filipe 1979). The expression of mucin like the histological appearances vary throughout the tumour. Studies which cite "higher proportion" or "predominantly" one type of mucin associated with one type of tumour must be viewed cautiously (Lauren and Sorvar, 1969; Paginini and Rugge, 1983). No simple relationship such as, diffuse tumours secreting only neutral mucin and intestinal tumours secreting O-acetyl or sulphomucin in a similar pattern to intestinal metaplasia has ever been demonstrated.

IMMUNOCYTOCHEMISTRY OF PRECANCEROUS STATES AND GASTRIC CARCINOMA

General principles

Immunocytochemistry is the study of tissue sections with labelled antibodies.

The basis of immunocytochemistry is the union between antibody and its corresponding antigen. The crucial part of the antigen is the epitope normally a small fraction of the whole antigenic macromolecule which binds with the antigen binding site of the antibody. A single epitope can combine with several antibodies with different antigen binding sites.

Injection of a foreign antigen into animals results in stimulation of plasma cells and the formation of plasma cell clones, each producing antibodies with differing antigen binding sites to one or more epitopes of the injected foreign antigen - polyclonal response. The antibodies may cross-react with other antigens which share the same epitopes as the original foreign antigen. This cross reaction may reduce the value of the anti

serum for identifying the original antigen.

The production of monoclonal antibodies (ie identical antibody with identical antigen binding sites) by Kohler and Milstein in 1975 was a major achievement. The fusion of antibody producing cells from the spleen of appropriately immunised animals with myeloma cells to produce "hybridoma" producing a perpetual supply of specific antibody was a brilliant conceptual and technological advance.

Application of immunohistology

Immunocytochemical techniques have been used to study three main aspects of gastric pathology.

(1) Antibodies to specialised cells.

(2) Alterations of cell function.

(3) Acquisition of oncofetal antigens.

1. Antibodies to specialised cell types

Two antibodies, intrinsic factor and pepsinogen, directed against the specialised epithelial cells of the stomach, the parietal and peptic cells have been studied. The theoretical value of such antibodies is that the specialised cells which they identify are predominantly located in the body of the stomach, and therefore such antibodies might be expected to identify tumours of "true" gastric origin (ie diffuse type) rather than those with possible histogenesis from intestinal metaplasia. In addition to specialised epithelial cells, immunohistology has been used to identify the specialised cell types of the neuroendocrine system.

Pepsinogen

Two immunologically distinct types of pepsinogen exist within

the human gastric mucosa, pepsinogen I (PGI) and pepsinogen II (PGII). PGI is restricted to the mucus neck cells and chief cells of the fundus, whereas PGII is produced by these cells and also by the antral and cardiac glands (Samloff and Townes, 1970; Samloff and Liebman, 1973). Neither of the pepsinogens are present in intestinal metaplasia. Pepsinogen I and II would appear to be a marker for gastric epithelium - however immunocytochemical studies of gastric tumours shows a mixed picture with a third of intestinal type tumours staining positively for PGII and 5-15% of diffuse and intestinal type tumours staining positively for PGI (Reid et al., 1983; Stemmerman et al., 1985). Adenocarcinomas from other organs showed no evidence of pepsinogen (Reid et al., 1983). It is extremely difficult from this type of data to comment on any theory of histogenesis of gastric carcinomas.

Intrinsic factor

In the adult the parietal cell produces both hydrochloric acid and the glycoprotein intrinsic factor (Levine et al., 1980). Like the chief cells, the parietal cells are classically regarded as being located exclusively in the body of the stomach. Recent work has shown that parietal cells though fewer in number are also distributed in the antrum (Tominaga, 1975). The parietal cell represents the first differentiated cell to appear in the fetal stomach and intrinsic factor antibodies specifically identify parietal cells (Aitchison and Brown, 1988). Preliminary work in a small number of gastric carcinomas identified positive staining for intrinsic factor within diffuse type tumours but not intestinal type tumours (Brown, 1982).

Neuroendocrine system

The recognition by Feyrter in 1938 of a gastrointestinal system of clear cells (Helle Zellen) with an endocrine or paracrine function has led to the current concept of a diffuse endocrine and neuro-endocrine system (Pearse and Takor, 1979). The origin of the cells of the diffuse endocrine systen were originally thought to be from neural crest (Pearse, 1969). This narrow origin, however, has been extended to embryonic epiblast with special neuro-endocrine programming (Pearse and Polak, 1971). This concept has also been challenged (Sidhu, 1979) and the possibility of endodermal derived epithelium itself giving rise to endocrine cells, along with mucous cells and the other specialised cell types, ie. parietal, has been proposed.

The antral region of the stomach contains neuro-endocrine cells which secrete the neuropeptides gastrin and somatostatin, the endocrine cells in the body secrete vaso-active intestinal polypeptide, glucagon and 5-hydroxytryptamine (Dawson, 1984). The duodenum and jejunum contain endocrine cells that secrete gastrin, somatostatin, vasoactive intestinal polypeptide (VIP), enteroglucagon, gastrin inhibitory polypeptide (GIP), motilin and bombesin (Bryant and Bloom, 1979).

The occurrence of endocrine cells within metaplastic intestinal epithelia in the stomach has been clearly demonstrated (Bordi and Ravazzola, 1979; Ho et al., 1984). Immunocytochemical studies have identified somatostatin, gastrin, cholecytokinin, secretin, GIP and enteroglucagon cells in foci of intestinal metaplasia (Bordi and Ravazzola, 1979; Ho et al. 1984).

Tsutsumi et al. 1983, identified several interesting features in the gastric mucosa endocrine cell population. The gastric mucosa affected by atrophic gastritis shows a relative increase in G cells (gastrin), areas of intestinal metaplasia show a decrease in the number of somatostatin and gastrin containing cells paralleled by an increase in enteroglucogan containing cells. These authors also noted that in the "incomplete type" of metaplasia the numbers of endocrine cells appeared greater than in the complete type. Mingazzini et al., 1984, compared sulphated versus sialomucin containing intestinal metaplasia and found no difference in the number of endocrine cells between the two types but only small foci were examined.

A variety of polypeptide hormones have been identified in gastric carcinoma (Ho et al., 1984), namely gastrin, somatostatin and enteroglucagon. Some authors have identified a higher number of endocrine cells in poorly differentiated tumours (Azzopardi and Pollock 1963) while others describe a higher number in well differentiated tumours (Ho et al., 1984).

2. Alterations in normal function

Secretory immunity in normal, inflamed, metaplastic and neoplastic gastric mucosa is the main aspect of normal function that has been examined by immunocytochemical techniques. Secretory component and IgA are well demonstrated in tissue sections by immunocytochemistry. The function of the secretory immunoglobulin (Ig) system is to transport dimeric IgA secreted by mucosal plasma cells through the epithelial cells to the mucosa surface (Braendtzaeg, 1981). The epithelial transport of IgA is

mediated by secretory component synthesised by the mucosal epithelial cells (Braendtzaeg, 1981). Secretory component is an epithelial glycoprotein of approximately 83,000 daltons (Brandtzaeg, 1974). The incorporation of J chain into IgA induces a configurational fit with secretory component in the baso-lateral surface of the epithelial cell. The SC-IgA complexes are then transported into the gland lumen in cytoplasmic vesicles (Braendtzaeg, 1981).

Secretory component is normally expressed on the plasma membrane of columnar epithelial cells of the small and large intestine (Braendtzaeg 1974). Normal gastric body mucosa shows no evidence of secretory component, and only scattered lamina propria plasma cells stain for IgA (Isaacson 1982). As material for studies is obtained from gastrectomy specimens Isaacson in 1982 drew attention to the fact that "normal" antral mucosa is never seen in such material as there is always a slight degree of superficial gastritis. With this degree of inflammation the foveolar mucous cells stain negatively for secretory component but became faintly positive in the isthmus or mucous neck region. The presence of secretory component was accompanied by an increasing number of IgA positive plasma cells (Isaacson, 1982).

In gastritis the mucus neck cells of the body stain positively for secretory component and the distribution of staining was similar to that of the antrum, with the foveolar mucous cells show no staining. Intestinal metaplasia demonstrates strong staining with secretory component similar to that of the crypts of intestinal villi (Isaacson, 1982).

Dysplasia diagnosed on the basis of Morson's criteria (Morson et al., 1980) was also examined by Isaacson (1982). Dysplasia showed intense staining for both secretory component and IgA.

The expression of secretory component and IgA by the cells of gastric carcinoma appears to be related to the differentiation of the tumour. Well differentiated tumours have an approximately 80-90% incidence of SC immunoreactivity on tumour cells regardless of the stage of the tumour (Sumiyoshi et al., 1984). Poorly differentiated tumours show considerably less expression of SC, in the region of 40% (Isaacson, 1982; Sumiyoshi et al., 1984). Ultrastructural studies demonstrate that the SC is expressed over the entire surface of the neoplastic cells rather than on the basolateral surface as occurs in the normal glandular epithelial cells (Nagura et al., 1983).

The pattern of IgA staining paralleled that for SC. IgA positive plasma cells were present in large numbers in well differentiated tumours and the tumour cells themselves showed IgA immunoreactivity. In poorly differentiated and anaplastic carcinomas the IgA immunoreactivity was markedly reduced or completely absent (Nagura et al., 1983; Sumiyoshi et al., 1984).

Isaacson , 1982, divided his series of gastric carcinomas into diffuse and intestinal type according to Lauren's classification. Intestinal type and diffuse type showed positive staining for both secretory component and IgA only in well differentiated tumours of both types.

3. Acquisition of oncofetal antigens

The appearance of embryonal antigens in extracts of tumour

tissues has been described in a variety of epithelial tumours. Several embryonal antigens have been detected in gastric carcinoma.

Carcino-embryonic antigen

The major oncofetal antigen of the human gut, carcino embryonic antigen (CEA) was extracted from human fetal intestine and adult colon cancer tissue by Gold and Freeman, 1965A; 1965B. CEA is a single glycosylated peptide with the antigenic determinants apparently residing in the peptide portion of the molecule (Turberville et al., 1973A; Westwood et al., 1978). Antisera to CEA however are known to cross-react with other glycoproteins. CEA antisera cross-react with non-specific cross- reacting antigen (Von Kleist et al., 1972) biliary glycoprotein antigens of the ABO - blood group system and non-specific cross-reacting antigen 2 (NCA_2) (Burtin et al., 1973A; 1973B; Holbourn et al., 1974). The latter antigen is present in gastric juice . These cross-reactions have led to considerable confusion in the literature. The source and specificity of antisera used in various studies is poorly defined and has led to the term "CEA-like material" being coined to describe positive staining with CEA antisera and the cross-reacting antigens.

CEA-like material has been reported to be present in normal stomach by some authors (Ejeckman et al., 1979) and to be absent by others (Burtin et al., 1973; Nagura et al., 1983). The CEA-like material is found in the mucous neck cells in gastric mucosa affected by intestinal metaplasia or with a marked infiltrate of IgA labelled plasma cells. It would appear that mucosa affected by gastritis expresses CEA-like material whereas normal mucosa

expresses little or none (Borch et al., 1987).

CEA-like material is expressed by intestinal metaplasia (Burtin et al., 1973; Ejeckman et al., 1979; Nagura et al., 1983). ultrastructural immunoperoxidase studies (Nagura et al., 1983) have shown that the CEA-like material is expressed on the apical surfaces and intracellularly in columnar cells of intestinal metaplasia but not within the goblet cells. The intensity of the immunocytochemical reaction for CEA-like material is strong (Ejeckman et al., 1979; Nagura et al., 1983) and appears to be related to the severity of the metaplasia (Nagura et al., 1983).

CEA-like material can be demonstrated in the majority of gastric carcinomas (Burtin et al., 1973; Ejeckman et al., 1979; Nagura et al., 1983; Borch et al., 1987). The antigen is seen clearly on the surface lining of tumour acini and ultrastructurally the microvillous, basolateral and intracytoplasmic structures show positive staining for the CEA-like material (Nagura et al., 1983). The relationship between the histological type of tumour and CEA expression has not been extensively studied but CEA expression has been demonstrated in both the intestinal and diffuse type of Lauren (Borch et al., 1987), "signet ring cell carcinoma", and anaplastic carcinoma (Ejeckman et al., 1979).

A recent study examining in detail the cross-reactivity of commercially available anti CEA sera has demonstrated that CEA expression in the normal stomach is due to cross-reaction with the NCA_2 antigen. To what extent the CEA expression determined in intestinal metaplasia, dysplasia and carcinoma outlined in the studies above is due to the presence of NCA_2 rather than true CEA

is impossible to determine.

Duodenal and colonic high molecular weight antigens

Bara et al., 1981 demonstrated a high molecular weight antigen associated with intestinal metaplasia and the intestinal type of gastric carcinoma. In further studies they identified three antigens, M3 Small Intestine, M3 Duodenum, M3 Colon, derived from the injection of high molecular components of duodenal or colonic mucosa into rabbits (Nardelli et al., 1983) . Immunocytochemical studies of normal adult and fetal gastrointestinal tissues demonstrated that M3D reacted primarily with the goblet cells of the duodenum, M3SI with the goblet cells in the small intestine and proximal colon and M3C with the goblet cells of the colon. M3D and M3SI was associated with goblet cells in the duodenal fetal mucosa at all gestational ages, whereas the M3C antigen was detected only after three months' gestation.

In intestinal metaplasia from stomachs resected for benign disease only the M3D and MSI antigens were observed. This finding was interpreted by the authors as indicating the small intestinal origin of such metaplasia. Metaplasia adjacent to carcinomas contained the M3C antigen in addition to the M3D and M3SI antigens. This pattern of expression was repeated in the intestinal type of tumours and the common differentiation features displayed by the metaplasia and adjacent intestinal type carcinoma was regarded as supporting the role of intestinal metaplasia in the histogenesis of the intestinal type of carcinoma.

Second trimester fetal antigen

Extracts of second trimester fetal lung, liver, kidney and

large bowel were injected into rabbits and the resulting anti serum isolated and absorbed with adult lung, liver, kidney and colon, and colonic carcinoma cell lines. The absorbed antibody designated anti STF a/b was found to be unreactive with normal adult gastrointestinal tissue but to react with gastric, oesophageal and rectal carcinomas. Immunohistochemical studies performed on normal, inflamed and metaplastic and dysplastic gastric epithelium showed increasing reactivity on the epithelial cells with anti STF a/b with the aforementioned sequence of gastric epithelial alterations. Differing types of gastric carcinoma and fetal tissue were not studied (Higgins et al., 1984).

Human chorionic gonadotrophin (HCG)

Human chorionic gonadotrophin is a glycoprotein secreted by the placental syncytiotrophoblast. Ito et al., (1983) studied 164 gastric carcinomas using immunoperoxidase techniques and demonstrated a 10% incidence of immunoreactivity for HCG. There was no difference in the incidence of HCG positive cells between well and poorly differentiated carcinomas.

SUMMARY

Despite the advances in technology that electron microscopy mucin histochemistry and immunocytochemistry represent, these techniques have done little to advance our understanding of the histogenesis of gastric carcinoma. The majority of studies have tried to identify a single factor ie. sulphomucin positivity, CEA positivity which will act as a marker for pre-malignancy. The studies have often used complex classification systems for intestinal metaplasia and gastric carcinoma in an attempt to prove

the authors conceived hypothesis. Added to this is the lack of standardisation of antibodies and their specificities and the development of antibodies ie. duodenal antigens, fetal antigens which are available only to the workers concerned.

PART 5

CELL KINETICS AND EXPERIMENTAL MODELS

NORMAL CELL CYCLE

Cells pass through a series of distinguishable phases between one mitosis and the next. The period of DNA synthesis is designated the S phase. The interval between the previous mitosis and the S phase is called the G_1 phase, and the interval following S and leading to the next mitosis is called the G_2 phase. During G_1 the cell may manifest its characteristic function and enter a resting phase - G_0.

TECHNIQUES TO STUDY CELL KINETICS IN HUMAN GASTRIC MUCOSA

Tritiated thymidine has been shown to be electively incorporated into DNA during the premitotic S phase (Taylor et al., 1957). The tritiated thymidine incorporated into DNA synthesising cells can be visualised using autoradiography. The Labelling Index (LI) can be derived by counting the number of labelled cells and the total cell number in the cell population under consideration. The Labelling Index is expressed normally as a percentage viz:

$$LI = \frac{N_l}{N_t} \times 100$$

N_l = number of labelled cells

N_t = total cell number of the given cell population

The Labelling Index provides only a static picture of the cell cycle, and does not provide any indication of the duration of the S phase.

The techniques of pulse labelling with tritiated thymidine or

in vivo double labelling with ^{3}H and ^{14}C thymidine allows for calculation of the length of the S and G phases of the cell cycle. These in vivo techniques are ideally suited for experimental animals but ethical considerations preclude their general adoption in humans. The double labelling technique is difficult to interpret on histological sections (Hansen et al., 1975).

The Labelling Index may be assumed to be proportional to the rate of cell proliferation if the DNA-synthetic phase (S phase) is constant. The S phase has been measured by continuous in vitro labelling and the results indicate that the S phase has a constant duration in normal gastric mucosa and in patients with different degrees of gastritis (Hansen et al., 1979). Alterations in the Labelling Index in gastric mucosa are interpreted in the majority of published work in this field as reflecting changes in the rate of cell turnover.

Techniques for the in vitro labelling of gastric biopsy material have been developed by incubating the specimens in tissue culture medium with labelled DNA precursors and good agreement has been demonstrated between the in vivo and in vitro estimates of kinetic parameters in experimental animals (Willems et al., 1970). The majority of published work in humans has used the in vitro thymidine incubation technique. Biopsies obtained using fibreoptic instruments are ideal for the study of cell proliferation in the human gastric mucosa using the in vitro thymidine incubation technique.

The small biopsies are difficult to orientate and

longitudinal sections of the foveolae are difficult to obtain in sufficient numbers for satisfactory estimation of the labelling indices, although some authors appear to be able to achieve this (Assad et al., 1980). Hansen et al., 1975, describe a technique in which labelled cells in cross sections of foveolae are counted. Serial sections are counted and the labelling indices are thus based on cell counts throughout the whole of the proliferative zone. This technique has been shown to be highly reproducible with a small observer error. This technique of counting cross sections of foveolae generates an estimated labelling index which may be corrected using the equation $(1-p)^n = 1-p/LI$ (see appendix). This technique is easier to perform as it requires no orientation of material. However, the disadvantage with this technique is that as cross sections are used changes in the site of the proliferative zone within the crypts cannot be easily detected.

NORMAL SITUATION

The gastric mucosa as with the remainder of the gastrointestinal tract is a steady state cell population, the equilibrium maintained by a regular cell loss from the mucosal surfaces and a sustained cellular production (Stevens & Leblond, 1953; Creamer et al., 1961;

The site of cell synthesis in the gastric mucosa of the body lies at the level of the mucous neck and lower part of the foveolae ie the isthmus of the gland. Undifferentiated cells in the isthmus area incorporate tritiated thymidine, and pulse labelling studies demonstrate that the cells produced by the undifferentiated

stem cells migrate upwards and reach the surface epithelium in 48-54 hours (Winawer and Lipkin, 1969). The undifferentiated cells also act as stem cells for the parietal cells which differ only by the route of migration in a downwards direction (Willems and Lehy, 1975). Peptic cells have also been shown to originate from the undifferentiated cells in experiments using gastric explants (Matsuyama and Suzuki, 1970). The origin of the peptic cells is disputed by some authorities and radio-autographic studies in normal mucosa in experimental animals have claimed to show that peptic cell renewal depends on slow mitotic activity of existing peptic cells (Willems and Lehy, 1975).

A similar kinetic situation is present in the antrum; the undifferentiated stem cells lie at the isthmus of the glands and by continual cell proliferation give rise to newly formed cells which migrate upwards to the gastric surface (Creamer et al., 1961) and downwards to form the glandular cells (Hattori and Fujita, 1979).

PROLIFERATION KINETICS OF GASTRIC MUCOSA IN DISEASE

Gastritis

Using an in vitro incubation technique with ^{3}H-thymidine Hansen et al., (1975) studied gastric biopsies from patients with atrophic gastritis. A significantly higher labelling index was demonstrated in both the antral and body mucosa in patients with endoscopic evidence of atrophic gastritis. In a further study (Hansen et al., 1977) a significant correlation between the severity of the gastritis and the labelling index was demonstrated.

Intestinal metaplasia

Winawer and Lipkin, 1969, reported on an vivo human experiment on a patient with marked intestinal metaplasia of the gastric mucosa. The labelling index in the intestinalised mucosa was higher than normal gastric mucosa and the abnormal mucosa showed incorporation of thymidine into cells at or near the luminal surface.

The increased labelling index in intestinal metaplasia has been confirmed by other authors (Hattori and Fujita, 1979). An elegant morpholgical study demonstrated that the generative cell zone in intestinal metaplasia shifts from the isthmus to the base of the gland resulting in a situation identical to that of the small intestine. The incorporation of thymidine into cells in the surface epithelium was not found by these authors. This discrepancy may be explained by the different thymidine labelling patterns that have been shown when the sub-types of intestinal metaplasia are examined. The variant of intestinal metaplasia defined as the incomplete type (absence of alkaline phosphatase activity) (Matsukara et al., 1980A) shows an extended generative zone from the base to the middle of the glands compared with the complete type (alkaline phosphatase activity) which possesses a generative zone localised to the base of the glands (Hashimoto et al., 1983). This extension of the labelled zone did not involve the surface epithelium and so the incorporation of thymidine into this area described by Winawer and Lipkin, 1969, remains unconfirmed by other workers.

Gastric carcinoma

The in vitro thymidine index labelling of biopsy specimens from gastric carcinomas show a wide variation from 1.4 to 40.8% (Sasaki et al., 1984). The labelling index has not been shown to be related to the stage of the tumour, the histological type or degree of differentiation.

The labelled cells appear to be distributed haphazardly amongst the tumour cells. Signet ring cells do not label with thymidine and nests of tumour cells within the lymphatic channels have a higher labelling index than surrounding tumour (Sasaki et al., 1984).

In vivo studies on human gastric carcinoma are technically difficult to perform. Such a study using the metaphase arrest technique (vide infra) reported a widely varying cell production rate between carcinomas ranging from 3-24 cells/1000 cells/hour in keeping with the variation in labelling index detected in the thymidine labelling studies (Wright et al., 1977).

Effect of surgery on gastric cell kinetics

There are at least three factors which may theoretically influence cell kinetics in the post-operative stomach; the changes in gastrin levels, the effect of vagotomy and the development of gastritis.

The surgical excision of the antrum combined with vagotomy results in a decrease in available gastrin (McGuigan et al., 1972). Gastrin has been shown to be trophic to body epithelium (Pearl et al., 1966), and the post-operative reduction in gastrin might be

predicted to decrease epithelial proliferation.

The effect of vagal sectioning on cellular proliferation in the gastric mucosa is unclear. In experimental animals both a negative and a positive trophic effect after vagotomy have been described (Ley et al., 1973). Proximal gastric vagotomy results in an increase in circulatory levels of gastrin. Gastrin is trophic to body epithelium but appears to have an inhibitory effect on cell proliferation in the antral mucosa (Pearl et al., 1966).

An increase in the frequency and severity of gastritis has been described after antrectomy and vagotomy and vagotomy alone (Roland et al., 1975; Pulimood et al., 1976; Watt et al., 1983). The increased rate of epithelial cell renewal associated with gastritis suggests that this change is likely to result in an increased Labelling Index in the mucosa of post-surgical patients.

Hansen et al., (1978), examined the Labelling Index in patients prior to partial gastrectomy or vagotomy and within the first post-operative year. The Labelling Index increased in the body mucosa after antrectomy and in both body and antral mucosa after vagotomy alone. Patients who had undergone vagotomy and antrectomy for duodenal ulcer disease between 4 months and 14 years previously were also shown to have increased labelling indices in the body mucosa (Assad and Eastwood, 1980) and the proliferative zone expanded to occupy a greater fraction of the gastric crypts. Both these studies (Hansen et al., 1978; Assad and Eastwood, 1980) attributed the increase in Labelling Index in the post-operative stomach to the gastritis found in these stomachs.

The gastritis following gastric resection may be due to reflux of bile and pancreatic secretions. These secretions in addition to causing the gastritis may also have a direct effect on the progenitor cells and increase cell proliferation (Weser et al., 1977). The aetiology of the gastritis following proximal gastric vagotomy alone is unclear.

In summary, the situation in the post-operative stomach is complex. Theoretical effects of changes in gastrin levels or vagal sectioning on cell proliferation seem of secondary importance compared with the post-operative development of post-surgical gastritis. Not only does the labelling index increase but the proliferative zone itself appears to expand. The role of the foveolar hyperplasia and relative absence of inflammatory changes described by Dixon et al., 1986 in reflux gastritis has not been considered in the published material on post-operative kinetic changes.

Effect of drug therapy

The effect of H_2 receptor antagonist cimetidine on gastric mucosal cell proliferation has been extensively studied in experimental animals. Chronic ingestion of this drug has been shown to have no effect on cell proliferation in either the antral or the body region in rats (Eastwood and Forrest, 1983). Studies in humans, however, have shown an increase in labelling index in both antral and body mucosa after cimetidine therapy when the degree of gastritis remained the same (Fich et al., 1985; Svendson et al., 1986).

EXPERIMENTAL GASTRIC CARCINOGENESIS

The induction of tumours in experimental animals has theoretically many advantages for the investigation of pre-malignant stages in carcinogenesis. There are considerable constraints in extrapolating data from animal models to the human situation. Animal experiments do allow for the investigation of the critical step from pre-cancerous lesion to a frankly malignant state; a sequence which is extremely difficult to examine in the human.

Early models for induction of gastric cancer in rodents involved the direct intramural injection of a polycyclic hydrocarbon, 20-methyl cholanthrene (Stewart et al., 1958). This results in neoplastic transformation of glands within four days and the development of adenocarcinomatous and sarcomatous lesions. Sugimura and Fujimira (1967), developed an animal model involving administration of a carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water. Administration of MNNG for 12 months led to the development of adenocarcinoma in 70% of the animals. The concentration of carcinogen used in these early experiments (83 ug/l) led to erosion, regenerative hyperplasia and adenomatous hyperplasia (Saito et al., 1970) and finally adenocarcinoma, a situation not thought analogous to human carcinogenesis. Such a sequence of events would appear to indicate that the carcinogen has a direct toxic effect on the gastric mucosa. To avoid this direct injury experiments have been performed using a lower concentration of the carcinogen for a

shorter duration (Matsukara et al., 1980B; Tatematsu et al., 1983). In rats treated for a shorter duration with (83 ug/ml) MNNG for two to four months although erosions and regenerative epithelium were still present it was noted that intestinal metaplasia also developed (Matsukara et al., 1980B). By reducing the concentration of carcinogen (MNNG) in drinking water to 50 ug/ml and exposure to four months this resulted in the induction of intestinal metaplasia and gastric carcinoma without ulcerative or regenerative changes (Tatematsu et al., 1983). In a similar manner, the administration of N-propyl-N-nitro-N-nitrosoquanidine (PNNG) the propyl derivative of MNNG, and weaker carcinogen resulted in the induction of intestinal metaplasia after one month of treatment with PNNG and adeno-carcinomas after 12 months treatment, without ulcerative or regenerative changes (Sasajima et al., 1979). Thus a model exists for the induction of intestinal metaplasia and carcinoma without causing ulceration and regenerative change by administration of a low concentration of MNNG (50 ug/ml) or its weaker propyl derivative PNNG.

The advantage of such an experimental model is that more sophisticated kinetic parameters can be measured than in the constraints of a human situation. Autoradiography can be used to measure the Labelling Index. The measurement of this kinetic parameter is time consuming and laborious and although the Labelling Index allows the proportion of cells in a given population proliferating to be established, it does not allow for changes in phase durations ie. the mitotic duration and the

duration of DNA synthesis.

The metaphase arrest or stathmokinetic technique establishes the kinetic parameter, the rate of entry of cells into mitosis (also known as the cell birth rate or mitotic rate). The basis for the technique is the addition of stathmokinetic agents which cause cells to accumulate in the metaphase stage of mitosis by interference with the formation of the metaphase spindle. With the addition of a stathmokinetic agent and fixation of samples of the cell population over a period, the progressively rising metaphase index can be determined and the rate of entry of cells into mitosis can be found.

For tissue in a kinetic steady state -

$r_m = I_m/t_m$ r_m = rate of entry into mitosis

I_m = mitotic index

t_m = duration of mitosis

The stathmokinetic equation is derived from this -

$r_m = I_{met}/tA$

where I_{met} is the metaphase index at the end of the arrest period tA. As during mitosis one cell generates two progeny, r_m gives the birth rate K_B. In a steady state situation experimental results are plotted as shown in figure 9. r_m is represented by the slope of the graph, the cell cycle time (tca) is given by the reciprocal of the slope ie. I/r_m.

Bowel crypts act as closed kinetic systems from which arrested metaphases are unable to escape. The estimation of

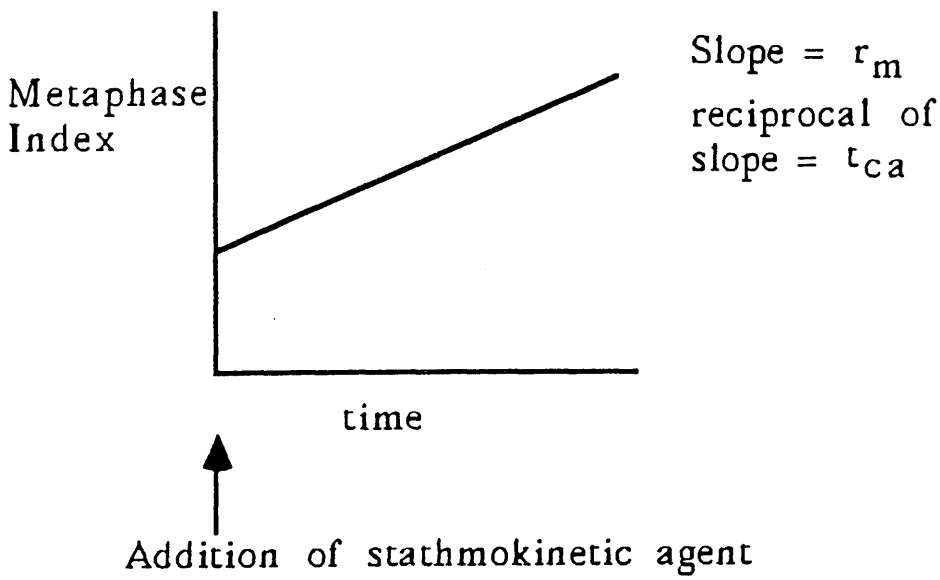


Figure 9. Accumulation of metaphases after addition of stathmokinetic agent. Slope of graph represents rate of entry into mitosis (Cell birth rate).

arrested metaphases within crypts allows for the metaphase data to be expressed in the form of crypt cell production rates (CCPR).

PART 6

IMMUNOLOGY

The inflammatory response in the gastric mucosa is manifest histologically by the appearance of gastritis. Gastritis is regarded by some authorities as the initial and fundamental step in the histogenesis of gastric carcinoma (Correa, 1984). The immunological responses that occur in the gastric mucosa during the development of gastritis, metaplasia and neoplasia are important in the understanding of gastric carcinogenesis.

The human immune system is complex. This section provides a brief summary of the salient points of relevance to the work of this thesis.

CURRENT KNOWLEDGE

Gastric Mucosa

The histological classification of gastritis (Whitehead et al., 1972) is based on a combination of the density of the inflammatory cell infiltrate in combination with degree of mucosal atrophy. The humoral component of the inflammatory response has been extensively investigated (Crabbe and Heremans, 1966; Kreunig et al., 1978; Valnes et al., 1986). The gastric mucosa contains a preponderance of IgA producing plasma cells which increase in number with increasing severity of gastritis (Valnes et al., 1986). IgM, IgG, IgD and IgE producing plasma cells are also present but in small numbers. The IgG isotype shows a greater relative increase in numbers with gastritis compared to IgA (Valnes et al., 1986).

In areas of intestinal metaplasia the number of inflammatory cells is seen to be reduced on conventional histology and this has been reported to be due to a decrease in IgG labelled plasma cells predominantly (Tsutsumi et al., 1984), compared with the others.

The humoral immune response in the stomach has been investigated as described above but until recently there has been relatively little knowledge of the cell mediated immune response within the human gut. Experimental animal work has shown that the gut associated lymphoid tissues differ from the systemic immune system with respect to cellular constituents, effector functions and the modulation of these functions. The gut provides the major barrier between the internal environment and the antigens of the external environment many of which are non-pathogenic. Thus there is a need for inhibition of most immune responses in the gut in contrast to the amplification of the response to parenterally encountered antigens in the systemic immune system.

The majority of published work in the field of gastro-intestinal immunology relates to the small intestine and disease states such as inflammatory bowel disease and coeliac disease. The intestinal mucosa appears to contain two distinct populations of T lymphocytes; those in the lamina propria and lymphocytes lying in the epithelial lining itself - intraepithelial lymphocytes (IEL). The majority of the lymphocytes in the lamina propria express the phenotype associated with helper/inducer T cells whilst the IEL express the phenotype associated with cytotoxic suppressor T cells (Selby et al., 1981; Cerf-Beunissan et al., 1983).

The intra-epithelial lymphocytes appear to be a highly specialised population of activated lymphocytes which possess natural killer activity (Mowat et al., 1983). The exact biological role and function of the intra-epithelial lymphocytes remains to be fully elucidated.

In the human stomach the pattern of distribution of lymphocytes appears to be similar to that of the intestine with a predominance of T helper/suppressor lymphocytes in lamina propria and T cytotoxic/suppressor cells in the intra-epithelial location. Natural killer cell phenotype is seen in a few lymphocytes in both locations. In areas of intestinal metaplasia there is a diminution in numbers of lymphocytes but the distribution of the T cell subsets appears to be the same to that of the gastric mucosa (Tsutsumi et al., 1984).

Class II histocompatibility antigens (HLA Dr) are genetically encoded molecules that restrict the immune reponse. The presence of such antigens is well known on B lymphocytes, macrophages and activated T cells. Recently the presence of such antigens has been detected on epithelial cells lining the gastrointestinal, urinary and respiratory tracts (Daar et al., 1984).

The Class 11 MHC antigens detected by anitbodies to HLA Dr have an interesting pattern of distribution. In gastric mucosa without inflammation there is virtually no expression but with increasing degrees of gastritis HLA Dr expression is seen on the epithelial cells particularly those in the area adjacent to lymphoid aggregates (Spencer et al., 1986). This has led to the

suggestion that the epithelial cells expressing HLA Dr antigens may play a role in presenting antigens from the gut lumen to lymphocytes in the adjacent lymphoid follicles (Spencer et al., 1986).

Gastric Carcinoma

The relationship between the immune response and neoplasm has provoked speculation for over 100 years (Underwood, 1974), yet despite the recent advances in immunology this field remains to be completely elucidated. Lauren (1965) in his initial description of intestinal and diffuse type of tumour noted differences in the inflammatory response between the two types. There have been sporadic reports in the literature (Monafo et al., 1962) that suggest that the degree of lymphocyte response to human gastric carcinomas is associated with the prognosis. Such studies have involved only conventional assessment of the lymphocytic infiltrate on light microscopy.

Modern immunocytochemical techniques have shown that the infiltrate surrounding tumours are heterogenous and consist of mononuclear phagocytes, various subsets of lymphocytes and other cell types - plasma cells and mast cells. The role of individual elements of the immune system in immune surveillance and the response to malignant change is complex and not fully explained. The diagram illustrates a summary of the interactions in the immune system which are currently thought to be involved in tumour immunity (figure 10).

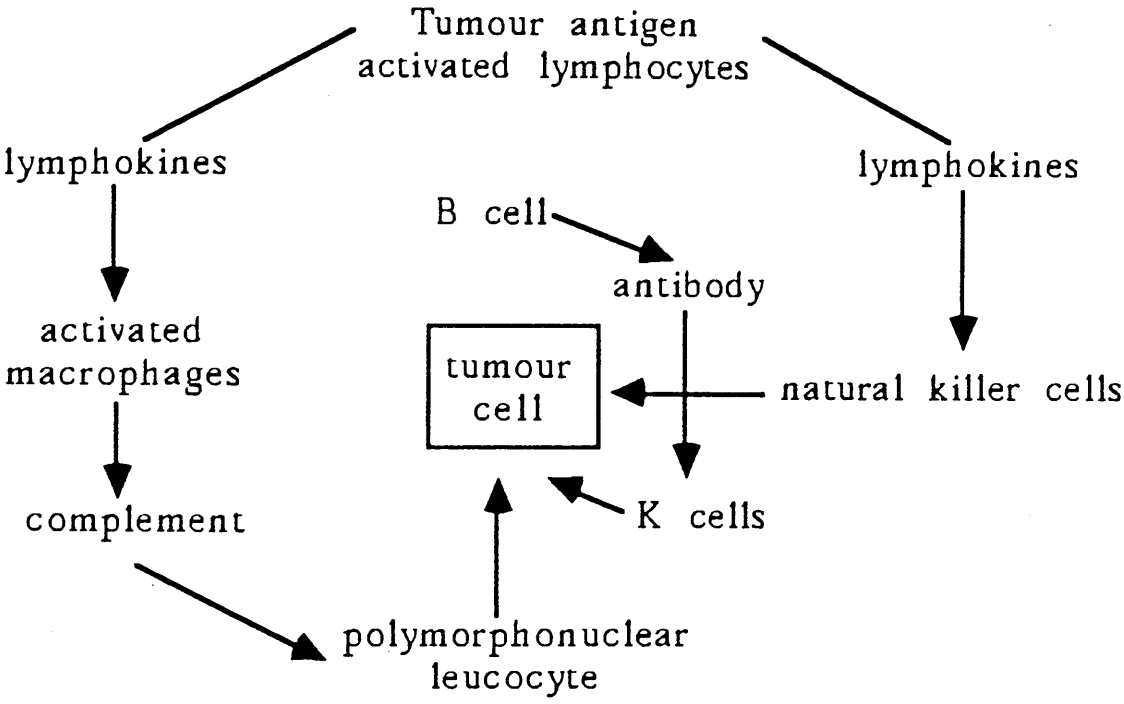


Figure 10. **Simplified summary of interactions of the immune system involved in tumour immunity.**

PART 7

HUMAN FETAL STOMACH

INTRODUCTION

Several features of the embryonic development of the gastric epithelium are of direct relevance to gastric carcinogenesis. The first feature is the original interpretation of intestinal metaplasia as a congenital heterotopia by Taylor in 1927. Taylor recognised that intestinal epithelium might arise from regenerative changes around gastric ulcers but regarded areas of mature small intestinal epithelium in the absence of inflammation as placing the "congenital nature beyond doubt". Magnus in 1937 examined 12 fetal stomachs from 6-9 months gestation and failed to find any intestinal epithelium and discarded the concept of congenital heterotopia. A more recent study (Salenius 1962) identified goblet cells in the pyloric canal at 11 weeks gestation. Goblet cells and cells with a striated border were seen in both the pyloric canal and the cardia from 20 weeks until term. A single neonatal stomach was examined and goblet cells and intestinal striated border cells identified in both cardia and pylorus.

Salenius's (1962) work raises the possibility that the presence of intestinal epithelium in the stomach may to some degree be due to a congenital rather than acquired phenomenon.

The second feature of interest is the presence of antigens common to the embryonic gut and gastro-intestinal tumours. Gold and Freedman in their original description of carcino-embryonic antigen in 1965 identified the antigen in fetal gut, liver and pancreas between 2-8 months gestation. The immunocytochemical

localisation of CEA has not been determined in the embryonic stomach.

The third aspect of interest in the embryonic stomach is the expression of mucin by the developing gastric epithelium and the relationship to the abnormal mucin histochemistry in disease states in the adult stomach. Two studies have been reported (Stemmerman 1967; Lev 1968) in the literature which have identified sialomucins in the fetal stomach although only small numbers of cases were studied.

DEVELOPMENT OF THE FETAL STOMACH

The stomach appears initially as a fusiform dilatation in the foregut in the neck, by the seventh week of gestation it descends into the abdomen. Circular smooth muscle appears in the eighth to ninth week at which time the epithelium forms as a single pseudostratified layer. The glandular pits form in rudimentary fashion at ten to eleven weeks gestation.

Succinic dehydrogenase activity can be detected in gastric homogenates at eight to nine weeks indicating the presence of developing parietal cells (Salenius, 1962). At the same time endocrine cells are present and by ten weeks all the currently identifiable endocrine cell types are present (Facer et al., 1989). Chief cells can be identified by 12 weeks by their high RNA content and granules staining black with iron haematoxylin. PAS-positive mucus neck cells appear at 13-14 weeks (Salenius 1962).

The antral body glands gradually increase in size from the 12th week of gestation and have an adult pattern by the 24th week of gestation. The circular muscle of the fetal stomach increases in thickness during the 12th to the 24th week of gestation.

PART 8

AIMS OF THESIS

A simplified diagrammatic representation of the possible histogenesis of gastric carcinoma is displayed in figure 11. A question mark can be placed beside each arrow and step in this sequence. The aim of this thesis is to attempt to investigate some of these questions. To obtain material for this study a prospective series of gastrectomy specimens from both benign and malignant cases was collected.

The first question to be addressed is the division of tumours into intestinal and diffuse types. The histological criteria of the Lauren system are investigated and a comparison with other classification systems undertaken.

The second question examined in the gastrectomy material is the relationship of intestinal metaplasia and its variants to the sub-types of gastric carcinoma. Several aspects of this relationship are examined. The amount of metaplasia in benign and malignant stomachs and the effect of other variables such as age, inflammation, ulceration and the presence of Helicobacter pylori are determined. The aim of this section of the thesis is to investigate if the relationship between intestinal metaplasia (and in particular Type IIb metaplasia) and carcinoma rather than representing a premalignant association might be explained by other factors.

The third aspect of the histogenetic sequence to be evaluated is dysplasia. The interpretation of dysplastic change is difficult and highly subjective. The investigation of dysplasia

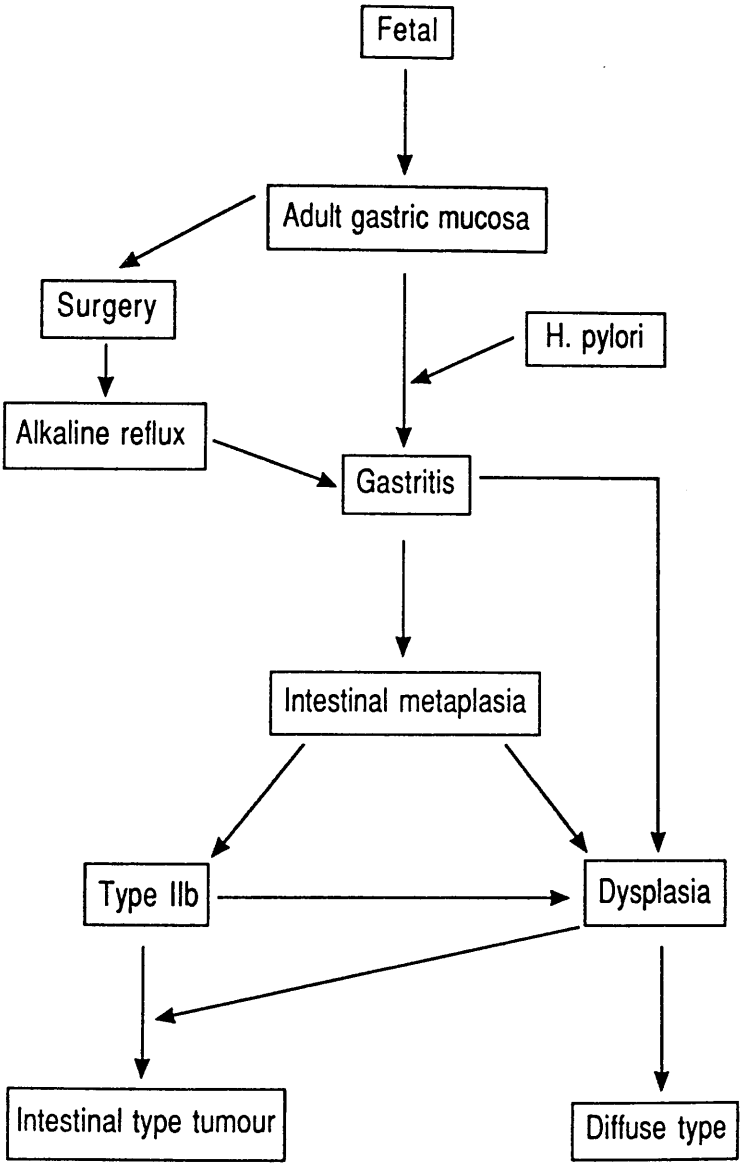


Figure 11. Hypothetical steps in the histogenesis of gastric carcinoma which are studied in this thesis.

is limited to documenting the incidence of dysplasia, the histological appearance and the relationship to benign and malignant changes in the gastrectomy material.

In conjunction with the collection of the histological material the clinicopathological data of the patients undergoing gastrectomy was collated. The aim of this section of the thesis was to document the methods of treatment, prognostic parameters and survival of patients with gastric carcinoma.

The histological assessment of the gastrectomy material outlined above results in the subtypes of metaplasia, types of tumour, dysplasia etc. being identified and classified. This material was then used to examine changes in the phenotypic expression of the epithelium at the various stages throughout the histogenetic sequence. Mucin histochemistry and immunocytochemistry were used to identify such changes. The aim of this part of the thesis was to determine if epithelial alteration in phenotype yielded any information to support or refute the possible steps in histogenesis. The phenotypic expression of the gastric tumours was also examined and the patterns of phenotypic expression that occurred in the different histological classification systems determined.

The next element to be examined was the cell kinetic changes that take place at different stages of the sequence. Material from the gastrectomy study was used in this part of the thesis and in addition epithelium from a selected group of patients who had undergone gastric surgery for benign disease was studied. The aim of this part of the thesis was to document the alterations in cell

kinetics that occur at various stages of the histogenetic sequence.

An animal experimental model of gastric carcinogenesis was used to study the cell kinetics of metaplasia and neoplasia using the stathmokinetic technique. The aim of this section of the thesis was to examine the more sophisticated parameters of the cell cycle and extend the human experimental observations.

The studies listed above are primarily concerned with epithelial changes. The cell mediated immune response within the lamina propria of the gastric mucosa was examined in the next part of the thesis. The aim of this was to determine the interaction between the immune response and the epithelial alterations.

The final section of the thesis involves a study of the fetal stomach. The aim of this section was to determine the histological development and phenotypic expression of the embryonic stomach and compare this with the changes that take place in the adult.

CHAPTER 2

SUBJECTS, MATERIALS AND METHODS

SUBJECTS, MATERIALS AND METHODS

SUBJECTS

Human Gastrectomy Study

A prospective collection of gastrectomy specimens submitted to the Department of Pathology, Western Infirmary, Glasgow, was initiated in December 1984 and continued to December 1987. The Pathology Department serves both the Western Infirmary and Gartnavel General Hospital. The two hospitals function as a single unit. There are ten Consultant General Surgeons.

A consecutive series of 94 gastrectomy specimens surgically resected for adenocarcinoma of the stomach and benign disease were collected, see table 9.

Mucin Histochemistry Study

The subjects studied included all the 94 patients who had a gastrectomy performed as described in the Human Gastrectomy Study.

Immunocytochemical Study

The subjects studied included all the 94 patients who had a gastrectomy performed as described in the Human Gastrectomy Study.

Cell Kinetic Study

Three groups of patients were studied.

Medical Treatment Group

Patients undergoing routine upper gastro-intestinal endoscopy for diagnostic or treatment purposes. This group was designated - MEDICAL TREATMENT GROUP.

The patients for this part of the study were drawn from the endoscopy clinic of a Consultant General Surgeon (WRM). The

Human Gastrectomy Study

Diagnosis	Number	Pathology
Benign	22	9 gastric ulcer 13 duodenal ulcer
Malignant	72	Adenocarcinoma

Table 9: Gastrectomy specimens collected between December 1984 and December 1987. Pathological condition resulting in gastrectomy detailed.

endoscopy clinic provides a diagnostic and therapeutic service for medical and surgical patients. The indications for endoscopy fall into two main categories; investigation for upper gastro-intestinal tract symptoms and follow-up of patients with both gastric and duodenal ulceration. The biopsy material was collected from 48 patients over an 18 month period, see table 10.

Surgical Treatment Group

Sixteen patients who had undergone gastric surgery for benign peptic ulceration between 4-25 years previously, see table 11. Biopsies were taken 1 cm, 3 cm and 5 cm from the gastro-enterostomy stoma when present. This group was designated SURGICAL TREATMENT GROUP.

Gastrectomy Group

Thirty seven patients who underwent gastrectomy, 30 for malignant disease and seven for benign disease from the Human Gastrectomy Study described previously. The gastrectomy specimens were sampled at three sites; A: the tumour, B: the gastric mucosa immediately adjacent to the tumour/ulcer within 1cm, C: the gastric mucosa at a distance of at least 5 cm from the tumour/ulcer.

Animal Experimental Model

Ninety five male Wistar rats aged between 4-6 weeks were studied.

Immunological Study

Twenty patients from the Human Gastrectomy Study were examined in this study. Twelve patients had a gastrectomy performed for malignant disease and eight for benign disease. The

Medical Treatment Group

Mean Age	52.5
(range)	(19 - 80)
Diagnosis	11 gastric ulcer
	23 non-ulcer dyspepsia
	14 duodenal ulcer
Sex	26 female
	22 male

Table 10: Age, diagnosis and sex of patients undergoing routine upper gastro-intestinal endoscopy and biopsy for diagnostic and treatment purposes - Medical Treatment Group

Surgical Treatment Group

Age	Sex	Operation	Time since operation (years)
71	M	Truncal vagotomy and drainage	12
62	M	" " " "	11
53	F	" " " "	25
45	M	" " " "	9
63	F	" " " "	8
47	M	" " " "	24
59	M	" " " "	17
32	M	" " " "	4
50	F	" " " "	12
61	M	" " " "	8
65	F	" " " "	9
58	M	" " " "	13
63	M	" " " "	7
41	M	Billroth 11	12
39	F	Billroth 11	4
74	M	Billroth 11	10

Table 11: Age, sex, nature of operation and time since operation in sixteen patients who had undergone gastric surgery for benign disease - Surgical Treatment Group.

gastrectomy specimens were sampled at three sites. A: tumour/ulcer, B: the gastric mucosa immediately adjacent to the tumour/ulcer within 1 cm, C: the gastric mucosa at a distance of at least 5 cm from the tumour/ulcer.

Fetal Stomach

Forty fetal stomachs, obtained from legal and spontaneous abortions, and four neonatal stomachs were studied.

MATERIALS AND METHODS

Human Gastrectomy Study

The gastrectomy specimens were collected from theatre immediately after resection, opened along the greater curvature and a macroscopic examination of the stomach and a description of any mucosal lesion made. The stomachs were then pinned on a cork board, inverted and fixed in 10% formol saline for 24-48 hours. Blocks were then taken in a standard fashion from the tumour and the length of the greater and lesser curves (see figure 12). A minimum of five blocks were taken from the tumour and adjacent mucosa. The length of the sections taken ranged from 2-3 cm.

The tissue was processed to paraffin blocks and serial 4 um sections were cut. The sections were stained with haematoxylin and eosin, the Cresyl Violet technique and the mucin histochemical procedures outlined below.

Mucin Histochemical Techniques

	Neutral	N-acetyl	O-acetyl	Sulpho
Diastase-periodic acid Schiff (D-PAS)	Red	Red	-	-
Perodate-borohydride (PB KOH PAS)	-	-	Blue	-
Alcian Blue AB pH 2.5/PAS (AB/PAS)	Red	Blue	Blue	Blue
High iron diamine/AB pH 2.5 (HID/AB)	-	Blue	Blue	Brown/ Black

Sampling of Gastrectomy

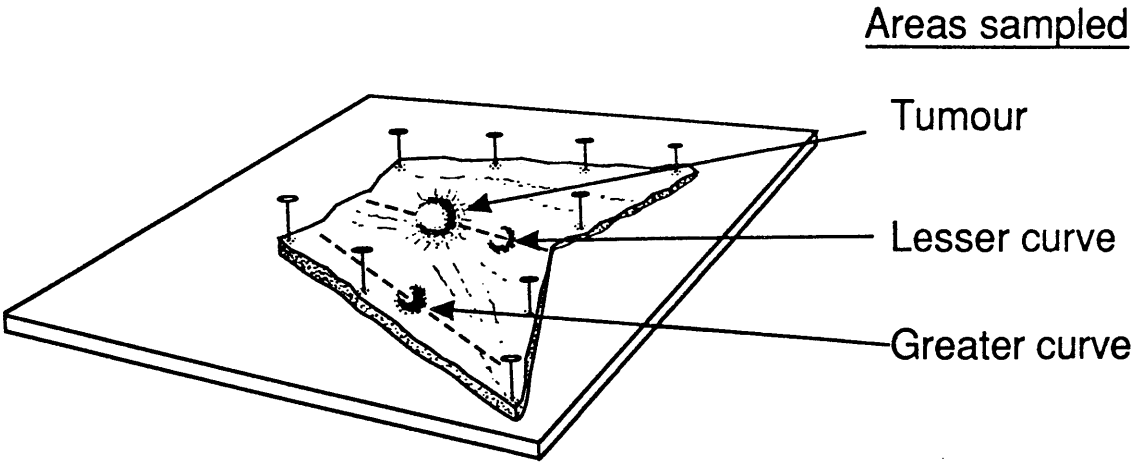


Figure 12. Technique of fixation and sampling of gastrectomy specimens.

The following flow chart illusrates the technique used to identify the mucins.

H & E identify tissue

D-PAS identify typical goblet cells and mucous cells

AB/PAS identify neutral mucins (red) or mixture acidic plus neutral purple

HID/AB identify sulphomucins (black) and sialomucins (O-A and NA) blue

PB/KOH/PAS identify type of sialomucin (O-Acetyl-blue).

The material from the Human Gastrectomy Study was examined in four main topics; clinico-pathological features, classification of tumours, intestinal metaplasia and dysplasia.

Clinico-pathological

The following clinico-pathological variables were noted for each patient.

Age

Sex

Presenting symptoms

Duration of symptoms

Drug history

Type of surgical resection

Length of post-operative survival

Size of tumour

Distance to nearest resection margins

Number and pathology of lymph nodes identified

Depth of penetration of tumour (T stage)

Presence of distant metastasis

Classification of tumours

Classification procedure

On the basis of the histological appearance the malignant gastrectomy specimens were classified into the various sub-groups outlined in the five classification systems in Chapter 1. The classification into the Lauren system was performed by two observers, the author and a Consultant Pathologist. The remaining classification systems were interpreted by the author alone.

(1) WHO

(2) Lauren

(3) Mulligan and Rember

(4) Ming

(5) The Jass modification of the Lauren system.

Evaluation of Lauren System

In order to examine the heterogeneity of gastric tumours and the implications on the Lauren system, a detailed analysis of the histological features of the gastric tumours using a scoring system was employed. Each tumour was examined in detail and all areas of the sections surveyed. The four main histological features of the Lauren classification were then assessed and a score assigned. (A score of +1 was assigned for each intestinal type histological feature, conversely a score of -1 was assigned for each diffuse type feature. A tumour displaying all four histological features of the intestinal type derived a score of +4. If however there were areas of single cells with poor cohesion in addition to well defined glandular lumina and all the other features of an intestinal type tumour then a score of 4+ -1 = 3) (see table 12).

Scoring System for Lauren Classification

Variable	Histological Features	Score
General structure	glandular lumina, cell cohesion	+1
	single cells, clusters of cells with poor cohesion	-1
Cell structure	large, variable morphology, mitotic figures	+1
	uniform, poorly defined, regular nuclei	-1
Secretory cells	few, extracellular mucin	+1
	plentiful, intracellular mucin	-1
Mode of growth	well defined, peripheral edge, polymorphonucler response in invaded tissue	+1
	poorly defined, peripheral edge, lymphocyte response and connective tissue proliferation in invaded tissue.	-1

Table 12. **Scoring system for Lauren Classification. On the basis of the four histological variables described by Lauren, tumours are assigned positive mark for intestinal feature, negative for diffuse.**

Intestinal Metaplasia

Quantification and classification of intestinal metaplasia

All sections from each gastrectomy specimen were examined. The number of sections ranged from 12-23 with a median of 17. The number of gastric crypts and crypts of intestinal metaplasia were counted. The total number of crypts in each specimen examined was thus determined and the percentage of the total which were of gastric type or metaplastic types was calculated for the intestinal, diffuse and benign gastrectomy specimens. The sub-types of intestinal metaplasia as defined by the morphological and histochemical criteria of Jass were identified using the serial sections stained for mucins. The percentage of each sub-type as a total of all crypts in each specimen (ie. gastric and metaplastic) and the percentage of each sub-type as a total of the metaplastic crypts only were calculated. Using the results of the quantitative analysis the amount of the gastric mucosa replaced by metaplastic epithelium was graded: little or none <5%, moderate 5-20%, extensive 20-100%.

The crypt counting technique was performed by one observer. Ten gastrectomy specimens were selected at random and recounted. Analysis of Variance between the two counts was performed to determine the validity of using the results from the counting technique in a statistical analysis.

Relationship of variables within the gastrectomy specimens to intestinal metaplasia

Six variables were assessed in relation to intestinal metaplasia.

(i) Diagnostic group - using the data from the previous section the gastrectomies were subdivided into intestinal, diffuse and benign groups according to Lauren and intestinal, "gastric" and benign, according to Jass. In determining the relationship between type IIb and the histological type of tumour as defined by Lauren (1965) all the gastrectomy specimens were analysed. However in the analysis of type IIb and the histological types of tumour defined by Jass (1980) only those stomachs which showed extensive IM were used as this was the selected group in which Jass (1980) described a significant relationship between type IIb and intestinal type of tumour.

(ii) Presence of ulceration - ulceration was defined as the breach of the mucosal layer to the level of the sub-mucosa at least.

(iii) Inflammation - the severity of the inflammation present within the gastric mucosa was graded on a scale of 0 - 6 using standard photographs after the method described by Watt et al., 1983. The method was modified to include gastritis and atrophy on a single scale rather than examining these features independently. The score depended on the quantity of inflammatory cells in the lamina propria and degree of atrophy. Standard photographs illustrating scores of 1, 3 and 5 were used. Score 1 showed a minimal infiltrate of cells in the superficial layer of the lamina propria; 3 indicated a heavy infiltrate of chronic inflammatory cells throughout the mucosa Specimens with score 3 and score 5 showed some degree of atrophy and were

roughly equivalent to chronic atrophic gastritis and active chronic atrophic gastritis. The severity of gastritis varied in different areas sampled.

The area with the most severe inflammatory changes was used to determine the grade for each stomach.

(iv) The presence of Helicobacter pylori in the Cresyl Violet stained sections was determined.

(v) The age of the patients undergoing gastrectomy was also determined.

(vi) The sex of the patient undergoing gastrectomy was determined.

The data were analysed using Chi square analysis with Yates correction to test the association between intestinal metaplasia and the subtype IIb and the diagnostic group.

The question of whether the presence of intestinal metaplasia or the subtype IIb is associated with any of the factors, ulceration, inflammation, diagnostic group, age, or any combination of these was investigated using the statistical package GLIM (Generalised Linear Interactive Modelling). In determining significant combinations of factors this technique investigates the probability of the presence of intestinal metaplasia on the factors and then, for any combination of factors found to be significant, provides estimates of the probability for any specific case.

Dysplasia

The sections taken from the area of the tumour/ulcer were examined by one observer. A subjective description of the variants of dysplasia was made. The criteria used to assess dysplasia were those outlined by Morson et al., 1980.

The frequency of the variants of dysplasia occurring in the diagnostic groups of the Lauren (1965) classification and benign

disease was determined. The tumours were classified into well and poorly differentiated categories on subjective histological grounds by one observer and the occurrence of dysplastic variants determined for these two groups.

Mucin Histochemistry Study

Using the flow chart outlined in the mucin histochemical techniques the serial sections from the human gastrectomy study were examined.

The mucin histochemistry of the gastrectomy material was examined in four ways.

(1) The mucin expression in normal and inflamed gastric mucosa in both the benign and malignant groups was determined. The presence or absence of mucins was solely determined; no attempt was made to grade the amount.

(2) The mucin profile of intestinal metaplastic crypts of subtypes I, IIa and IIb from gastrectomy specimens containing benign, intestinal type and diffuse type tumours of the Lauren (1965) classification. Ten gastrectomy cases from each group were selected randomly and 100 crypts of each subtype which were axially sectioned were studied. The mucin content of the goblet cells in type I and the goblet cells and columnar cells in type IIa and type II recorded. The presence or absence of a particular mucin within the goblet or columnar cells of the individual crypts was noted; no attempt was made to quantify the number of cells expressing a specific mucin within a crypt.

(3) The presence or absence of the mucin subtypes within the

tumours was determined by examining all the sections taken from each tumour. The mucin content was related to the classification systems and the degree of differentiation.

(4) The sulphomucin content of the tumours was related to the presence or absence of type IIb metaplasia in the mucosa of the gastrectomy specimen.

Immunocytochemical Study

Epithelial cell and oncofetal antibodies

Blocks were selected from each case from the human gastrectomy study to include representative portions of tumour/ulcer, adjacent mucosa and areas of intestinal metaplasia. Axially sectioned crypts of intestinal metaplasia were examined in order to determine the distribution of the immunoperoxidase staining throughout the crypts. Specimens which showed less than perfect fixation were excluded. The antibodies used were against carcino-embryonic antigen (CEA), secretory component (SC), IgA and intrinsic factor (IF). The source and dilution of the antibodies is shown in table 13.

Production of Intrinsic Factor Antibody

Antibodies to hog intrinsic factor were produced by immunisation of New Zealand white female rabbits with 75 ug purified hog intrinsic factor in aqueous solution in a 2 ml emulsion with Freund's adjuvant and boosted at 14 day intervals with Freund's complete adjuvant. Antibody to intrinsic factor was detected in the rabbit serum by its ability to block the binding of Vitamin B12 by intrinsic factor in vitro. The titre of antibody

varied but typically 1 ml of rabbit serum blocked the binding of approximately 200 ng of cyanocobalamin by intrinsic factor. In Ouchterlony plates a single precipitin line was detected between the rabbit anti-intrinsic factor and purified intrinsic factor. When the antibody was run against an intrinsic factor - vitamin B12 complex a faint accompanying line was detected, indicating the presence of antibody to intrinsic factor - vitamin B12 complex. Non-intrinsic factor vitamin B12 binding proteins did not cross-react with the antibody.

Absorption techniques with CEA antibody

NCA_2 was obtained from normal stomachs of cancer free patients. The stomach mucosa was stripped off and freeze dried and 50 mg of tissue powder used for absorption. Human spleen was prepared in a similar manner.

Absorption was performed by mixing the stomach and spleen powder with antiCEA serum, incubated at $4^{o}C$ overnight. The mixture was centrifuged at 100,000 revs for one hour and the supernatant extracted (Nap et al., 1983).

Neuro-endocrine cells

Selected blocks from formalin fixed paraffin wax material from benign and malignant gastrectomy specimens which included representative areas of normal, inflamed and sub-types of metaplasia, were stained using antibodies raised against.

Chromogranin

Glucagon

Gastrin

Vasoactive intestinal polypeptide (VIP)

Cholyecystokinin (CCK)

Glucose dependent insulinotropic peptide (GIP)

Calcitonin gene related peptide (CGRP)

Bombesin

Somatostatin

The source and dilution of the antibodies is shown in table 13.

Immunoperoxidase methods

4-5 um sections thick mounted on freshly polished slides, degreased in 99% alcohol for a minimum of 24 hours. Sections dried overnight at 37°C followed by 15 minutes at 60°C.

Peroxidase-antiperoxidase method

This technique was used for the anti CEA, IF, SC and Neuro-endocrine antibodies.

1. Dewax and hydrate sections
2. Removal of endogenous peroxidase with 0.5% Hydrogen Peroxide in methanol half an hour.
3. Ten minute exposure to normal inactivated swine serum, diluted 1:5 with Tris buffered saline. Excess drained off.
4. Treat with primary antibody.
5. Wash Tris buffered saline x 3
6. Swine anti-rabbit IgG for 30 minutes at room temperature
7. Wash Tris buffered saline x 3
8. Rabit antiperoxidase-peroxidase complex.
9. Wash Tris buffered saline x 3.
10. Visualisation using Diamino Benzidine Tetra Hydrochloride

Antibody	Dilution	Source	Incubation
CEA	1/5	DAKO	12 hrs 4°C
Secretory component	1/600	DAKO	1 hr room temp.
IgA	1/600	DAKO	1 hr room temp.
Intrinsic factor	1/500	S.A.P.U.	1 hr room temp.
Chromogranin (Human)	1/200	RPMS	12 hr 4°C
Glucagon	1/2000	RPMS	" "
Gastrin	1/5000	RPMS	" "
CCK (9-20)	1/200	RPMS	" "
GIP	1/5000	RPMS	" "
CGRP	1/2000	RPMS	" "
Bombesin	1/2000	RPMS	" "
Somatostatin	1/5000	RPMS	" "

SAPU = Scottish Antibody Production Unit

RPMS = Royal Postgraduate Medical School, Hammersmith

Table 13: Source, dilution and incubation period of antibodies used in immunocytochemical study.

Controls

(1) Pooled heat inactivated normal rabbit serum diluted at the same strength as the test serum.

(2) Section treated for endogenous peroxidase and DAB only.

(3) Known positive control sections.

Avidin-Biotin Technique

This technique was used for the anti IgA antibody using the ABC kit.

Interpretation of Immunocytochemical Preparations

The interpretation of immunoperoxidase staining is highly subjective. The simplest analysis involves the determination of presence or absence of staining within the particular tissue under study. The pattern and morphological distribution of the staining is of prime importance. In the neoplastic epithelium the following features of peroxidase staining were noted:

1) Cellular distribution ie cytoplasmic, apical, brush border.

2) Types of cells stained ie Signet ring cells.

3) Distribution of labelled cells within tumour - ie. focal or diffuse and the areas within the tumour stained.

4) Number of cells within tumour stained - objective assessment graded 0 - 4 . 0 = no cells, 1 = >20%, 2 = 20-50%, 3 = 50-80%

4 = 80-100% cells stained.

The peroxidase staining of the gastric and metaplastic mucosa was assessed in a similar fashion but with some modification.

1) Cellular distribution.

2) Types of cells ie goblet cells, columnar cells, parietal

cells.

3) Distribution of cells within the crypt and type of crypt, antral/gastric, type of metaplasia, and dysplasia.

4) Level of inflammation in the surrounding lamina propria according to the 0 - 6 scale outlined previously.

Quantification

The staining of the antibodies against SC, IgA and CEA was quantified in the subtypes of metaplasia. 1,323 crypts axially sectioned to include the base and luminal surface were examined. The number of crypts of each subtype which showed positive staining with the antibodies against CEA, SC and IgA were determined. The figure was expressed a percentage of the number of crypts examined of the three subtypes.

The staining pattern of the antibodies against the neuropeptides in normal, inflamed and metaplastic epithelium was also quantified. The quantification of neuroendocrine cells has been investigated by Pietroletti et al., 1986. These authors describe a technique of counting positively stained cells in a given morphological unit. This technique of counting was used with a morphological unit of six crypts. Using the antichromogranin antibody the number of endocrine cells positively stained per morphological unit was determined. The antibodies raised against specific neuropeptides did not stain cells in sufficient frequency to allow for a meaningful count to be made; often only one or two cells stained positively with the antibody against a specific neuropeptide in 30-40 crypts. The positive staining of cells with antibodies against specific neuropeptides is

presented as a list in descending order of frequency.

Cell Kinetic Studies

Tissue preparation

The material from the three groups of patients was handled in an identical manner. The tissue was diced into small fragments, incubated in medium containing tritiated thymidine, fixed, processed into paraffin blocks and 20 serial 4 um sections cut. Every third section was developed to visualise the incorporated thymidine. The intervening sections were stained with haematoxylin and eosin and High Iron Diamine/Alcian Blue to identify the subtypes of intestinal metaplasia. A Cresyl Violet stain was also performed to identify any Helicobacter pylori present. The tritiated thymidine technique is detailed below.

The tissue was diced into small pieces and placed in 5 ml of RPMI 1640 medium containing, foetal calf serum 10%, insulin 20 units, glutamine 1 ml (29.3 mgs), penicillin/streptomycin 2 mls conc 5,000 units/5,000 ug/ml) per 100 ml volume.

Fifty ul of tritiated thymidine was added to medium, concentration of thymidine 1 mCi/ml, giving concentration of 10 uCi/ml in medium. 95% O_2/5% CO_2 was bubbled into medium for 20 seconds. Specimen tubes were placed in 37^{o}C shaking H_2O bath for two hours.

Samples were then immersed in 10% buffered formalin and processed to paraffin wax. Serial 4 um sections were cut and placed on slides coated with gelatine, chrom. alum. Slides were dewaxed and rehydrated.

Slides were coated with Ilford K_2 liquid emulsion, placed in

light-tight box containing silica and crushed solid CO_2. The box was closed and placed in a cool place for two weeks. The slides were developed using D19 developer for 5 minutes, 30 second wash in deionised H_2O, Kodafix for 5 minutes and then washed in running tap water for 15 minutes. The sections were then stained with haematoxylin and eosin.

Histological variables

The mucosa was classified as antral or body type on the basis of histological examination. The severity of the inflammation was graded 0 - 6 as previously described. The reflux gastritis score was determined using the criteria outlined by Dixon et al., 1986. The subtypes of intestinal metaplasia were identified using Jass's criteria (Jass 1980). The presence of Helicobacter pylori was determined using the Cresyl Violet technique. The tumour material examined was not classified using the small tissue samples obtained for the thymidine incubation technique but by reference to the histological material taken for the Human Gastrectomy Study.

Counting techniques

Gastric mucosa

Cells were considered labelled if they had five or more grains over the nucleus. In the majority of the cases the crypts had been sectioned perpendicular to their axis resulting in cross-sectioning.

In the gastric mucosa labelled cells in all cross-sections of foveolae containing one or more labelled cells were counted. The total number of cells in all cross-sections and the number of foveolae were also counted. In each sample a minimum of 2,000

cells were counted and the percentage of labelled cells (Labelling Index (LI)) estimated. This counting technique tends to overestimate the LI as cross-sections through the progenitor region which do not have labelled cells will not be included in the estimation of the LI. This error was reduced by means of an equation for correction described by Hansen et al., 1975 described in the previous text. The LI quoted in the results are the corrected values.

In addition to the examination of cross-sections of gastric crypts, the biopsies were re-orientated and axial sections of the antral crypts cut. The axial sections were examined to determine the distribution of the labelled cells within the crypts and the relative lengths of the crypts in cell numbers counted and expressed as column length. To determine the column length the number of cells from the luminal surface to the base of the crypt were counted. It proved technically difficult to achieve axial sections which included the complete length of a crypt. In the gastric crypts the column length and the geographical position of the labelled proliferative zone was determined. The position of the labelled cells in the crypts were counted in defined positions. The cell at the luminal surface of the crypts was designated cell 1 and the cells numbered in increasing order towards the base (see figure 13). The number of labelled cells were counted in defined positions of the crypt; above the luminal surface, cell postion 1-5, cell position 6-10, etc. One hundred crypts were examined from selected biopsies from the Medical Treatment Group at gastritis scores 1, 3 and 5 and the cell counts pooled and expressed as number labelled cells at a defined cell position as a percentage of

DIAGRAM OF GASTRIC PIT

Cells numbered from cell at luminal surface.
Cell position 10 shown labelled.

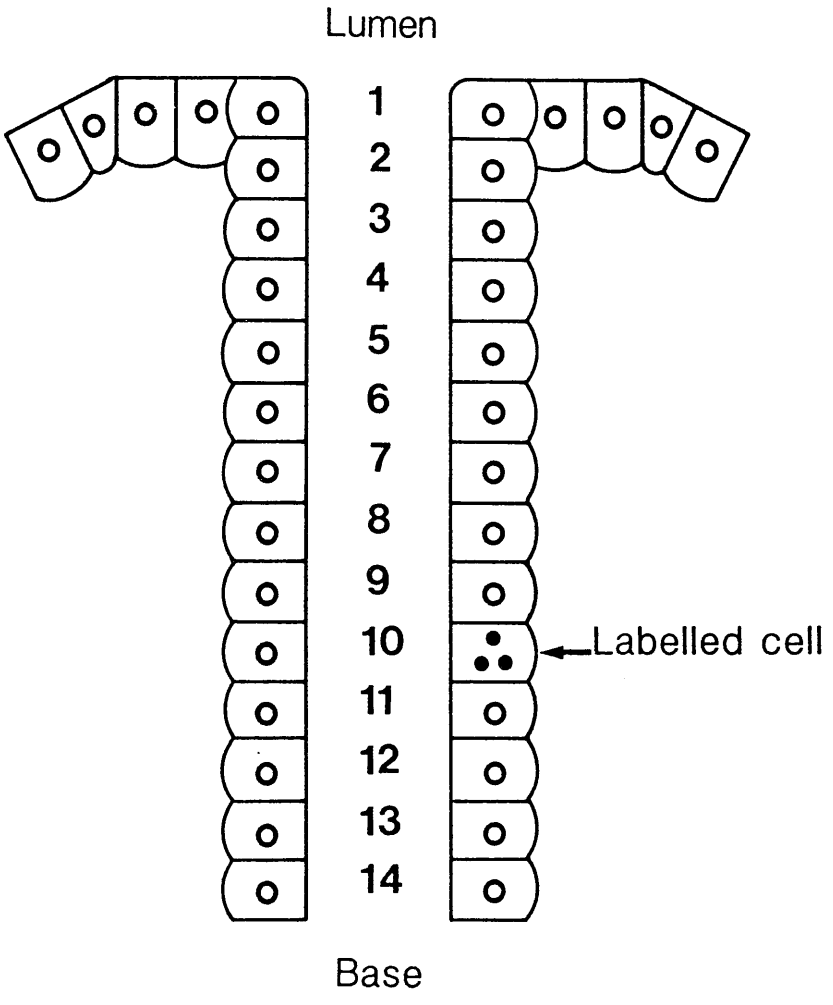


Figure 13. Diagrammatic representation of axially sectioned crypt and cell numbering technique. Cell at luminal surface of crypt is numbered cell 1 and adjacent cell 2 and so forth, towards the base of the crypt. Cell in position 10 is shown as labelled with thymidine.

the total number of labelled cells. Twenty five crypts from the Surgical Treatment Group were examined in a similar fashion. This allows for the position and distribution of the generative zone to be assessed in both the Medical and Surgical Treatment Groups.

Intestinal metaplasia

The labelling index of the intestinal metaplastic crypts was determined using a modified technique. It was not possible to determine the type of metaplasia in cross-sections so axial sections were examined. In these axial sections the labelling index was defined as the percentage of the labelled cells and counted in a total number of 2000 nuclei in the generative zone; the generative zone was defined as the region between the level of the uppermost labelled cells to that of the lowermost labelled cells.

Gastrectomy Group

The Labelling Index of the mucosal samples from areas B and C were determined by counting labelled cells in cross-sections of crypts as previously described.

The Labelling Index of the tumours was determined by counting the number of labelled cells and unlabelled cells in high power fields (x 400). A minimum of 2,000 cells were counted for each case and the LI expressed as a percentage of labelled/unlabelled cells. The type and distribution of the labelled tumour cells was assessed histologically.

Validation of Counting Technique in Thymidine Labelled Material

The objective of this study was to determine to what extent 'observer error', either in the form of a person being able to reproduce stable relative counts of labelled to unlabelled cells or

in the form of variability across different selected areas of tissue, would affect the results of the study.

Three pairs of counts (L,U) of labelled and unlabelled cells were obtained on ten cases from the endoscopic biopsy material (five from the Medical Treatment Group and five from the Surgical Treatment Group) and ten cases of gastric carcinoma from the gastrectomy material. The first pair of counts was on area 1, the second and third counts were on the same area 2. This provided information on the reproducibility of the count ratio L/U between two different areas of the same case and also on the ability of the investigator to reproduce relative counts in the same area. Using this data a statistical analysis was performed to determine if the observer error was small and insignificant to allow valid statistical comparisons to be made, or alternatively if the observer error was of such a magnitude to make statistical comparisons invalid.

For the purposes of discussion of the statistical analysis it is convenient to imagine that there are three 'observers' j = 1, 2, 3, with observer 1 being regarded as the investigator determining the counts on the first area, observer 2 the investigator at his first attempt on the second area and observer 3 the investigator on his second attempt on the second area.

For data of this compositional type a standard recommendation is that instead of the ratio L/U of labelled to unlabelled cells the logarithm of L/U (shortened to logratio) should be studied. Let x_{ij} denote the value of this logratio recorded for the ith case by observer j. For the given data set the maximum possible

explanation of the variability of the logratios is provided by a model M involving both case and observer effects as follows:

$$x_{ij} = u + \alpha_i + \beta_j + \text{error } e_{ij}$$

where u is the general mean, α_i is specific to the ith case and β_j is specific to the jth observer. Hypotheses of interest can then be posed within this model and set out in a lattice. The different levels of the lattic correspond to different degrees of complexity of explanation of the variability observed in the logratios log (L/U), with hypotheses at lower levels providing simpler explanations than those at higher levels. Level 0 corresponds to the simplest hypothesis H_0 that the logratios do not depend on either case or observer. At level 1 there are two hypotheses, H_1 that the variability depends on case only and H_2 that the variability depends on observer only. At level 2 there are again two hypotheses, H_3 that there is no difference between replicate count determinations within a given area and H_4 that there is no difference between replicate count determinations between different areas. Level 3 corresponds to the model M.

For the statistical testing of the various hypotheses within this lattice the statistical package GLIM (Generalized Linear Interactive Models) was used. For this particular problem the 'response variable', log (L/U), is 'continuous' and the hypotheses can be defined in terms of the two factors, 'case' at 10 levels and 'observer' at 3 levels. For the model and each hypothesis GLIM provides a fit to the data. For the model M and each hypothesis H a measure of the lack of fit is reported as a 'deviance' D_M or D_H, together with a number of 'degrees of freedom' d_M and d_H. The

number of degrees of freedom is the number n of observations less the number of 'parameters' required to define the model or the hypothesis, so that the more complex the explanation the fewer the number of degrees of freedom. The test of any hypothesis H within the model M then depends on the extent to which D_H is bigger than D_M and the critical value of the test quantity depends on the numbers of degrees of freedom d_N and d_M. The appropriate test is the well-known F-test used in standard analysis of variance: reject the hypothesis H within the model M at significance level P if

$$\frac{(D_H - D_M)/(d_H - d_M)}{D_M/d_M} > F_p$$

where F_p is the appropriate upper P percentage point of the F distribution with $d_H - d_M$ and d_M degrees of freedom. This test is carried out successively on the various hypotheses starting at level 0, proceeding to level 1 only if the hypothesis at level 0 is rejected, moving up to level 2 only if the two hypotheses at level 1 are both rejected, and so on. This process is stopped at the first level at which there is failure to reject a hypothesis, with all non-rejectable hypotheses at that level being regarded as possible explanations of the observed variability.

Animal experimental model

Ninety five male Wistar rats were divided into two groups. Group 1 received N-methyl-N'-nitro-N-nitrosoguanidine at a concentration of 50 μg/ml in their drinking water ad libitum for 16 weeks and thereafter normal tap water. Group II served as a control receiving unadulterated tap water. The carcinogen

was prepared freshly twice weekly and stored in a light proof container, the rats drinking bottles also being protected from light. The health of the rats was monitored by daily inspection and weekly weighing. Any animal showing significant deviation from normal health was humanely killed.

The animals were sacrificed at the following intervals shown in table 14.

The rats were injected intraperitoneally with 0.75 mg/kg vincristine sulphate and sacrificed at hourly intervals from time 0, 1 hour, 2 hours, 3 hours and 4 hours. In addition to this two rats in each group were injected intraperitoneally with 500 Uci thymidine and sacrificed at one hour post injection.

A post mortem examination was performed. The stomach was removed with a cuff of duodenum and oesophagus, opened along the greater curvature and pinned on a small cork board. The board was then inverted and placed in Carnoy's solution for 24 hours and then transferred to Cellosolve (C). The fixed material was then sectioned and processed to paraffin blocks. Four um sections were cut for haematoxylin and eosin staining and for autoradiography where appropriate.

Histological Examination

The haematoxylin and eosin sections were examined and the presence of ulceration, metaplasia and carcinoma noted.

Metaphase arrest technique

The metaphase arrest technique depends on correct application. Wright and Appleton, 1980, have highlighted certain

Time weeks	Control Group	Carcinogen exposed
0	5	5
20	10	14
40	10	14
60	10	10
80	4	5

TABLE 14: Sacrifice intervals and number of animals for experimental carcinogenesis study.

points and these are discussed below, in relation to the proposed experiment.

(1) Choice of metaphase arrest agent

(2) Dosage to be used

(3) Which cells to count

(4) Timing of readings

(5) Interpretation of results

(1) Which agent to use

Vincristine has been shown to be effective in the rat small bowel and this agent is regarded as the most suitable for gut epithelium when compared with vinblastine and colcemid (Tannock, 1965).

(2) Dosage to be used

Tannock's experiments (1965) identified the optimum dose for Vincristine for rat gut in the range 0.25 mg/kg to 1 mg/kg. The dose selected in this experiment was 0.75 mg/kg.

(3) Which cells to count

The number of metaphases should be counted and related to the total cell population. Bowel crypts act as closed systems from which arrested metaphases are unable to escape during the experiment. A technique of microdissection of whole colonic crypts is now widely accepted and this allows for the calculation of a crypt cell production. The advantage of this method is that it avoids the difficulty of correcting for the diameter of arrested metaphase and interphase nuclei in tissue sections (vide infra). The technique of whole crypt dissection has been widely applied in

colonic crypts but not for gastric crypts. An initial experiment was performed to examine the application of the technique in the stomach. Unfortunately the gastric crypts appeared too friable and fragmented into clumps of cells. It was therefore decided to use tissue sections. The number of cells in each crypt and the number of arrested metaphases in the crypt were counted. In counting tissue sections an important geometric factor has been identified by Tannock, 1965. Mitotic nuclei in the intestinal crypts are invariably situated nearer the axis of the crypts out of line with interphase nuclei. Using a simple cylindrical crypt sectioned along its axis, a mitotic nucleus of diameter d and at radius a will be counted in approximately d/2 a of the possible axial sections while an interphase nucleus of the same size will be counted in only d/b of these sections where b is the crypt lumen radius (see figure 14). Thus the metaphase index will be overestimated by a factor of b/a. To determine the factor b/a, 50 cross-sections of crypts were examined; the radius (b) and position of the metaphases (a) were measured using a graticule and expressed as the ratio a/b (see figure 15). The mean value was found to be 0.53 and this value for a/b was used as the Tannock correction factor for the gastric crypts.

(4) Timing of reading

Two errors may occur when taking readings (Wright and Appleton, 1980). Firstly, only two readings may be taken, one at time zero and the second after a given period. If the regression line is calculated using only these two points then any initial delay in the arrest of metaphases is not

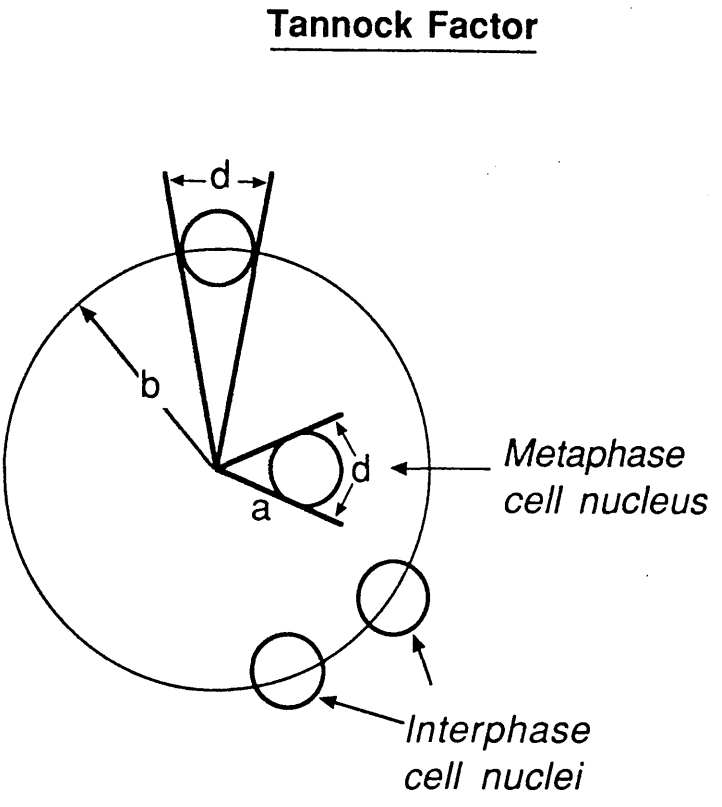


Figure 14. Diagrammatic explanation of Tannock Factor. Large circle represents cross section of intestinal crypt. Metaphase nuclei lie nearer the axis of the crypt compared with interphase nuclei. If an axial section of the crypt is taken a mitotic nucleus of diameter d and at radius a will be counted in only d/b of these sections where b is the crypt lumen radius

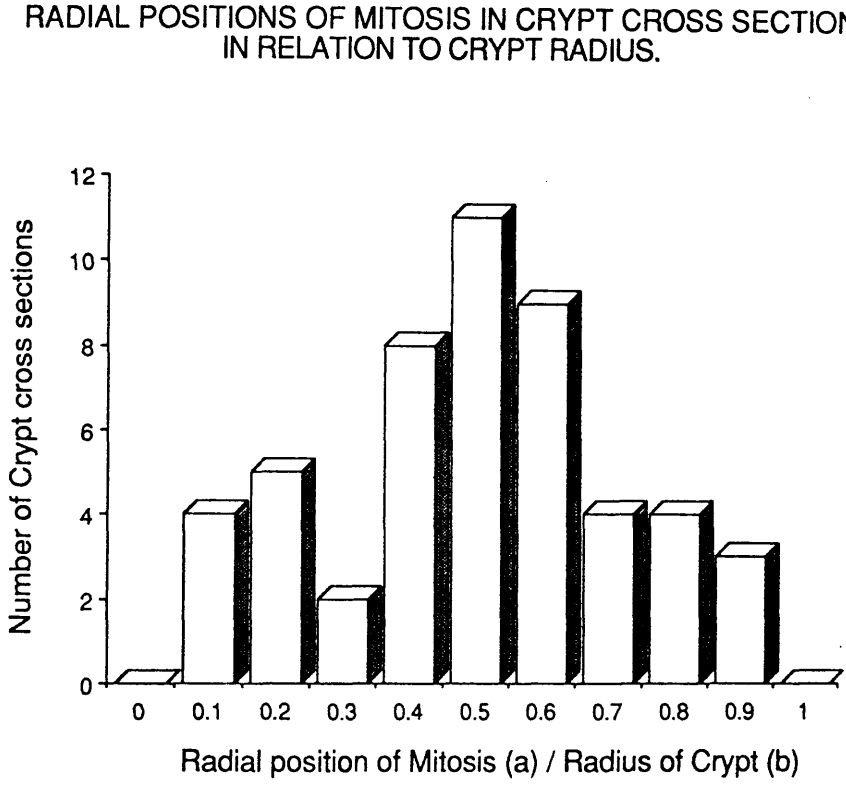


Figure 15. Radial position of mitosis (a) in crypt cross sections in relation to crypt radius (b) in 50 rat antral crypts. Mean value is 0.53.

taken into consideration and the rate of entry into mitosis will be underestimated. Secondly, readings may be taken over the non-linear portion of the curve ie. when metaphases have begun to degenerate. Wright and Appleton, 1980, regarded 0 - 4 hours as a relatively standard period in metaphase arrest experiments in mammalian tissue. Tannock demonstrated linearity of metaphase arrest with vincristine over a four hour period. A four hour period was selected for the present experiment; metaphase degeneration was not seen over this time scale using vincristine at a dose of 0.75 mg/kg.

(5) Interpretation of results

In the antrum ten crypts from each animal were assessed. The number of arrested metaphases and the number of cells in each crypt column were counted. Multiplication by a factor of two gives the number of cells in each crypt section and allows the metaphase index to be expressed as a percentage:

$$\frac{\text{number of arrested metaphases}}{\text{number of cells per crypt section}} \times \frac{100}{1} = \text{mitotic index.}$$

This was then corrected using the Tannock factor.

The accumulation of metaphases over the four hour experimental period was calculated. The slope of the line was fitted using the least squares method assuming a rectangular age distribution. The slope of the line is the rate of entry into mitosis R_m. The rate of entry into mitosis is otherise known as the cell production rate. The results can be expressed in the form of Crypt Cell Production Rate/Hour ie number of cells produced

per crypt per hour.

Thymidine Labelling Technique.

The sections were processed for autoradiography and counterstained as described in Human Cell Kinetic Study. Serial sections were examined and the number of labelled cells and non-labelled cells in the generative zone of the antral crypts were counted. A minimum number of 1,000 cells per animal were counted. The generative zone was defined as the section of the crypt from the uppermost labelled to the lowermost labelled cell. The Labelling Index was calculated as the number of labelled cells divided by the number of unlabelled cells and expressed as a percentage. The mean value of the LI of the two animals in each group studied is given in the results section. In view of the small numbers of animals no statistical comparison was attempted.

In addition to the estimation of the Labelling Index the site of the generative zone in the crypts was noted in each case. The site of the generative zone was classed as being in the lower, middle or upper third of the crypts.

Cell mediated immunity

The specimens were snap frozen in liquid nitrogen. Serial sections were cut 4 um in thickness. The sections were then stained using a panel of monoclonal antibodies outlined in table 15 using an alkaline phosphatase technique outlined below.

Alkaline Phosphatase Technique

Cryostat sections

1. Fix in acetone ten minutes

Antibodies	Cluster of differentiation	Specificity
Leu 1	CD 5	T lymphocytes, less 5% of B-lymphocytes
Leu 2	CD 8	Suppressor/cytotoxic T cell subset and natural killer cells
Leu 3	CD 4	Helper/inducer T cell subset; some peripheral blood monocytes, some macrophages and Langerhans cells
Leu 4	CD 3	Mature thymocytes, peripheral T lymphocytes.
Leu 7	? CD3/CD2/CD8	Subset of natural killer cells; neuro endocrine tissue prostate.
Leu 11b	CD 16	Low affinity F_c receptor on natural killer cells, neutrophils and some macrophages.
IL $2R_1$	CD 25	Interleukin 2 receptor
HLA-Dr	-	B lymphocytes, monocytes/ macrophages, Langerhans cells, interdigitaly reticular cells and activated T lymphocytes.

Table 15: Antibodies used to investigate cell mediated immune response in gastric material. Cluster of differentiation and specificity of antibodies shown.

2. Incubate with primary antibody (Dako) 1 hour room temperature
3. Wash Tris buffered saline. Incubate with rat anti-mouse Ig and alkaline phosphatase conjugate
5. Wash with Tris buffered saline
6. Visualise using substrate containing AS/TR phosphate in veronal acetate buffer pH 9.2 (contains fast red violet as diazonium salt and levamisol to reduce endogenous alkaline phosphatase).

Analysis of data

A quantitative analysis of T cell subsets within the lamina propria was performed. Three techniques of counting were attempted; (1) counts per high power field (2) counts per mucosal length and (3) point counting using a graticule. Analysis of variance was used to determine the observer variation using the statistical package GLIM outlined in the Thymidine Labelling section.

Fetal Stomachs

The crown-rump and foot length of the fetuses were measured and the gestational ages estimated from this according to the method of Streeter, 1927. The fetuses were dissected within 24 hours of death and the stomachs identified and removed with attached oesophagus and small intestine and fixed for 48 hours in 10% formalin. In fetuses less than 16 weeks the entire stomach was embedded in paraffin as a single sample. In fetuses older than 16 weeks tissue blocks were taken from the pylorus, antrum and body of the stomach and from the lower oesophagus and duodenum.

Serial 4 um sections were cut. The sections were stained with haematoxylin and eosin, PAS-diastase, PB/KOH/PAS, AB/PAS and HID/AB as outlined in the Mucin Histochemical Section. Immunoperoxidase stains were also performed using the antibodies raised against CEA and Intrinsic Factor as described previously.

Analysis

The fetal material was examined histologically to determine if any areas of metaplasia were present within the fetal stomach. The expression of mucin by the fetal stomach was determined by examination of the sections stained histochemically at the various gestational ages. The presence of intrinsic factor and CEA was determined at the various gestational ages by the presence of positive staining with the anti intrinsic factor and anti CEA antibodies.

CHAPTER 3

HUMAN GASTRECTOMY STUDY

INTRODUCTION

The work of this chapter is based on a histological and clinico-pathological assessment of the prospective series of gastrectomy specimens. The study is divided into four main areas; clinico-pathological, classification of tumours, intestinal metaplasia and dysplasia.

The literature review has highlighted the marked differences that are present between Europe and the USA compared with Japan regarding the prognosis and treatment for gastric carcinoma. A study of the clinical and pathological data over a three year period might be expected to illustrate some of the factors which cause these differences. This was the rationale behind the clinico-pathological section in this chapter.

The histological classification of gastric carcinomas is complex and several classification systems exist. The Lauren system (1965) is the most long established and well recognised. Although the reproducibility of the various classification systems has been addressed (Paginini and Rugge, 1982) a comparison between the systems has not been performed. Such a comparison would reveal any overlap between classification systems which could help rationalise this difficult area.

The heterogenous histological nature of gastric carcinoma has been emphasised in the literature review, and the variety of histological patterns that can appear within an individual tumour discussed. The crucial importance of the Lauren system (1976) is that it divides gastric carcinomas into two types intestinal and

diffuse with a possible difference in origin and biological behaviour between the types. If instead of using a subjective impression to apply the Lauren classification an objective scoring system were used this would allow for the co-existence of intestinal and diffuse features within a single tumour to be analysed.

The relationship of intestinal metaplasia to gastric carcinoma has been extensively discussed in Chapter 1. The collection of material from a prospective series of gastrectomy specimens allows for the classical studies of Morson (1955) to be repeated. The majority of studies of intestinal metaplasia have used a subjective assessment of the degree of metaplastic change and have frequently not taken into account other important variables such as age. The development of a simple quantitative assessment of intestinal metaplasia allows for the subjective element to be eliminated. Multivariate analysis can determine the effect of a variety of factors on the presence of intestinal metaplasia and this is the method chosen in the present study.

Although the association between Type IIb intestinal metaplasia and sub-types of gastric carcinoma described by Jass (1980) has been tested by other authors (Filipe et al., 1985; Ectors and Dixon, 1986) in endoscopic biopsy material the original work by Jass has not been repeated in gastrectomy material. In this present study the observations of Jass (1980) are performed in a larger number of cases and in addition a multivariate analysis is performed to determine the relationship of other variables to the

presence of Type IIb metaplasia.

The review of the literature concerning gastric dysplasia has demonstrated that although dysplastic change can be recognised the natural history and clinical significance is uncertain. The importance of dysplasia as a precancerous lesion has been emphasised by leading authorities (Morson et al., 1985). It seemed appropriate therefore to attempt to describe and document dysplastic change in the gastrectomy material and to relate this to the studies discussed in Chapter 1.

AIMS

Clinico-Pathological Study

Clinical

The aim of the clinical section of this study was to determine the age, sex, presenting syptoms and pattern of referral of the patients who underwent gastrectomy for malignant disease. The method of diagnosis prior to surgery and the survival of patients following surgery was also investigated in those patients with a two year follow-up available.

Pathological

The aim of the pathological section was to determine the T stage and N stage of the gastric tumours, and in conjunction with clinical data on the presence of metastases and the nature of the surgical resection, stage the tumours and assess the effect of surgical resection.

Classification of Gastric Tumours

There were three aims in this section.

(1) To classify the gastric tumours using the Lauren, Mulligan, Ming, Jass and WHO systems.

(2) To compare the latter three classification systems with the Lauren system.

(3) To evaluate the Lauren system using an objective scoring system described in Chapter 2 to determine relative heterogeneity or homogeneity of histological features within each individual tumour.

Intestinal metaplasia

The aims of this section were.

Quantitative data

1. Using the counting technique described in Chapter 2 to quantify the amount of the gastric mucosa replaced by intestinal metaplasia.

2. To determine if the observed amount of intestinal metaplasia was significantly greater in stomachs resected for malignant disease than benign disease or between the intestinal type and diffuse type tumours (Lauren).

3. To determine if the observed proportions of the intestinal metaplasia sub-types were significantly different between benign and malignant stomachs or between the intestinal type and diffuse type (Lauren).

Relationship to diagnostic group

4. To determine if the presence of intestinal metaplasia was significantly associated with malignant compared with benign specimens or with intestinal type rather than diffuse type tumours (Lauren).

5. To determine if the presence of Type IIb intestinal metaplasia was associated with malignant compared with benign specimens or with intestinal type compared with benign type tumours (Lauren).

6. To determine if Type IIb intestinal metaplasia was significantly associated with the intestinal type of tumours as defined by Jass.

7. To perform a multivariate analysis to assess the relationship of the presence of intestinal metaplasia within the gastric mucosa of a gastrectomy specimen to diagnosis, age, ulceration and inflammation.

8. To perform a multivariate analysis to determine the relationship of the presence of Type IIb intestinal metaplasia within the gastric mucosa of a gastrectomy specimen to diagnosis, age, ulceration and inflammation.

9. To determine the relationship between the presence of Helicobacter pylori and the observed amount of intestinal metaplasia within the resected specimens and the level of inflammation within the specimens.

Dysplasia

The aim of this section was to identify and describe the mucosal abnormalities adjacent to tumours or ulcers. The frequency of mucosal abnormalities in the benign, intestinal and diffuse groups (Lauren) were determined and also in relation to the degree of differentiation of the tumours.

RESULTS

Ninety four gastrectomy specimens were collected, 72 resected for malignant disease and 22 for benign disease. The results are presented in four sections. Firstly the clinico-pathological data of the 72 patients who underwent gastric resection for malignant disease. Secondly the classification of the gastric tumours. Thirdly the intestinal metaplasia data and finally, the dysplasia data. The clinico-pathological data of the 72 patients who underwent gastric resection for malignant disease is presented below.

CLINICO-PATHOLOGICAL FEATURES

Clinical data

Age and sex

The peak incidence was in the 60 - 79 age group with a male:female ratio of 3:2. The mean age and male:female ratio at all ages were 66.6 years and 1.6:1 respectively. The age and sex distribution of the patients with the gastrectomy specimens classified according to the Lauren system are shown in table 16.

Pattern of referral

There were three distinct referral patterns by the general practitioner; for investigation and treatment of symptoms, for further investigation when the general practitioner had begun investigation with a barium meal and this had suggested endoscopy, these first two categories were referred to either medical or surgical out-patients, and the third category in which patients had symptoms requiring emergency admission to hospital - dysphagia or

	Mean Age (yrs)	Sex Distribution	
Benign	54.1	16 M	6 F
Intestinal	67.2	26 M	11 F
Diffuse	66.1	16 M	15 F

Table 16: Mean age and sex distribution of benign and malignant gastrectomy specimens classified by Lauren criteria.

haematemesis (see table 17).

One patient was diagnosed as having gastric carcinoma at the time of laparotomy for an aortic bifurcation graft. The additional patient was referred directly to an endoscopy clinic for routine endoscopy following a cadaver renal transplant for chronic renal failure. All transplant recipients have routine endoscopies post-transplant because of the high incidence of peptic ulceration.

Symptoms

The presenting symptomatology is displayed (see figure 16). The duration of symptoms was judged from the time prior to the initial general practitioner consultation. The mean duration of symptoms was 3.7 months and ranged from less than 1 month to two years, only three patients had symptoms for more than one year.

Medication

At the time of referral by the general practitioner 37 patients (51%) were on no specific therapy for their upper gastrointestinal tract symptoms, 15 (20%) were on antacids or alginates, and 20 (29%) were on H_2 receptor blocking agents.

Investigation and diagnosis prior to surgery

The in-patient investigations and the method and numbers in which a tissue diagnosis was reached prior to resection, are outlined in table 18.

Pathological data

Microscopic assessment of stage

The depth of penetration of the tumour was easy to assess and is shown in table 19.

Indications for referral	No of cases
Investigations and treatment of symptoms	56
Abnormal findings on barium meal	9
Symptoms requiring acute admission	5

Table 17: Indications for referral of patients with gastric carcinoma to hospital by general practitioner.

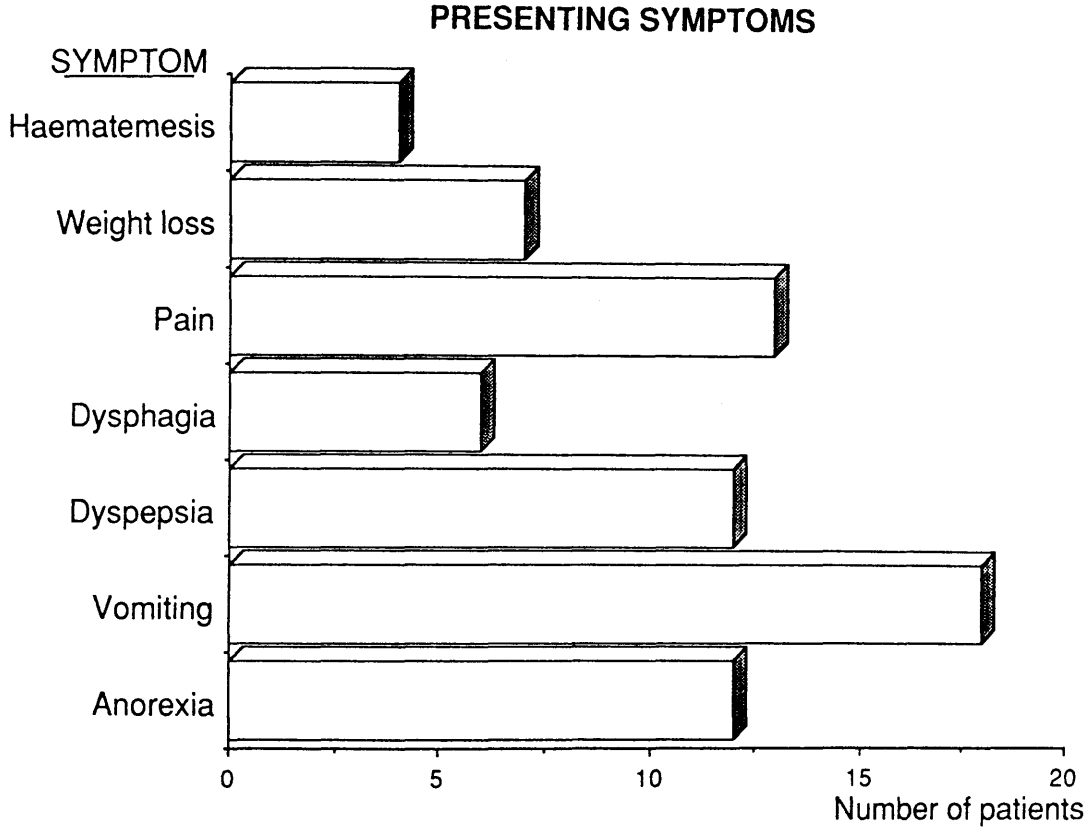


Figure 16. Main presenting symptoms of 72 patients undergoing gastrectomy for malignant disease

No of Patients	Investigations	Diagnosis prior to surgical resection
59	Endoscopy	Biopsy proven carcinoma (7 on repeat biopsy)
2	Endoscopy	Negative biopsy, clinical suspicion
2	Nil	Nil. Emergency resection for haematemesis
1	Incidental finding at laparotomy	Confirmed by frozen section
3	Palpable epigastric mass	1 confirmed by frozen section
5	Barium meal filling defect/ outlet obstruction	2 confirmed by frozen section

Table 18: Investigation and method of histological diagnosis of gastric carcinoma prior to gastrectomy in 72 consecutive patients.

T Stage	No of Cases	% of Total
T_1	3	4.2
T_2	3	4.2
T_3	47	65.2
T_4	19	26.4

Table 19: T stage (depth of penetration) of 72 consecutive gastrectomy specimens.

The nodal status was in contrast extremely difficult to determine. As stated previously the nodal status was gathered from the histopathology report routinely issued. In eight cases no mention was made of nodes, in nine cases an unspecified number of nodes were deemed to be either positive or negative. A further complicating factor was that there was no attempt to identify the site of origin of the nodes described by the pathologist and in only 14 cases were nodes sent separately by the surgeon and identified as node from coeliac axis etc.

With this deficiency in nodal status it was impossible to grade the cases into N_1 and N_2 accurately, the only distinction possible was between node positive and node negative patients. The other factor which may have increased the inaccuracy between the simple distinction between node positive and negative was the number of nodes detected. The number of nodes ranged from none to 18 but with a mean of 4.

Assessment of metastatic spread (M) was based on the findings at laparotomy. Ten patients had evidence of metastatic spread to liver or other organs. With the deficiencies in the N stage the tumours could not be staged accurately.

Site of tumour

This was assessed from the macroscopic resection specimen and classified into antrum (48), body (21), or cardia (3).

Surgical resection

In view of the inadequacy of the nodal staging it was not possible to ascribe surgical treatment into the curative or non-

curative groups. Twelve cases involved a total gastrectomy, two of these with splenectomy and hemipancreatectomy. The remainder involved sub-total gastrectomy and in four cases splenectomy. In only 14 cases were nodes separately identified and these were either from coeliac axis, sub diaphragmatic or porta hepatis. In no cases were splenic, justapyloric or retropancreatic nodes identified.

Resection margins

In ten cases the resection margin was involved with tumour. In 12 cases in which the tumour was situated in the body the resection margins were less than 5 cm.

Survival

Follow-up data was available on 50 patients followed for two years. A cumulative survival curve is shown (see figure 17). The operative mortality was defined as deaths within the first month following surgery and was 10% (n = 5). The two year cumulative survival for all cases surgically treated was 27%.

CLASSIFICATION OF GASTRIC TUMOURS

The classification of the 72 tumours is shown in Table 20. The composition of the sub-groups of four classification systems, Mulligan, Jass, Ming and WHO with respect to the intestinal, diffuse and unclassified types of the Lauren system are given.

Evaluation of Lauren System

The scoring system for intestinal and diffuse features outlined in the materials and methods section was used to assess the distribution of the histological features in the tumours. The

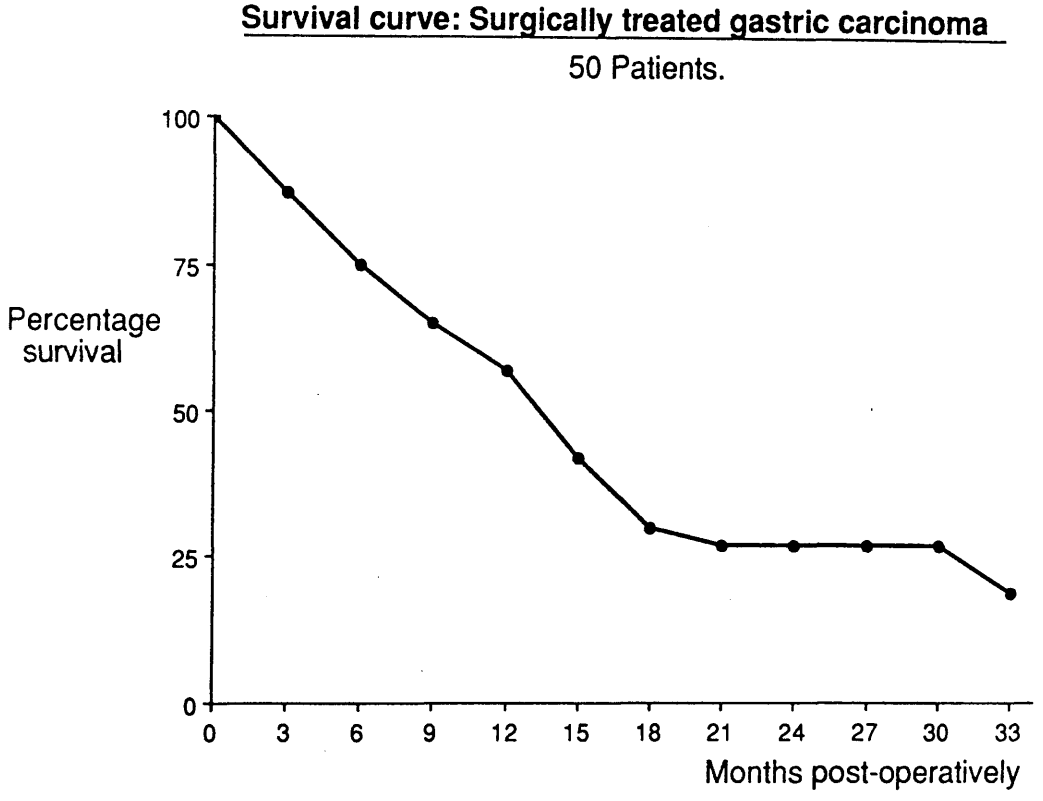


Figure 17. Percentage survival of 50 patients with surgically resected gastric carcinoma over a 33 month period calculated using Clinical Life Table Analysis.

Classification	No of cases	Comparison with Lauren System
LAUREN		
Intestinal	37	-
Diffuse	31	-
Unclassified	4	-
MULLIGAN		
Intestinal cell	49	33 intestinal, 15 diffuse, 1 unclassified
Mucinous	16	16 diffuse
Pylorocardiac	4	4 intestinal
Unclassified	3	3 unclassified
MING		
Expanding	37	22 intestinal, 14 diffuse, 1 unclassified
Infiltrative	35	15 intestinal, 17 diffuse 3 unclassified
JASS		
Intestinal	23	16 intestinal, 7 diffuse
Gastric	49	21 intestinal, 24 diffuse, 4 unclassified
WHO		
Tubular	37	24 intestinal, 13 diffuse
Papillary	8	8 intestinal
Mucinous	7	5 intestinal, 2 diffuse
Signet ring	14	14 diffuse
Adenosquamous	2	2 unclassified
Undifferentiated	4	2 unclassified, 2 diffuse

Table 20: Classification of 72 malignant consecutive gastrectomy specimens into Lauren, Mulligan, Ming, Jass and WHO system. The subtypes of the latter four systems are compared with the intestinal, diffuse and unclassified of the Lauren system.

results are displayed graphically (figure 18).

Using this scoring system 18 tumours (26.4%) showed all the histological features of both intestinal and diffuse, 39.8% displayed some aspects of both and 33.8% showed only intestinal or diffuse features exclusively. Although the numbers were not sufficient for statistical analysis there appeared to be a positive correlation between the number of sections examined in each tumour and the finding of intestinal and diffuse features in the same tumour. A greater proportion of the diffuse tumours showed homogeneity of the histological pattern compared with the intestinal group.

INTESTINAL METAPLASIA

Quantitative analysis

The coefficient of variance for the ten repeated counts ranged from 0-9% with a mean of 3.02% (see appendix). At this level of variance, statistical comparison utilising the data was regarded as valid.

The percentage distribution of the gastric crypts, and the three sub-types of metaplasia in the intestinal, diffuse and benign groups (Lauren classification) are displayed graphically (figure 19). The four unclassified tumours are excluded from the analysis. There was a wide range of values. The intestinal and diffuse group had a statistically significantly greater amount of Type I metaplasia ($p < 0.05$) than the benign group. There was no significant difference in amount of Type I metaplasia between the intestinal and diffuse groups. There was no significant

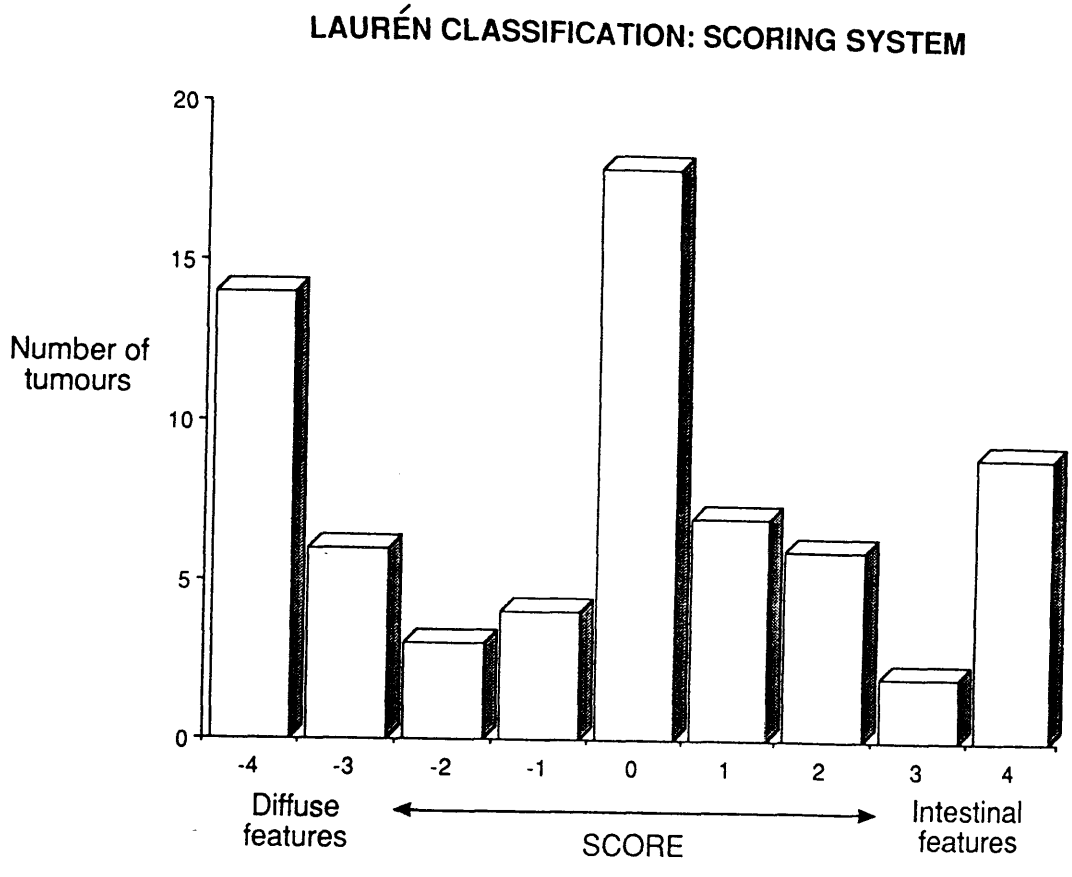


Figure 18. Results of the application of the scoring system, for intestinal and diffuse features based on the four main histological criteria of the Lauren system, to the 72 gastrectomy specimens. A score of +4 indicates that all four intestinal type features and no diffuse type features were present within a tumour. A score of -4 indicates that all four diffuse features and no intestinal features were present. Scores between -3 to +3 indicate that both intestinal and diffuse features were present in the same tumour to varying degrees.

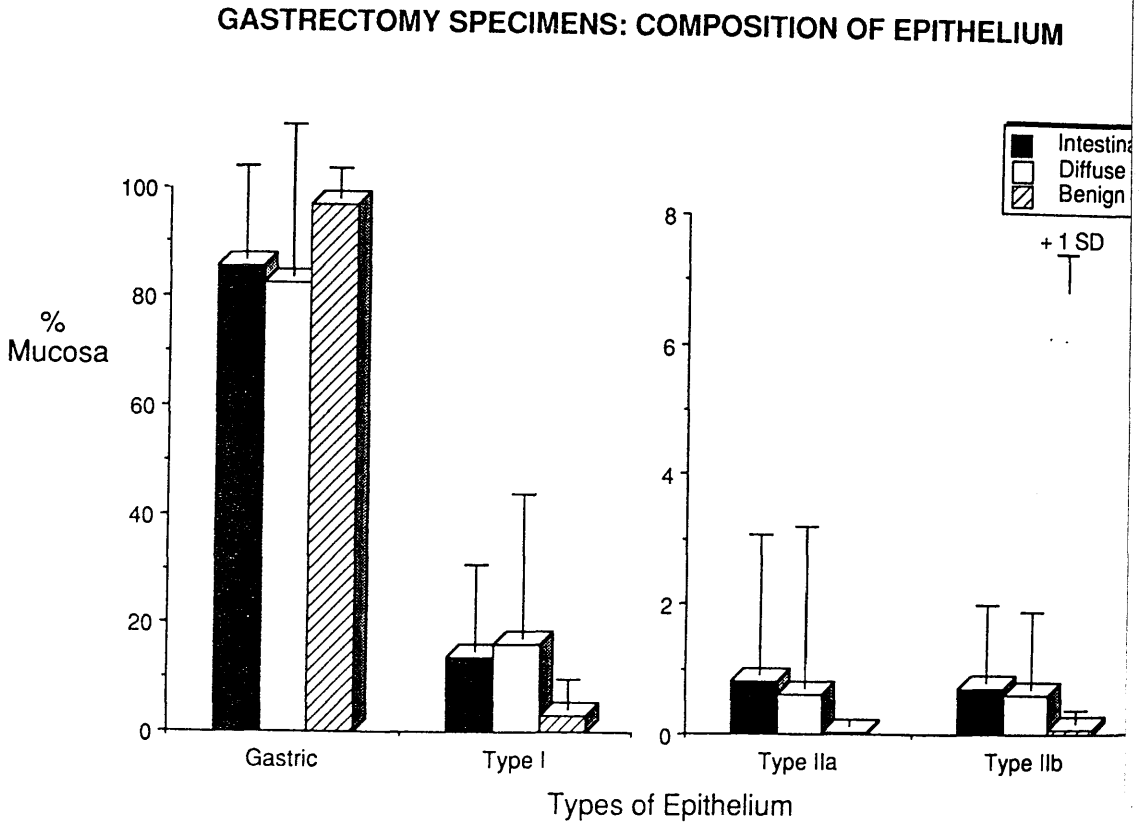


Figure 19. Percentage of mucosa from 90 gastrectomy specimens composed of gastric epithelium, Type I, Type IIa, Type IIb intestinal metaplasia in the three groups, intestinal type, diffuse type tumour (Lauren) and benign. Note the expanded scale for Type IIa and Type IIb. Four unclassified tumours are excluded from the analysis.

difference in the amount of Type IIa or Type IIb intestinal metaplasia between the intestinal, diffuse or benign groups.

The percentage distribution of the sub-types of intestinal metaplasia is shown in figure 20. There was no significant difference in the proportion of each sub-type between the intestinal,diffuse and benign groups. Figures 19 and 20 clearly show the small percentage of the mucosa that shows the specific features of type IIb.

Relationship to Diagnostic Group

Lauren Classification

The results are expressed numerically in table 21 for the presence or absence of the sub-types of metaplasia in the intestinal and diffuse type tumours (Lauren classification) and benign stomachs.

The presence of metaplasia of any type was significantly associated with the intestinal and diffuse tumours when compared with benign specimens (X^2 $p < 0.05$). No significant difference in the frequency of occurrence of intestinal metaplasia of any type could be demonstrated between the intestinal and diffuse groups. The presence of type IIb was not significantly associated with the intestinal type when compared with diffuse type and benign groups. Combining the intestinal and diffuse tumours into a single malignant group resulted in a significant association with the presence of type IIb when compared with benign ($X^2 = 5.2$ $p < 0.05$).

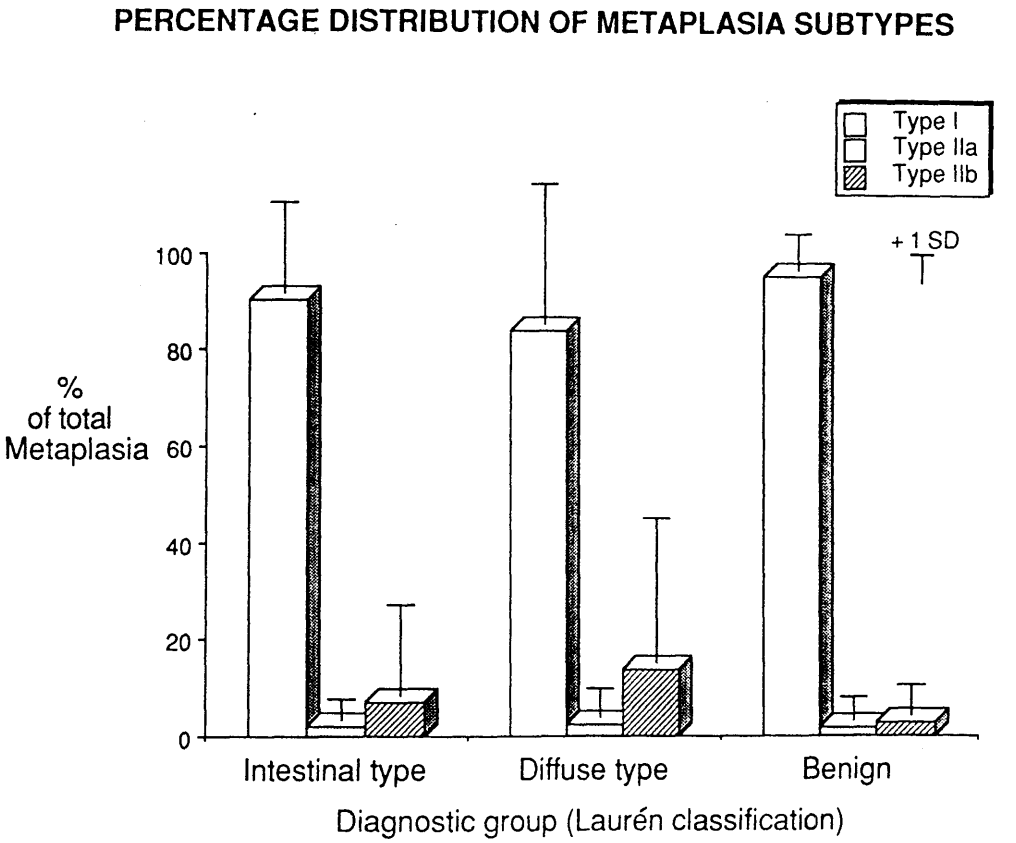


Figure 20. Distribution of intestinal metaplasia sub-types I, IIa and IIb as a percentage of the total metaplasia in 90 gastrectomy specimens divided into intestinal and diffuse types (Lauren) and benign. Four unclassified tumours excluded from the analysis.

	Subtypes of Metaplasia					
Lauren Classification	**Type I**		**Type IIa**		**Type IIb**	
	+	-	+	-	+	-
Intestinal	30	7	9	26	16	21
Diffuse	25	6	4	27	10	21
Benign	10	12	1	21	2	20

+ = subtype present - = subtype absent

Table 21: Presence and absence of subtypes of intestinal metaplasia in benign gastrectomy specimens and intestinal and diffuse type of tumours according to Lauren System.

Jass Classification

The tumours were classified into the intestinal and gastric type as defined by Jass and the relationship to the presence of type IIb analysed using Chi square analysis (see Table 22). Only cases in which extensive metaplasia was present were used in the analysis, as this was the selected group in which Jass demonstrated a significant association between type IIb and the intestinal type of tumour (Jass 1980). A significant association (x^2 19.5 $p < 0.01$) was demonstrated between the presence of type IIb and the intestinal type of tumour as defined by Jass.

Relationship between the presence of intestinal metaplasia and the variables diagnostic group, age, ulceration and inflammation.

Chi square analysis has demonstrated a significant association between the presence of metaplasia (of any type) in stomachs removed for carcinoma compared with those resected for benign disease. The benign and malignant groups differ not only in their diagnosis but also in mean age of patient, percentage of cases with ulceration and mean gastritis score (see table 23).

The GLIM analysis demonstrated a significant association between age, ulceration and gastritis score to the presence of intestinal metaplasia. The estimates of probability for these variables are displayed graphically and compared with diagnostic groups (intestinal or diffuse tumour as defined by Lauren or benign). Estimates of probability for combination of factors ie 50 year old with an ulcerated lesion, 55 year old with an ulcerated lesion and gastritis score = 3, 70 year old with grade 3 gastritis,

Type of Tumour	Type IIb present	Type IIb absent
Intestinal	19 *	4
Gastric	8	26

* $p < 0.01$ $\quad x^2$ 19.5

Table 22: Relationship of Type IIb metaplasia in 57 gastrectomy specimens with extensive intestinal metaplasia to the "intestinal" and "gastric" types of tumour as defined by Jass.

	Malignant	Benign
Mean age	66.6	54.1
% cases with ulceration	55	31
Mean gastritis score	3.63	3.09

Table 23: Comparison between benign and malignant gastrectomy specimens for the variables, age, ulceration and gastritis score.

are also shown in figure 21.

Relationship between the presence of Type IIb metaplasia and the variables diagnostic group, age, ulceration and inflammation

The GLIM analysis demonstrated a signficant association between age, ulceration, diagnostic group (Intestinal type, Gastric type tumour as defined by Jass, or benign) and gastritis to the presence of type IIb metaplasia. Estimates of probability for these variables are displayed graphically in figure 22. Estimates of probability for a combination of factors ie. 75 year old gastritis score = 3, 70 year old ulcerated lesion (benign or malignant) 60 year old ulcerated lesion (benign or malignant) gastritis score = 3.

Relationship between Helicobacter Pylori, intestinal metaplasia, diagnostic group and gastritis

Histology

The organisms lay close or in the mucus layer predominantly at the luminal surface of the gastric crypts. No organisms were seen in foci of metaplasia of any type nor in areas of malignant change.

Multivariate analysis

The GLIM analysis demonstrated no significant association between the presence of Helicobacter pylori and the presence of intestinal metaplasia. The relationship between the presence of Helicobacter pylori and diagnostic group according to the Lauren classification is shown in table 24. The presence of Helicobacter pylori was analysed with respect to two other factors. Firstly the

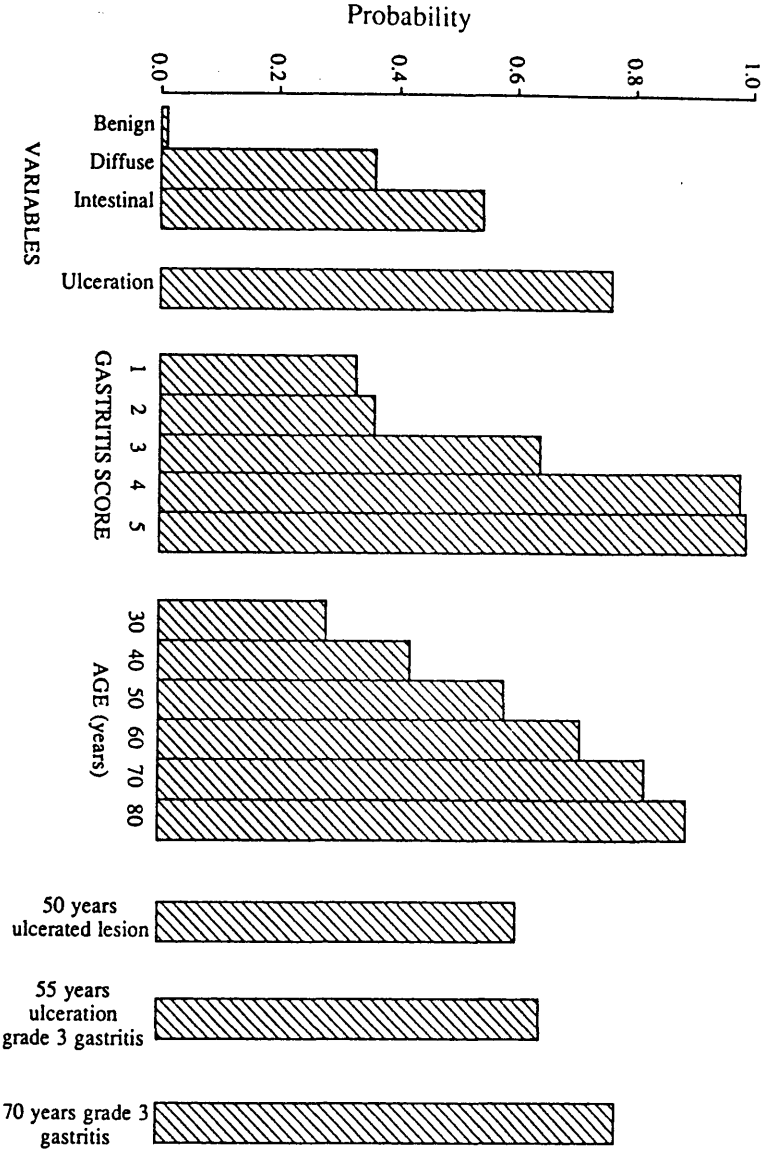


Figure 21. Probability that intestinal metaplasia of any type will be present in gastrectomy specimens in relation to diagnostic groups (intestinal or diffuse tumours (Lauren) or benign) ulceration, gastritis score and age of patient, and combinations of these variables.

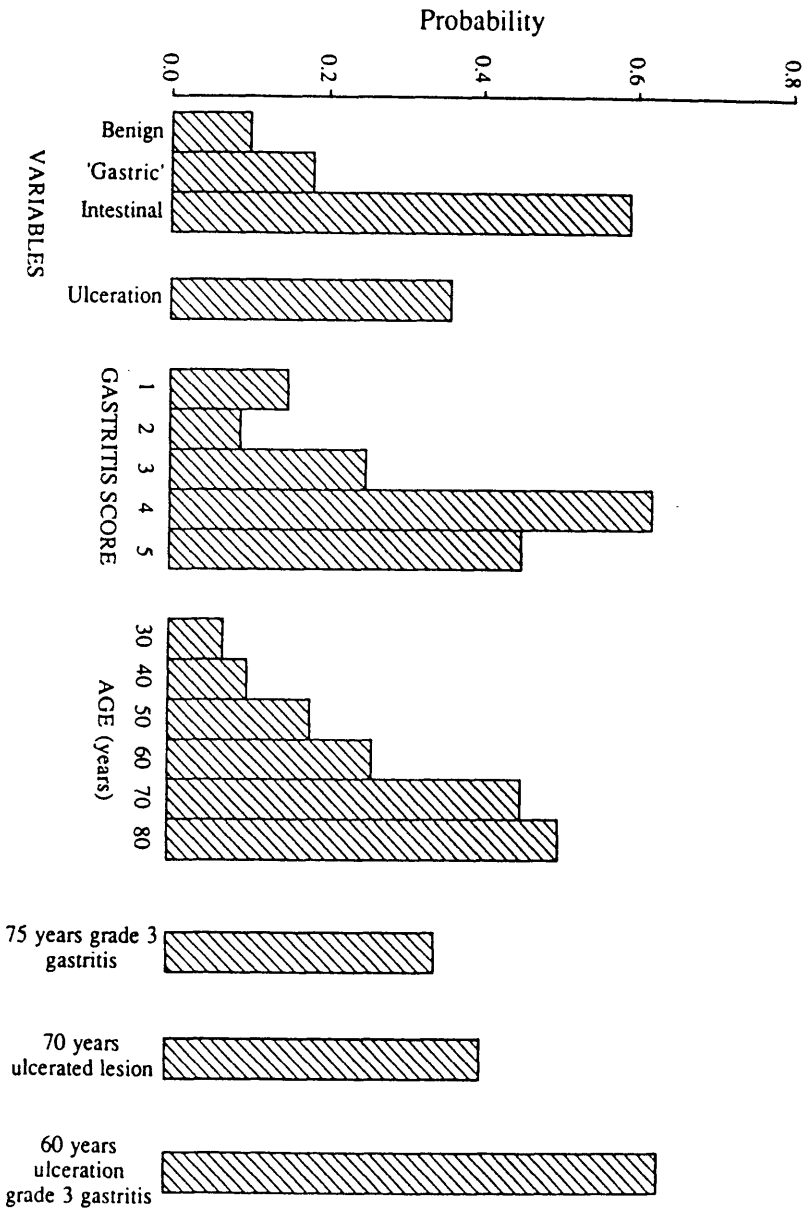


Figure 22. Probability that Type IIb intestinal metaplasia will be present in gastrectomy specimens in relation to diagnostic group (intestinal, gastric tumour (Jass Classification) or benign), ulceration, gastritis score and age of patient, and combinations of these variables.

Diagnosis	H. pylori present	H. pylori absent
Intestinal type	7	30
Diffuse type	2	29
Benign	10 *	12

* $p < 0.01$

Table 24: Relationship between presence and absence of Helicobacter pylori in the mucosa of 90 gastrectomy specimens and diagnostic group; benign, intestinal or diffuse type tumour (Lauren classification). H. pylori significantly associated ($p < 0.01$) with benign compared with other groups (Chi square analysis).

amount of the gastric mucosa replaced by intestinal metaplasia in the gastrectomy specimens and secondly the gastritis score of the gastrectomy specimens. No significant association was found between the presence of Helicobacter pylori and these two variables. The results are displayed in table 25 and table 26.

DYSPLASIA

Histological features

Three histological patterns were identified in the mucosa adjacent to carcinoma/ulcer.

(1) Regenerative hyperplasia

The constituent cells had basophilic cytoplasm, hyperchromatic nuclei with frequent motises and because of the associated inflammation intramural or intraglandular inflammatory cells. In mild to moderate inflammation there is an increase in cell numbers consistent with the term hyperplasia. However with epithelial loss in ulceration the glandular size is reduced and the epithelium forms rudimentary glands. The consistent feature of the epithelium in regenerative hyperplasia was the regular size and shape of the cells and their nuclei. In mild forms the cells showed maturation towards the luminal surface but with severe ulceration there was little or no maturation with reduction or absence of mucus secretion.

2. Atypical hyperplasia

This pattern of mucosal abnormality occurred predominantly in type IIb metaplasia. The incomplete metaplastic crypts

	H. pylori present	H. pylori absent
	Amount of metaplasia (mean value %)	
Intestinal tumour	13.8	19.7
Diffuse tumour	3.4	3.9
Benign	3.9	1.5
All groups	10.5	12.5

Table 25: Relationship of Helicobacter pylori to amount of gastric mucosa replaced by intestinal metaplasia as a percentage of the mucosa examined in 90 gastrectomy specimens classified into intestinal and diffuse tumours (Lauren) and benign.

Diagnosis	H. pylori present	H. pylori absent
	mean gastritis score	
Intestinal type	3.4	3.9
Diffuse type	2	3.7
Benign	3.5	3

Table 26: Relationship between Helicobacter pylori and mean gastritis score in 90 gastrectomy specimens classified into benign, intestinal or diffuse type tumours (Lauren).

showed marked architectural abnormalities. The crypts extended from the muscularis to the luminal surface. The lower third contained a preponderance of goblet cells and Paneth cells were absent, the lower third joined the middle third at sharp angle and above this level there was marked papillary infolding pseudostratification of nuclei and columnar cells containing apical mucus and absent brush border. The constituent nuclei did not show any marked atypia in the majority of the glands. These histological features are equivalent to those classified by Jass as grade 0 dysplasia.

(3) Dysplasia

The two types of dysplasia described by Jass were identified. The description of the two types of dysplasia given by Jass are complete in all details and cannot be significantly added to (Jass 1983).

Frequency of Atypical Hyperplasia, Regenerative Hyperplasia and Dysplasia in Gastrectomy Specimens

The frequency of dysplasia, atypical hyperplasia and regenerative hyperplasia in the gastrectomy specimens is shown in table 27.

The relationship of the types of dysplasia to the differentiation of the tumours is shown in table 28.

DISCUSSION

Clinico-Pathological

This series of patients who underwent gastric resections for

	DIAGNOSTIC GROUP		
	Benign	Intestinal	Diffuse
	n = 22	n = 37	n = 31
MUCOSAL ABNORMALITY			
Regenerative hyperplasia	12	20	14
Atypical hyperplasia	1	18	2
Dysplasia Type I	2	16	6
Type II	3	14	9

Table 27: Frequency of mucosal abnormalities present in 90 gastrectomy specimens resected for benign conditions (22 cases). Intestinal (37 cases) and diffuse tumours (31 cases). Mucosal abnormalities classified according to Jass (1983).

Mucosal abnormality	Well differentiated	Poorly differentiated
Atypical hyperplasia	10	8
Dysplasia Type I	7	9
Type II	4	10

Table 28: Frequency of dysplasia subtypes and atypical hyperplasia in well and poorly differentiated intestinal type tumour (Lauren classification).

carcinoma is not an attempt to provide an epidemiological survey of gastric carcinoma in the West of Scotland but provides a small sample to enable certain clinico-pathological features to be examined. The series only includes cases of gastric carcinoma that underwent resection - no estimate of inoperable cases has been made. The data generated has been used to illustrate certain points but no statistical comparisons have been made.

The age distribution of the patients is similar to previous studies (Weed et al., 1981). The male to female ratio is lower than in other studies at 1.6:1 compared with 1.8-1.85:1 (Weed et al., 1981).

The symptomatology of the patients is comparable to previous series. The interesting feature is the relatively short duration of symptoms (>80% less than six months) admitted to by the patients. Although almost half the patients were on therapy for upper gastrointestinal symptoms at the time of referral only three patients could be identified who had received therapy for longer than one year. One patient had been treated empirically without any investigation, one patient had a barium meal reported as showing no abnormality which in retrospect showed clearly a filling defect, and the remaining patient had a gastric ulcer biopsied and reported as malignant but this fact was not noted by the hospital clinician. The implications behind these findings are that firstly, patients tend not to seek medical advice until they have significant symptoms and by that time the disease is at an advanced stage. Secondly, that in the majority of patients the general

practitioner although he may prescribe therapy to alleviate the symptoms tends to refer these patients for investigation.

The two years survival rate of 27% in this series is not strictly comparable to other published data as the other studies publish five year rates. The five year survival rates in the Western literature, range from 7.4-20% (Weed et al., 1981). It is likely that in a further three years this series will be at the lower part of the range.

One of the aims of the clinico-pathological study was to observe the staging of the tumours by the pathologists. This has clearly demonstrated that the nodal status is poorly assessed by the pathologists.

Initially one of the other aims of the study was to determine the effect of palliative or "curative" surgery. However as the study progressed it became obvious that due to the inadequacy of pathological staging and the surgical philosophy that this was not possible. From the operative data it was impossible to determine in the majority of cases whether the surgeon considered a curative or palliative procedure had been performed. This is not just a failing of the West of Scotland as most of the European and American series have inadequate clinical and pathological staging.

The reasons for the deficiencies in surgical and pathological management of gastric carcinoma are probably multifactorial. The three main factors influencing the surgical and pathological management are the medical staff (both surgeons and pathologists), the nature of the disease process and the patients themselves.

Within the Western Infirmary/Gartnavel complex there are ten Consultant Surgeons none of whom has a particular interest in gastric carcinoma. With the number of operable cases of gastric carcinoma presenting each year each of the Surgeons will on average perform only three gastrectomies per year for malignant disease. A total of five Consultant Histopathologists are routinely involved in the reporting of surgical specimens and so by a similar extrapolation will each examine on average six gastrectomy specimens per year. During the period of the present study there were no clinical trials being undertaken in the treatment of gastric carcinoma in the Western/Gartnavel complex. These factors in conjunction with the advanced stage of disease at presentation and the elderly age of the majority of patients may partially explain the apparently nihilistic approach of treatment represented in this study compared with that reported in Japan.

CLASSIFICATION OF TUMOURS

The number of cases of gastric carcinoma in this study bear no comparison with the large series reported from Japan but are similar in magnitude (and in some cases greater) than many Western series (Morson 1955, Jass 1979, Paginini and Rugge, 1983).

A retrospective analysis of material on file would have rapidly yielded a large number of cases, the sampling of the tumour and the gastric mucosa undertaken for routine histological diagnosis would not have been sufficient for the purposes of the study.

The proportions in the WHO and Lauren classifications are

comparable with previous published series (Paginini and Rugge, 1983). The results of the Mulligan classification in this study display a preponderance of intestinal cell tumours and a deficit of pylorocardiac tumours compared with the series reported by Mulligan (Mulligan and Rember, 1972). The explanation for this is the difficulty in distinguishing the pylorocardiac type from the intestinal cell; this defect in the Mulligan system has been highlighted (Day and Morson, 1978). Another problem with the Mulligan system is co-existence of pylorocardiac-like areas within tumours of predominantly intestinal cell differentiation. Paginini and Rugge (1983) described this mixture of types in almost half of the pylorocardiac cell tumours, in this series 16 of the 49 tumours classified as intestinal cell had some areas with pylorocardiac features.

Two published series of the Ming classification appear in the literature; the original series by Ming, 1977, and the study described by Paginini and Rugge, 1983. The two series divide the tumours into expanding and infiltrative type in the proportion 2:1 and 2:3 respectively. These contradictory results are further questioned by the approximately equal numbers of both types in the present study.

Comparison of classification systems

Although the reproducibility of the classification systems has been examined (Paginini and Rugge, 1983), a comparison has not been studied. The Lauren system is the most extensively used and widely accepted system and this has been used as the standard and

an examination of the sub-groups of tumours in other systems which fall into the three categories intestinal, diffuse or unclassified, is shown in the results section.

The Mulligan system compared with the Lauren system equates mucinous tumours with diffuse tumours, pylorocardiac tumours are variants of intestinal type and the intestinal cell an admixture of intestinal type and diffuse type.

The Ming classification appears to contain a mixture of both intestinal and diffuse type in the expanding and infiltrative groups. As the Ming classification primarily examines the mode of growth it might be expected that Lauren diffuse type would comprise the majority of the infiltrative and the intestinal type the majority of the expanding group. The mode of growth represents only one of the histological parameters used in the Lauren classification. The results of this study suggest that when used alone, the mode of growth, without consideration of the structure of the tumour does not divide the tumours into the same sub-groups as the Lauren system.

The results of the comparison of the classification systems are difficult to interpret. The Mulligan and the WHO system recognise two main sub-groups which correspond to the intestinal and diffuse type of Lauren. This applies to the Jass system to a limited extent. The plethora of sub-types within the classification systems is a reflection of the heterogenous nature of the histological appearance of gastric carcinoma. To some extent each classification system appears to use the histological

features identified by Lauren but to apply them in different ways.

The rationale behind employing a classification system is to divide tumours into groups which have some biological significance. This significance may take the form of a prognostic difference or some difference in histogenesis or both of these factors. The overall survival in this present study was so low at two years and the numbers of patients were too small to allow a valid statistical comparison to be made between tumours in the various classification sub-groups. The possible difference in histogenesis is investigated in the subsequent chapters on Mucin and Immunohistochemistry.

Critical Appraisal of the Lauren Classification

The results of this section of the study depend on the accurate classification of the tumours into intestinal and diffuse types. For this reason the tumours were classified using two observers, the author and a Consultant Pathologist (ILB) with considerable experience in gastro-intestinal pathology.

The two sub-types, intestinal and diffuse, have differing morphologies as outlined by Lauren, but the crucial importance of this system is the proposed difference in the aetiology and pathogenesis of the two groups. The main difficulty in accepting this concept of a separate histogenesis is the finding in a large proportion of cases that both types of pattern are present in the same tumour. This phenomenon had led authors to use the predominant histological feature for classification (Paginini and Rugge, 1983). If this technique of classification using the Lauren system is employed then the tumours are sub-divided on the basis of

an overall subjective impression rather than an objective assessment of the four histological features outlined by Lauren in his original description.

The method employed in the present study was to score each individual histological feature present in a tumour with a positive mark for intestinal and a negative mark for diffuse features. The aim of this scoring system was to determine the frequency with which the histological features of both intestinal and diffuse tumours co-existed in the 72 tumours in the study. A bimodal distribution might be expected if the tumours showed exclusively intestinal or diffuse features. The results of the present study do not show this expected distribution but demonstrate that two-thirds of the tumours show features of both types of tumour. Although in most tumours the features of one type of tumour predominate the value of the scoring system has been to illustrate the frequent co-existence of intestinal and diffuse features within the one tumour.

The basis of the Lauren classification is the identification of two tumour types with different histological features and by implication a different histogenesis. The concept of a separate histogenesis for intestinal and diffuse types of tumour is difficult to accept as the present study shows that such a high proportion of tumours contain histological features of both types. The term "histogenesis" in tumour pathology implies a putative "cell of origin" of a given neoplasm. The origin of the intestinal type of tumour has been regarded as intestinal metaplasia and diffuse type from gastric epithelium. However

intestinal metaplasia is derived from the transformation of gastric epithelium. Both types of tumour are therefore derived from the same cell population and this may explain the frequent co-existence of histological features in the same tumour. The ability of neoplasms to change their pattern of differentiation during their lifespan has been well demonstrated (Gould et al., 1981) and this multidirectional differentiation may also explain the appearance of small areas of diffuse type features in a predominantly intestinal type tumour for instance.

The results of the present study in themselves cannot be used to confirm or refute any concept of histogenesis of gastric carcinoma. The study does illustrate the difficulties that might be encountered in using any immunocytochemical marker to investigate the histogenesis of gastric carcinoma. As such a substantial histological overlap has been demonstrated this might also be reflected in a considerable overlap in the expression of a particular antigen by the tumours.

Intestinal Metaplasia

Quantitative analysis

The counting technique employed in the study although tedious and time consuming was simple to perform and analysis of variance has demonstrated that it was reproducible by one observer. The counting technique provides more objective data than the scoring systems employed by other authors (Morson 1955; Stemmerman and Hayashi 1968; Jass 1980). The limitation of any form of objective or subjective scoring system is that only a sample of the

gastric mucosa is studied. The standard sections taken in the present study ensured that the same geographical areas in each stomach were examined thus providing a representative sample of the gastric mucosa from each specimen. The quantitative values obtained in the present study provide only an estimate of the amount of gastric mucosa replaced by the various sub-types of intestinal metaplasia. No attempt as made to determine the geographical distribution of the intestinal metaplasia within the individual specimens as this macroscopic distribution has been well described by other authors Morson 1955; Stemmerman and Hayashi 1968; Sugimura et al., 1982.

The percentage of the gastric mucosa replaced by Type I intestinal metaplasia in the intestinal, diffuse and benign groups quoted in the results section are mean values for each group. Although the mean value of both the intestinal and diffuse groups are significantly higher than that of the benign group, a wide range of values in all groups was observed. The intestinal and diffuse groups differ significantly from the benign group with respect to age. The amount of intestinal metaplasia present in gastrectomy specimens has consistently been shown to increase with age (Morson 1955; Stemmerman and Hayashi 1968; Lauren 1965). The significantly greater amount of Type I metaplasia in the specimens resected for malignant disease cannot be directly ascribed to the presence of malignant disease without taking into account this age factor. The relationship between age and intestinal metaplasia is discussed further in the section on variables and intestinal

metaplasia.

The present study has not demonstrated any significant difference in the percentage of the gastric mucosa replaced by Type I intestinal metaplasia in the stomachs resected for intestinal compared with diffuse type tumours. This finding is at variance with those of other studies (Lauren 1965; Rubio et al., 1985) which have reported a larger amount of intestinal metaplasia in the intestinal group compared with the diffuse group. In the present study the mean age of the two groups was remarkably similar at 67.2 and 66.1 years for the intestinal and diffuse groups respectively. Lauren (1965), although demonstrating that the incidence of intestinal metaplasia was greater in the intestinal group compared with diffuse at given age groups, did not make a similar age adjusted comparison for the amount of intestinal metaplasia. The figures quoted by Lauren 1965 of 66% of intestinal type and 24% of diffuse type showing "profuse" or "fairly profuse" intestinal metaplasia of the gastric mucosa refer to an intestinal group with a mean age of approximately ten years greater than that of the diffuse group. Rubio et al., 1985, in their quantitiative study of intestinal metaplasia demonstrated a significantly higher amount of metaplasia in the intestinal group compared with diffuse. However the mean ages of the two groups in this study was significantly different.

The quantitative analysis clearly demonstrates that Type I intestinal metaplasia is the predominant variant of intestinal metaplasia in all the gastrectomy specimens examined. Although

(as discussed in the next section) the frequency of occurrence of the Type IIb variant is significantly greater in malignant than benign stomachs the mean amount of the gastric mucosa replaced by this variant is not significantly different between benign and malignant stomachs.

Relationship of Intestinal Metaplasia and Sub-types to Lauren and Jass Classification

The quantitative analysis demonstrates that only a small percentage of the mucosa sampled (on average less than 1%) is replaced by type IIb metaplasia. The implication of this finding is important in relation to screening for gastric carcinoma by endoscopic biopsy surveillance. Proponents of the theory that type IIb intestinal metaplasia has malignant potential have undertaken a prospective study to assess the prognostic implication of the intestinal metaplasia sub-types using endoscopic biopsy surveillance (Filipe et al., 1985). In this study (Filipe et al., 1985) three to four random biopsies with dimensions of 3 X 2 mm are taken. Such biopsies can only represent a tiny proportion of the total mucosal surface area, and given that type IIb is present in such small amounts as shown by the quantitative analysis in the present study, a high false negative biopsy rate is likely to occur. This lack of sensitivity of endoscopic biopsy material has been demonstrated by one study (Silva and Filipe, 1986) which compared the frequency of type IIb in stomachs from which endoscopic biopsies had been taken with the material from the subsequently resected specimen. This study found that type IIb

was identified twice as frequently in the gastrectomy material compared with the endoscopic biopsies from the same specimens.

Analysis of the data in a non-parametric fashion (ie presence or absence of metaplasia and sub-types in relation to diagnostic groups) produced unexpected results in view of previous studies on intestinal metaplasia. Although the presence of intestinal metaplasia of any type was significantly associated with malignant stomachs compared with benign, there was no significant association with Lauren's intestinal type compared with the diffuse type. Similarly type IIb intestinal metaplasia although significantly associated with malignancy was not significantly associated with intestinal type compared with the diffuse type of Lauren's classification. However when the tumours were reclassified into the "intestinal" and "gastric" types using Jass's criteria and only cases with extensive intestinal metaplasia examined then type IIb did show a significant association with the "intestinal" type.

The failure to find a significant association between the presence of intestinal metaplasia of any type and Lauren's intestinal type of tumour would appear to be contradictory to Lauren's original observation (Lauren 1965) and various epidemiological studies which have shown an increased incidence of intestinal metaplasia in the intestinal type of tumour (Correa et al., 1970; Stemmerman et al., 1977; Munoz and Matko, 1972). Lauren's description (1965) of a higher incidence of intestinal metaplasia in intestinal compared to diffuse tumours although in age matched material was based on the mucosa adjacent to the tumour

rather than on sampling the entire specimen as in the present study. The epidemiological surveys cited differ from the present study in several factors. Firstly there are differences in ages between the intestinal and diffuse groups whereas in the present study the groups are of a similar age. Secondly the patient population is selected from areas with a high incidence of gastric carcinoma. Finally the interpretation of the material appears to be different as a qualitative assessment of intestinal metaplasia is made, little or no intestinal metaplasia being regarded as absent (Stemmerman et al., 1977) whereas in the present study the presence of even a single crypt of intestinal metaplasia was regarded as a positive finding.

A second conflicting result in the present study was the significant association of type IIb metaplasia with intestinal tumours of the Jass classification but not with this group in the Lauren classification.

Multivariate Analysis

In view of complicated and conflicting nature of the results a form of multivariate analysis was performed. The aim of this form of analysis was to determine the relationship between several important variables firstly, the presence of intestinal metaplasia of any type and secondly, the presence of the variant type IIb.

Six variables were identified which were possibly of importance; diagnostic group, age, sex, gastritis score, presence or absence of ulceration, presence or absence of Helicobacter pylori.

The variable "diagnostic group" was the category into which the gastrectomy specimen was classified. Intestinal, diffuse (Lauren classification) or benign categories were used in the analysis regarding the presence of intestinal metaplasia. "Intestinal", gastric (Jass classification) or benign categories were used in the analysis of the type IIb variant.

The frequency and extent of intestinal metaplasia have repeatedly been shown to increase with age (Morson 1955; Lauren 1965; Filipe et al., 1985; Rubio et al., 1985; Rothery and Day, 1985; Silva and Filipe, 1987), so this appears to be an important variable. Little attention has been directed to differences between males and females regarding intestinal metaplasia but because of the different sex ratios described between the intestinal and diffuse tumours (Lauren, 1965) this was included as a variable. The presence and severity of gastritis is inextricably linked to the development of intestinal metaplasia as observed by Magnus 1937 in his original paper and this was included as a variable. The severity of the gastritis was judged using a scoring system with standard photographs in order to gain some objective measurement. The presence of ulceration was also included as a variable because of recent histological studies which indicated that the development of intestinal metaplasia was related to disordered regeneration in the healing process of gastric erosions (Mukawa et al., 1987). The presence or absence of Helicobacter pylori in the gastrectomy material was included as a variable in view of the hypothesis that the development of intestinal metaplasia may be a response to longstanding infection with the organism (Ectors and Dixon, 1986) on the basis that foci

of intestinal metaplasia are not colonised by the organism (Steer, 1984).

The multivariate analysis demontrates that four variables age, diagnostic group, ulceration and gastritis score are all significant factors in relation to the presence of intestinal metaplasia of any type. If the results are expressed in terms of probability (ie probability of 1 = 100% likelihood of event) then if only the variable diagnostic group is considered the intestinal type of tumour is most likely to have associated intestinal metaplasia compared with the benign or diffuse groups. If however the other variables are considered independently the probability that intestinal metaplasia will be present increases with age and increasing gastritis score and is closely linked to the presence of ulceration. The multivariate analysis allows for the combination of variables to be calculated and with this ability the probability of a 70 year old with grade 3 gastritis having intestinal metaplasia present is clearly greater than the probability of intestinal metaplasia being present with a diagnosis of intestinal type tumour.

The results of the multivariate analysis for type IIb show that when the diagnostic group is considered in isolation the likelihood of type IIb being present is much greater in the "intestinal" group compared with "gastric" or benign groups. However when the other variables are assessed the likelihood of type IIb being present is greater in a 60 year old with grade 3 gastritis and ulceration than in "intestinal" group.

The results of the multivariate analysis suggest that the significant association often demonstrated in the literature between the presence of intestinal metaplasia and gastric cancer and the presence of type IIb intestinal metaplasia with the intestinal type tumour can be explained by differences in age, ulceration and inflammation rather than by any causal link between intestinal metaplasia and gastric cancer. On the basis of the present study intestinal metaplasia and the type IIb variant appear to be an epiphenomenon in relation to gastric carcinogenesis.

The alternative hypothesis that type IIb metaplasia is related to the variables age, ulceration and inflammation rather than related to the intestinal type of tumour can be used to explain the fact that type IIb variant was not significantly related to intestinal type of tumour by Lauren classification but was significantly associated with the intestinal type as defined by Jass, 1980. This curious finding may be explained by the use of metaplasia in the mucosa adjacent to the tumour as one of the main criteria used by Jass to classify tumours into the intestinal type. By redefining the classification in this way Jass has selected a group of patients who are older, have a higher percentage of ulcerated lesions and with a greater degree of gastritis (see table 29). As all of these factors are related to the presence of type IIb as shown by the multivariate analysis, Jass (1980) has selected an "intestinal" group which shows a significant association with type IIb.

"Intestinal Tumours" Lauren and Jass Classification

	LAUREN	JASS
Mean age	67.7	71.1
% cases ulcerated	30.5	76.1
Mean gastritis score	3.83	4.28

Table 29: Comparison of the mean age, % of cases ulcerated and the mean gastritis score in the gastric mucosa betweeen the "intestinal tumours" as defined by Lauren (1965) and as defined by Jass (1980).

Relationship of Helicobacter pylori to the presence of Intestinal Metaplasia

Although the multivariate analysis did not show any significant association between the presence of Helicobacter pylori and intestinal metaplasia some interesting features of the relationship did appear.

In the gastrectomy group the incidence of H. pylori (10 out of 22 stomachs) in the benign group was of the same magnitude as in other published series (Marshall 1984; Forrest 1984). The incidence of H. pylori in the malignant group was markedly lower for both types of tumour at 15%. The severity of gastritis in the malignant group was higher in the stomachs without H. pylori and there was no significant difference in the quantity of intestinal metaplasia between the groups with or without the organism.

The hypothesis that infection with Helicobacter pylori may cause gastritis leading to the formation of metaplasia as a protective response is not supported by the crude analysis of the percentage of the gastric mucosa replaced by metaplasia between "infected" and "non infected" gastrectomy specimens, as no difference has been shown. The natural history of Helicobacter pylori infection is unclear; the development of gastritis and intestinal metaplasia may in some individuals be sufficient to eradicate the organism whilst in others there may be a persistent infection. Therefore examining the area replaced by the metaplastic change may be too simple an analysis and other variables ie. pH may require to be evaluated.

Dysplasia

Gastric dysplasia occurring in either gastric or metaplastic epithelium although identified as the potential pre-malignant lesion in gastric carcinoma (Morson et al., 1980) has not been extensively studied.

The results of any study on dysplasia must be interpreted carefully. The three points which must be considered are the source of the material studied, the relationship between dysplasia and carcinoma and the subjective variability in the interpretation of dysplastic change.

The study of gastric dysplasia in man can be performed on two sources of material; endoscopic biopsies or human gastrectomy specimens. Both sources have advantages and limitations. Endoscopic biopsy material relied on a relatively small amount of tissue which may be subjected to biopsy artefact. Invasion can be detected on biopsy material but the absence of invasion does not exclude this possibility in such material (Ming, 1984). The major criticism of many studies on endoscopic material is that repeated biopsies may not be sampling the same area. It is impossible to sample the same area on repeated biopsy over a time interval.

The use of gastrectomy material allows for the study of a large area of gastric mucosa and the relationship to the gastric neoplasm can be assessed.

The differences between the information gathered about the two types of material highlights the deficiencies. In endoscopic material changes in the gastric mucosa can be examined over a time

course but the same area cannot be assessed accurately. In gastrectomy material the area adjacent to the tumour can be examined extensively but the inference that mucosal abnormalities surrounding a neoplasm represent the source of malignant change is made. It is difficult to confirm or refute such an assumption.

The histological assessment of dysplasia is highly subjective. Observer variation is large: one study of dysplasia of the colon showed agreement between observers as low as 34% (Brown et al., 1985). Variations in nuclear size and architectural abnormalities are the two main features which contribute to the subjective impression of dysplasia (Tosi et al., 1987). These two components of dysplasia are well represented in Jass's classification. The important finding of this study is that the sub-types of dysplasia as described by Jass have been identified. This suggests that to a certain extent the Jass classification is reproducible.

Jass's histological sub-types of gastric dysplasia can be identified in the gastrectomy material. His elegant description of type I and type II dysplasia cannot be faulted or improved on. The association that he describes with particular types of tumour does not appear to be supported by this study. Neither does his hypothesis for the role of the sub-types of dysplasia in the histogenesis of tumours.

The result when expressed in terms of well and poorly differentiated tumours do not show any preponderance of type II dysplasia in the latter group as Jass describes (Jass 1983). The

other features which are also at variance are the high proportion, approximately two-thirds of cases in which type I and type II co-exist and the presence of both types of dysplasia in the diffuse tumour group.

The last discrepancy may be explained by differences in the classification of the tumours. Jass appears to have included in the diffuse group of tumours only signet ring cell type and non-mucin producing tumours (Jass, 1983). This interpretation of Lauren's classification (Lauren, 1965) might tend to transfer some diffuse type of tumours in this series into the poorly differentiated intestinal type. This would result in dysplasia being almost exclusively restricted to the intestinal type. This point emphasises the difficulty in the use of the Lauren system which was illustrated in the previous section.

Jass has propounded a complicated hypothesis regarding the origin of the two types of dysplasia. The two cell types - goblet and columnar cell in type II dysplasia and the association with poorly diferentiated tumours provide the basis for his theory. Severe forms of type II dysplasia show only one cell type and no association with poorly differentiated tumours has been demonstrated with this study. A simpler hypothesis is that all the forms of dysplasia result from a single cell type. Two-thirds of the intestinal tumours in which dysplasia was found in the adjacent mucosa showed atypical hyperplasia type I and type II dysplasia. This supports the concept of an unstable epithelium immediately adjacent to tumour. The unstable epithelium might originate from

a cell or population of undifferentiated cells which may transform into dysplasia of either type in some cases through an intermediate stage of type IIb metaplasia.

CHAPTER 4

MUCIN HISTOCHEMISTRY

INTRODUCTION

Four different mucins can be readily identified using the mucin histochemical techniques outlined in Chapter 2, namely neutral, O-acetyl sialomucin, N-acetyl sialomucin and sulphomucin. The work of this chapter involved a study of the gastrectomy specimen material discussed in Chapter 3 processed for mucin histochemistry. The broad aim of this study was to analyse the changes in mucin histochemistry that occur in the various stages of the histogenetic sequence portrayed in figure 11.

The review of the literature has shown that there have been few systematic studies on the mucin histochemistry of inflamed gastric mucosa. Although a variety of changes have been described in inflamed gastric mucosa adjacent to tumours (Gad 1969, Jass and Filipe, 1979) it is not clear if these changes are related to neoplasia or inflammation alone. The inclusion of both benign and malignant specimens in the present study has allowed this question to be addressed.

The mucin histochemistry of intestinal metaplasia is complicated with a variety of classifications based on mucins contained within the cells. The Jass system (Jass, 1980) combines both morphology and mucin histochemistry and has been most extensively studied in the recent literature. Several aspects however of the Jass system require further study. Are the descriptions of the mucin content of Type I, IIa and IIb described by Jass 1980 accurate when a large number of crypts are examined? Is there any difference in the mucin content of the intestinal metaplasia sub-types between benign and malignant stomachs?

Most authorities agree that gastric carcinomas contain a mixture of the four mucins mentioned above (Lev 1965; Filipe 1979; Montero and Segura, 1980). What is not clear is if the intestinal and diffuse types of the Lauren system show any significant differences in mucin content. The mucin content of the tumour sub-types of other classification systems have also not been studied systematically. Another aspect which has not been evaluated is the effect of the degree of differentiation of the tumours on mucin secretion. These questions have been addressed in this present study.

The mucin histochemical hallmark of Type IIb intestinal metaplasia is the presence of sulphomucin in the columnar mucus cells. If as Jass suggests (Jass, 1980) type IIb represents a possible premalignant variant then the sulphomucin present in the metaplastic crypts might be reflected in sulphomucin secretion by the intestinal type of tumours. By combining the mucin analysis of the intestinal metaplasia and carcinomas this hypothesis has been investigated.

AIMS

Gastric Mucosa

The aim of this study was to determine the frequency of occurrence of the types of mucin in the gastric mucosa of the benign and malignant gastrectomy specimens.

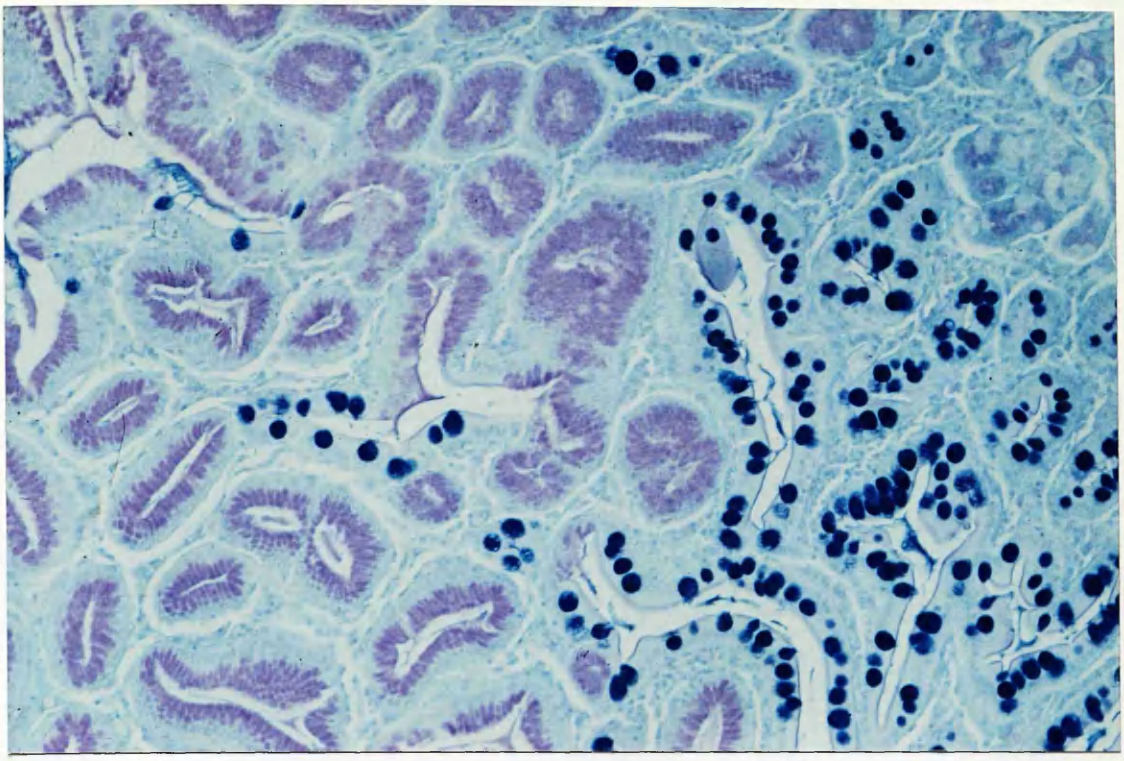
Intestinal Metaplasia

The aim of this investigation was to determine a mucin profile of the sub-types of intestinal metaplasia. One hundred crypts of each sub-type from benign, intestinal and diffuse types

of tumour were examined to see if any differences were apparent in the mucin profile of the intestinal metaplasia sub-types between these groups.

Gastric Carcinoma

The aim of this section was to analyse the mucin content of the gastric carcinomas to determine if the tumours when classified into the Lauren, Mulligan and WHO systems or into well or poorly differentiated categories displayed any particular features.



Photomicrograph 1. Antral mucosa with Type I intestinal metaplasia (Jass classification). AB/PAS x 60.

RESULTS

Gastric Mucosa

Neutral mucins predominated in the gastric mucosa in both benign and malignant gastrectomy specimens. Mucosa with significant inflammatory change and in particular areas of regenerative hyperplasia showed evidence of N-acetyl and sulphomucin in the columnar cells. O-acetyl mucins were not detected in the gastric epithelium. The number of cases in which Neutral mucins, N-acetyl and sulphomucins were present is shown in table 30.

Intestinal Metaplasia

The mucin profile of 100 crypts of the three sub-types I, IIa and IIb for the benign gastrectomy specimens and the intestinal and diffuse types of tumour of the Lauren Classification are shown in table 31. As an illustrative example the mucin profile of the metaplasia sub-types in the intestinal type of tumour are displayed graphically (figure 23).

No significant differences were present (Chi Square analysis) in neutral, O-acetyl, N-acetyl and sulphomucin content of Type I, IIa and Type IIb metaplasia respectively in the stomachs resected for benign disease, intestinal type or diffuse type of tumour.

Mucin content of the gastric carcinomas

The mucin content of the gastric tumours of all types and the sub-divisions according to the Lauren, Mulligan and WHO classifications are shown graphically. The mucin profile of well differentiated as compared to poorly differentiated is also shown

Mucin	Benign		Malignant	
	number	(% of total)	number	(% of total)
Neutral	22	(100)	72	(100)
N-acetyl	12	(54.5)	41	(56.9)
Sulphomucin	13	(59)	34	(47.2)

Table 30. Frequency of mucin types in the gastric mucosa in 94 gastrectomy specimens resected for benign and malignant disease.

Gastrectomy specimen diagnosis	Intestinal Metaplasia sub-type	Columnar Cell				Goblet Cells			
		Mucins							
		N	N-A	O-A	S	N	N-A	O-A	S
Intestinal	I	11	98	23	18				
Diffuse	I	8	100	25	17				
Benign	I	10	100	21	15				
Intestinal	IIa	24	100	22	33	95	8	22	14
Diffuse	IIa	30	100	18	31	87	11	10	16
Benign	IIa	27	100	25	30	85	6	12	11
Intestinal	IIb	10	100	18	13	21	6	14	96
Diffuse	IIb	12	95	14	14	19	8	10	88
Benign	IIb	14	100	21	17	26	7	17	98

Table 31. **Presence of mucins in goblet and columnar cells in 100 crypts of Type I, IIA and IIb metaplasia from benign, intestinal and diffuse (Lauren classification) gastrectomy specimens**

Key: N = Neutral mucin

N-A = N-acetyl

O-A = O-acetyl

S = Sulphomucin

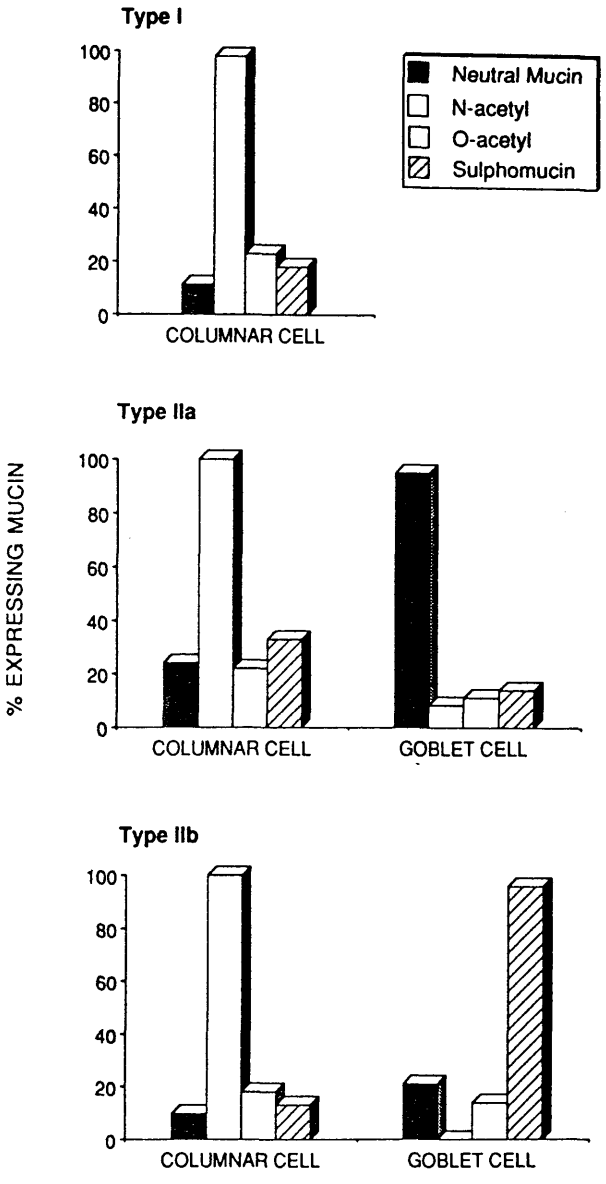


Figure 23. Mucin profile of 100 crypts of Type I, IIa and IIb metaplasia in stomachs containing intestinal type tumours. The number of crypts with columnar cells (Type I) and columnar and goblet cells (Type IIa and Type IIb) which express neutral, N-acetyl, O-acetyl and sulphomucin are shown.

(figures 24 and 25).

The expression of each individual mucin by the tumours in the classification systems was compared using the Chi Square test. The Signet Ring cell in the WHO classification had a significantly higher frequency of N-acetyl secretion than the other tumours in the WHO systen ($x^2 = 6.712$ $p < 0.05$). The number of pyloro-cardiac tumours in the Mulligan System was too small to make a statistical comparison. No significant differences were demonstrated between the intestinal and diffuse type of the Lauren System or between well and poorly differentiated tumours.

Relationship of type IIb metaplasia to sulphomucin content of gastric carcinomas

The presence of the sulphomucin containing variant of intestinal metaplasia - type IIb, to the presence of sulphomucin in the gastric carcinomas is shown in Table 32.

DISCUSSION

The results of this study demonstrate that in approximately half of the gastrectomy specimens from benign and malignant cases the columnar cells of the gastric mucosa express N-acetyl sialomucin and sulphomucin.

The mucin profile of intestinal metaplasia conforms with previous published work in that N-acetyl sialomucins predominate.

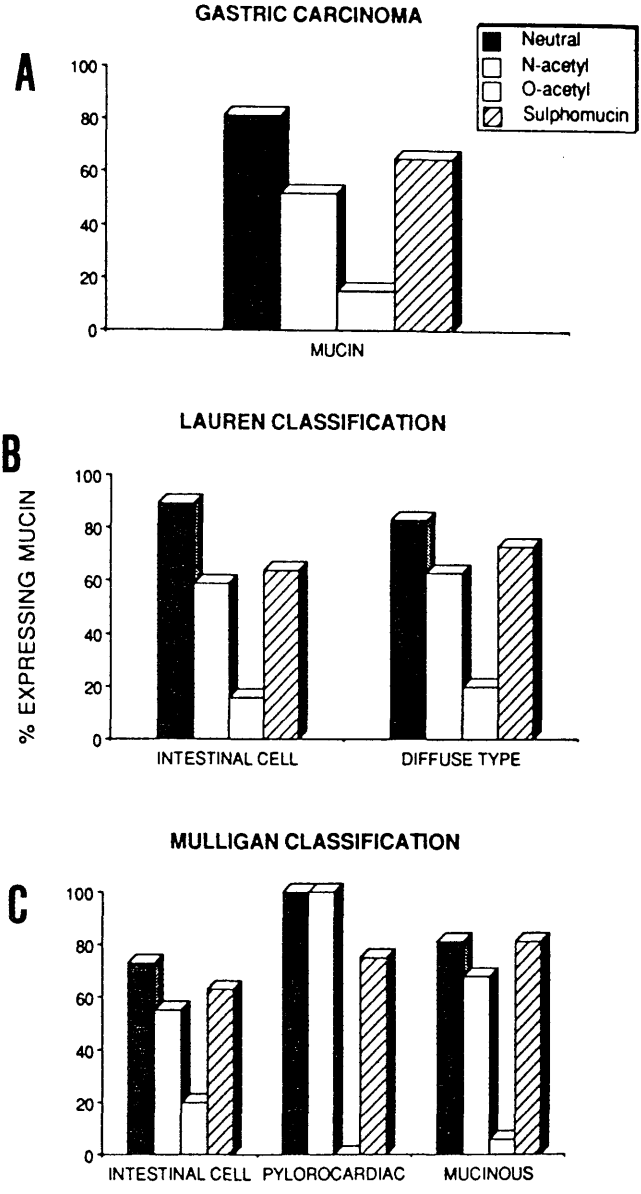
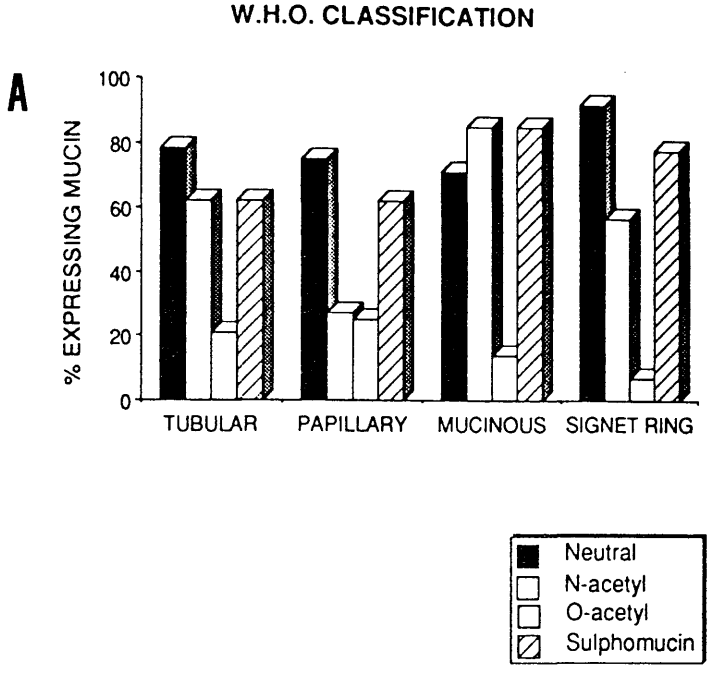


Figure 24 A. Percentage of gastric tumours expressing neutral, N acetyl, O-acetyl and sulphomucin.

B. Percentage of gastric tumours classified into intestinal and diffuse types (Lauren) expressing neutral, N-acetyl, O-acetyl and sulphomucin.

C. Percentage of gastric tumours classified into intestinal cell, pylorocardiac and mucous types (Mulligan) expressing neutral, N-acetyl, O-acetyl and sulphomucin.



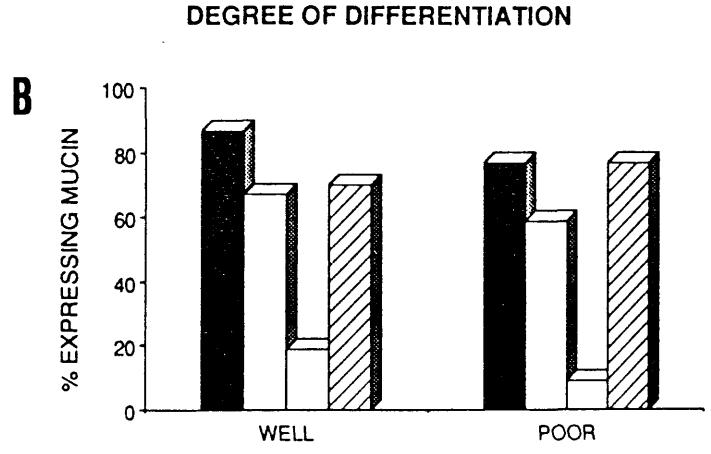


Figure 25 A Percentage of tumours classified according to WHO expressing neutral, N-acetyl, O-acetyl and sulphomucin.

B Percentage of well and poorly differentiated tumours expressing neutral, N-acetyl, O-acetyl and sulphomucin.

Tumour mucin content	Type IIb present	Type IIb absent
Sulphomucin present	17	29
Sulphomucin absent	9	17

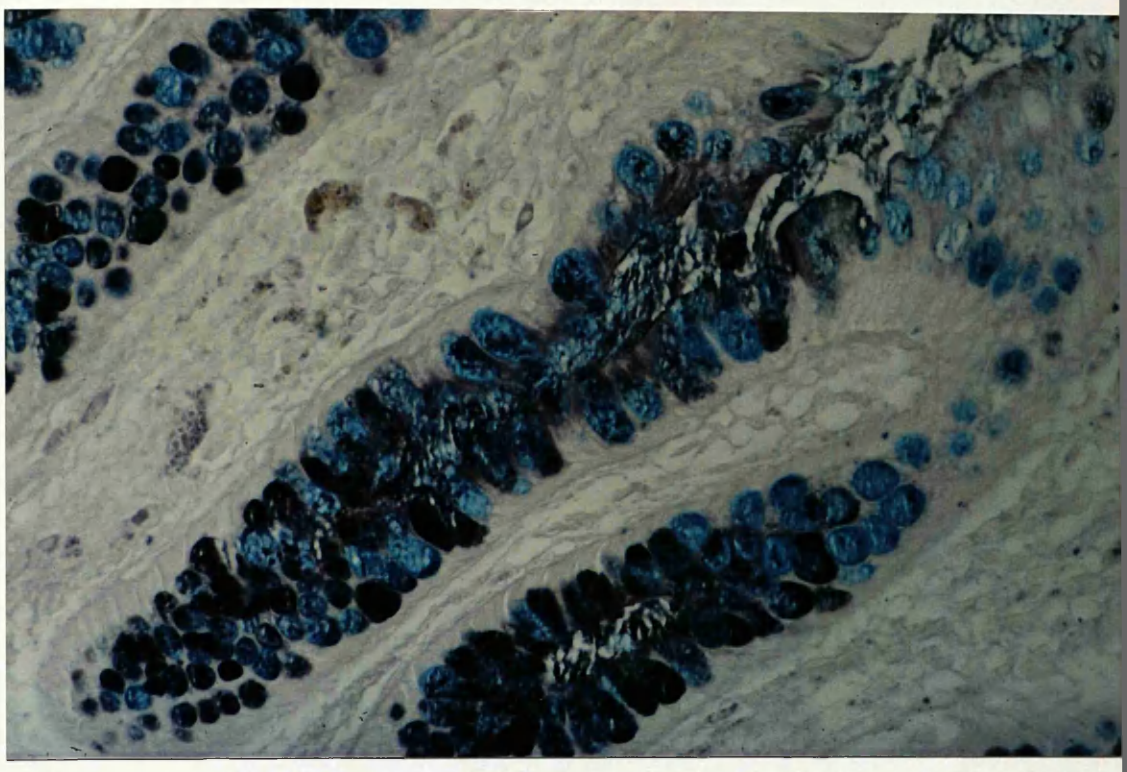
$x^2 = 2.709$ No significant difference

Table 32. Relationship of sulphomucin content of gastric tumours to the presence of type IIb intestinal metaplasia

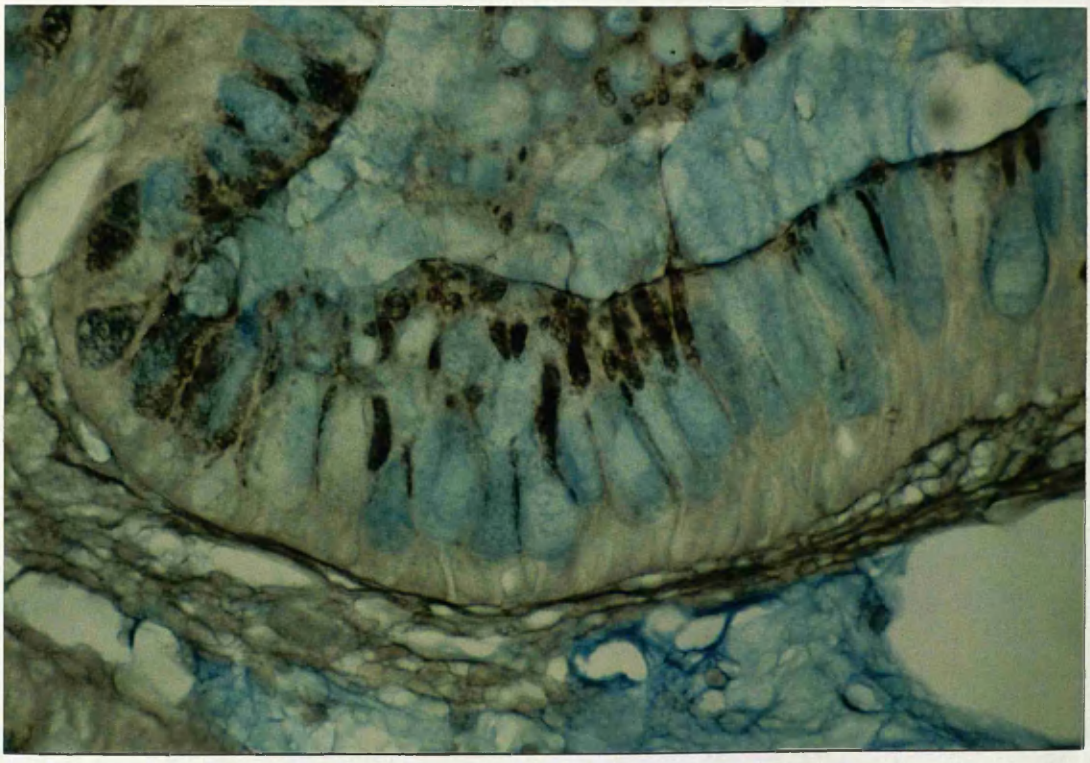
The results however show a significant proportion of Ø-acetyl and sulphomucin. When analysed with respect to Jass's sub-types several interesting features emerge (Jass, 1980). Firstly that type I metaplasia shows an appreciable content of sulpho and O-acetyl sialomucin and secondly that in type IIb variant although N-acetyl sialomucin and sulphomucin predominate in the columnar and goblet cells respectively Ø-acetyl sialomucin is still present in approximately 10% of crypts. In 1980 Jass's original description of the sub-types of metaplasia describes the sub-types as secreting mostly acid mucins in the goblet cells of type IIa and type IIb respectively, with an absence of O-acetyl sialomucin. The findings of this study suggest that the distribution of mucins between the three sub-types is not so clear cut with a significant overlap, in the presence of sulpho and O-acetyl sialomucin.

When mucin profile of the sub-types of intestinal metaplasia are compared between benign stomachs and malignant stomachs and between intestinal and diffuse type tumours, only slight differences are seen between the groups, which are not statistically significant.

The presence of sulphomucin within intestinal metaplasia has been promoted by some authorities as a possible indicator of pre-malignancy (Jass and Filipe, 1979; Matsukara et al., 1980). Endoscopic biopsy studies have emphasised the relatively high sulphomucin content of type I metaplasia (Rothery and Day, 1985; Filipe et al., 1985). The results of this study confirm the presence of sulphomucin in type I metaplasia and demonstrate



Photomicrograph 2. Type IIb intestinal metaplasia (Jass classification). HID/AB x 400.

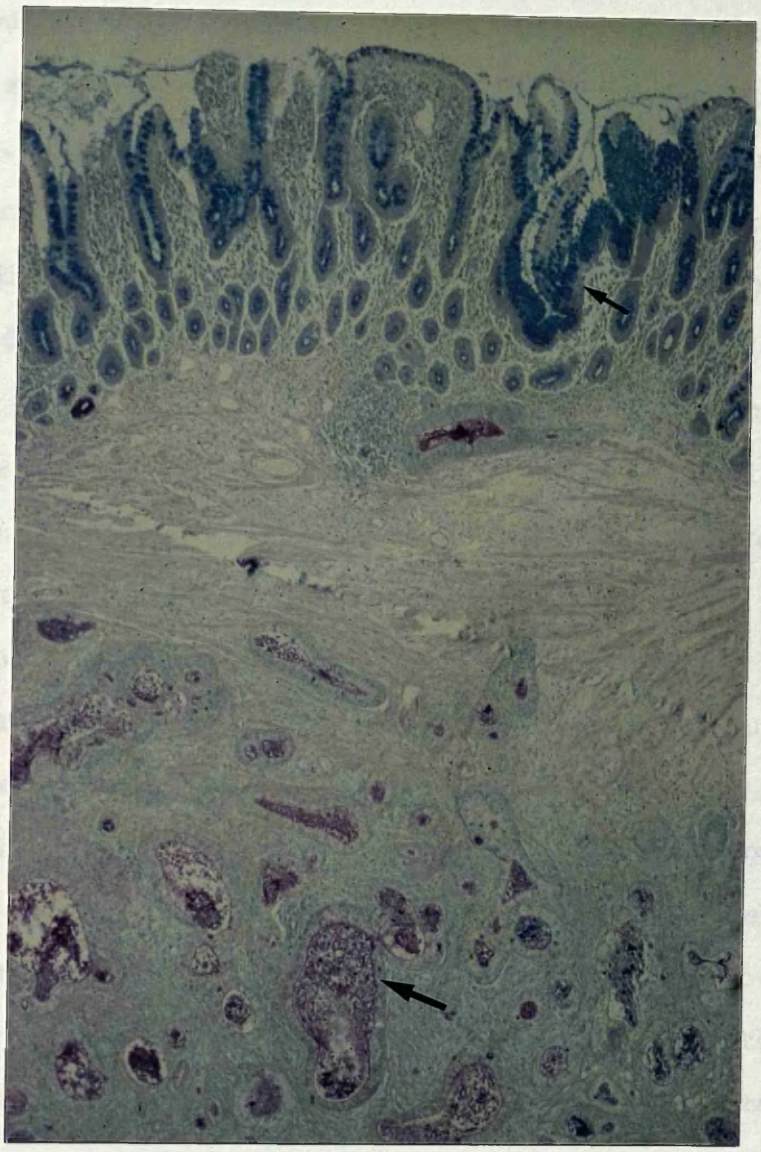


Photomicrograph 3. Type IIb intestinal metaplasia (Jass classification). HID/AB x 600.

numerically that sulphomucin is present in approximately 18% crypts in this variant. Sulphomucin positivity per se in endoscopic biopsy material is unlikely to be of any benefit in the prediction or identification of malignancy. The application of mucin histochemistry requires to be interpreted in conjunction with the morphology of the crypts as emphasised by Jass, 1980, and subsequent studies (Rothery and Day, 1985).

This study confirms that gastric carcinomas contain a mixture of neutral, O-acetyl and N-acetyl sialomucin and sulphomucin. The results are also in keeping with Cook's 1982, of a high neutral mucin content of gastric adenocarcinoma. Fifteen per cent of the gastric carcinomas in this study show O-acetyl sialomucin secretion which is comparable to the original description of 10% Culling et al., 1975, in a smaller series.

This study has failed to find any significant difference in mucin expression between the intestinal and diffuse types in the Lauren classification. Lauren and Sorvar, 1969, described originally a higher incidence of neutral mucin content in diffuse compared with intestinal type carcinoma. This finding has been extrapolated by Paginini and Rugge, 1983, into the hypothesis that the mucin type in cancers arising from normal gastric mucosa is likely to be different from that observed in cancers developing from metaplastic mucosa. These authors have postulated that the diffuse type of tumour having origin from the gastric mucosa may be identified as the minority of tumours that secrete neutral mucins only. These authors also postulated that the intestinal type of



Photomicrograph 4. Low power view of intestinal type tumour (large arrow) invading stomach secretory mainly neutral mucins and intestinal metaplasia (small arrow) containing N-acetyl, O-acetyl and sulphomucins. AB/PAS x 10.

tumour secretes a mixture of sialo and sulphomucins typical of colonic cancer secretion. This hypothesis based on morphology and mucin secretion would appear theoretically to be a logical extension of Lauren's original concept (Lauren 1965). The authors however do not specify the number of tumours showing neutral mucin secretion alone. In the present study only two such tumours could be identified in the 81% tumours that were positive for neutral mucin. In addition to this when the tumours were classified into intestinal and diffuse types on morphological grounds, a smaller percentage of the diffuse type of tumours were neutral mucin positive. The second major criticism of this hypothesis is the use of the term "colonic cancer secretion". Colonic tumours show a predominance of sialomucins against sulphomucins in contrast to that seen in normal colonic mucosa, however this is only seen in two-thirds of cases (Montero and Segura, 1980). The other feature of colonic tumours is the presence of O-acetylated sialomucins in two-thirds of the cases. (Filipe, 1979). Paginini and Rugge, 1983, described a sialomucin to sulphomucin ratio of approximately 75 to 52% in intestinal type tumours, a similar ratio for both intestinal and diffuse type of tumours can be obtained from the present study if O-acetyl and N-acetyl positive tumours are counted together. Thus both intestinal and diffuse tumours show this so-called "colonic type" secretion but the type of sialomucin is different with O-acetyl contributing only a small percentage.

The mucin histochemistry of the tumours classified according to the system of Mulligan and Rember (1972) show two interesting

features; the pylorocardiac tumours all express neutral mucin and the PB/KOH/PAS stain fails to reveal any O-acetyl sialomucin. The number of pylorocardiac tumours is however small and N-acetyl and sulphomucins are also present. Two other aspects of the mucin histochemistry of gastric carcinomas were studied in relation to classification; the descriptive WHO classification and the degree of differentiation of the tumours, either well or poorly differentiated. In the mucin expression according to the WHO classification there was a higher frequency of neutral mucin in the signet ring cell type compared with the other types. This may relate to the "true gastric type" of tumour expounded by Paginini and Rugge, 1983, but again there was a substantial expression of other types of mucin by this type of tumour.

The results of this study show a relative decrease of sialomucin and an increase in sulphomucin content in the poorly differentiated tumours. Gad 1969, and Paginini and Rugge 1983, describe the reverse, whereas Montero and Segura 1980, agree with the results of this present study. The present study involves a larger number of cases of gastric carcinoma than the previous reports. The increase in sulphomucin content is from 70 to 77% and the decrease in sialomucin is of the same order. The alteration in sialo and sulphomucin content was not statistically significant.

The characteristic histochemical feature of type IIb metaplasia is the presence of sulphomucin. The results of this study show no clear relationship between the presence of

sulphomucin in the tumours with the presence of type IIb metaplasia in the adjacent mucosa.

Several authors (Montero and Segura, 1980; Paginini and Rugge, 1983) have used isolated aspects of the pattern of mucin expression in normal gastric mucosa intestinal metaplasia and gastric tumours to constrict theories of the histogenesis of gastric carcinoma.

The present study has not demonstrated any significant differences in mucin histochemistry between the intestinal and diffuse type tumours of the Lauren classification to support a concept of a different histogenesis for these two types of tumours.

CHAPTER 5

IMMUNOCYTOCHEMICAL STUDIES

INTRODUCTION

General points

The review of the literature in Chapter 1 concentrated on three aspects of immunocytochemistry in relation to gastric pathology namely antibodies to specialised cell types, alterations in normal function identified using immunocytochemical techniques and oncofetal antigens. The work of this chapter was performed on the human gastrectomy material discussed in Chapter 3 and involves the investigation of the three aspects of immunocytochemistry listed above in relation to the histogenetic sequence outlined in figure 11.

Antibodies to specialised cell types

A polyclonal antibody raised against intrinsic factor was selected for study. The reason for this choice of antibody was an original observation made by Brown in 1982 that gastric tumours of the diffuse type (Lauren 1965) displayed immunoperoxidase staining with this antibody whereas intestinal type tumours did not. This preliminary observation was interpreted as possibly indicating the origin of the diffuse type of tumour from gastric epithelium. The intrinsic factor antibody was therefore included in the present study of a greater number of gastric tumours to determine if this pattern of immunoperoxidase staining was consistent in the gastric tumours and if any of the pre-neoplastic states displayed immunoperoxidase staining.

A second group of antibodies to specialised cell types in the gastric epithelium have also been studied namely the neuropeptides.

The literature review in Chapter 1 revealed that few studies existed concerning the neuro-endocrine cell population in inflammation and metaplasia of the gastric mucosa. The present study has concentrated on two particular aspects of the changes in the numbers of neuro-endocrine cells and the changes in specific neuropeptides.

Alterations in normal function

The secretory immunity in normal, inflamed, metaplastic and neoplastic gastric mucosa is the aspect of normal function examined in the present study. The stages in the histogenesis of gastric carcinoma have been examined systematically and changes in the stomachs resected for malignant disease compared with those resected for benign disease. The importance of this comparison is the need to discriminate between changes in normal function that occur in response to inflammation alone rather than pre-neoplastic or neoplastic change. The inclusion of "control" material in the present study (ie. benign specimens) has often been lacking in other studies.

Acquisition of oncofetal antigens

The literature review has illustrated marked confusion concerning the immunocytochemical demonstration of CEA in gastric mucosal alterations. The reason for this confusion appears partly to be due to the presence of cross reactions with other glycoproteins in commercial CEA antisera. The present study has addressed this problem by comparing the immunoperoxidase staining pattern with and without the abolition of these cross reactions. As with the studies on secretory immunity outlined above the steps

in the hypothetical sequence of gastric carcinogenesis have been examined systematically using the anti-CEA antibody to determine the nature of the epithelial alterations associated with acquisition of this oncofetal antigen.

AIMS

1. To examine the expression of intrinsic factor, secretory component, IgA and carcino-embryonic antigen (with and without the abolition of cross reacting antigens in the antisera) in normal and inflamed gastric mucosa, intestinal metaplasia sub-types, and dysplasia in human gastrectomy specimens resected for both benign and malignant disease.
2. To examine the expression of intrinsic factor, secretory component, IgA and carcino-embryonic antigen in gastric tumours sub-divided into the intestinal and diffuse types (Lauren) and well and poorly differentiated tumours.
3. To quantify the neuro-endocrine cell population in normal antral and body crypts and the numerical changes that occur in gastritis and the intestinal metaplasia sub-types.
4. To identify the specific neuropeptides in the neuro-endocrine cells in normal antral and body mucosa, inflamed antral mucosa and the intestinal metaplasia sub-types.

RESULTS

Intrinsic factor, secretory component, IgA, CEA

Eight tumours showed extensive necrosis with poor fixation and were not included in the analysis (4 unclassified, 3 diffuse type, 1 intestinal type). To quantify the staining pattern of SC, IgA and secretory component in the sub-types of metaplasia 1,323 crypts which were axially sectioned to include the base and luminal surface were analysed.

Intrinsic factor

Gastric mucosa

Immunoperoxidase staining was limited to the parietal cells situated below the level of the neck of the gastric crypts. The parietal cells were abundant in body type mucosa but an appreciable number were identified in the antral mucosa. The pattern of staining within the cells was characteristic: there was a strong immunoreactivity in the luminal and supranuclear cytoplasm in a band-like configuration. Although the number of parietal cells tended to diminish with increasing severity of inflammation and atrophy, the staining pattern did not change. No difference in pattern was noted in the staining from benign or malignant stomachs.

Intestinal metaplasia and dysplasia

No immunoreactivity was noted in any of the sub-types of intestinal metaplasia, regenerative hyperplasia or dysplasia.

Carcinoma

No immunoreactivity was noted in any of the cases examined.

Secretory component

Gastric Mucosa

Normal antral and body mucosa showed no immunoreactivity for secretory component. Mild superficial gastritis resulted (gastritis score = 1) in slight immunoreactivity in the columnar cells at the neck of the antral crypts. More severe degrees of gastritis (gastritis score 3 - 5) led to increasing numbers of cells within the gastric crypts in both antral and body mucosa staining for SC. Severe chronic atrophic gastritis (gastritis score = 5) particularly in areas adjacent to tumour and ulcer showed immuno-peroxidase staining in almost all of the cells within the gastric crypts above and below the neck region. The columnar cells alone were stained; the parietal and peptic cells showed no immunoreactivity. The staining was present predominantly on the apical surface of the cells.

Intestinal metaplasia

Type I

The columnar cells throughout the length of the metaplastic crypts were immunoreactive with secretory component.

Type IIa

This showed a variable pattern of expression with cell crypt demonstrating immunoreactivity at the neck but faint staining throughout the remainder of the crypts. In approximately half of the crypts SC was expressed by the columnar cells at the neck of the crypts only, in the remainer there was immunoreactivity in columnar cells at the neck and the base.

Type 11b

Secretory component was expressed predominantly by columnar cells at the base of the crypts, in a few crypts there was faint staining in cells at the neck.

Regenerative hyperplasia

The constituent cells in areas of regenerative hyperplasia displayed intense and widespread staining for secretory component.

Atypical hyperplasia

As with type IIb the immuno-peroxidase staining for secretory component was limited predominantly to cells at the base of the crypt.

Dysplasia

Type I and II dysplasia displayed staining for secretory component in the columnar cells distributed throughout the gland, from base to luminal surface.

IgA

The pattern of IgA expression mirrored that of secretory component in normal, inflamed and type 1 and type 11a metaplasia and dysplasia. In type 11b metaplasia often staining for IgA staining was absent and when present tended to be extremely faint and restricted to the base of the crypts. The number of crypts showing IgA and secretory component staining is shown in table 33.

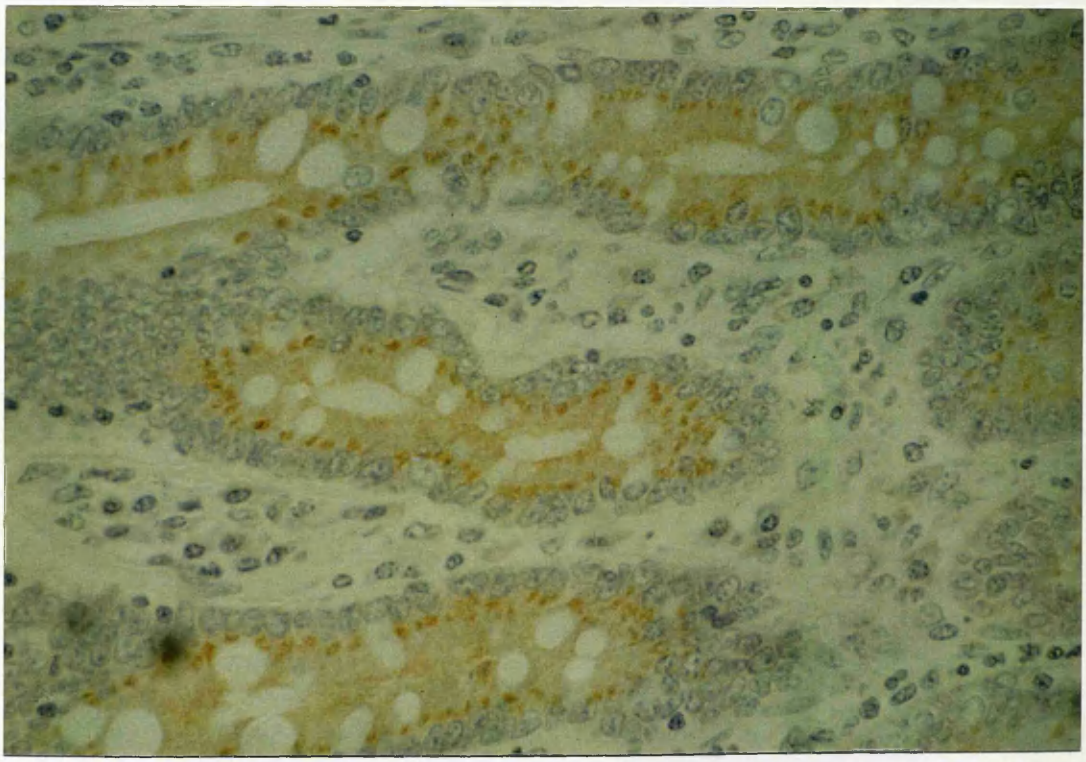
IgA and secretory component in gastric carcinomas

IgA and SC expression within the tumours was highly variable in respect of cellular distribution, types of cells, distribution and the number of cells within the tumour. The most reproducible feature was the co-existence of scanty IgA and secretory component

Type of Metaplasia	Type 1	Type 11a	Type 11b
Number of crypts	1195	51	88
Percentage of crypts expressing antigen			
SC	100	100	85.3
IgA	100	100	40.6
CEA	17.3	27.4	69.3

Table 33. **Staining pattern of intestinal metaplasia sub-types with antibodies to secretory component, IgA and carcino-embyronic antigen.**

Photomicrograph 5. Cross section of antral epithelium with positive staining for secretory component. PAP method x 350.



Photomicrograph 6. Type I intestinal metaplasia with positive staining for secretory component. PAP method x 350.

expression within the same tumours. The cellular distribution was different to that seen in the gastric mucosa and intestinal metaplasia; immunoreactivity was distributed on the apical surface of cells within tumour acini and in addition to this on the basolateral surface and apparently within the cytoplasm. The expression of SC and IgA was not limited to acinar cells but was found in signet ring cells and within other malignant cells not within acini. The staining tended to be localised to discrete areas within the tumour. Some areas within the tumour showed strong immunoreactivity whilst areas which did not appear histologically different displaying a marked lack of staining. The number of tumours stained is shown in table 34.

Carcino-embryonic antigen

Normal mucosa

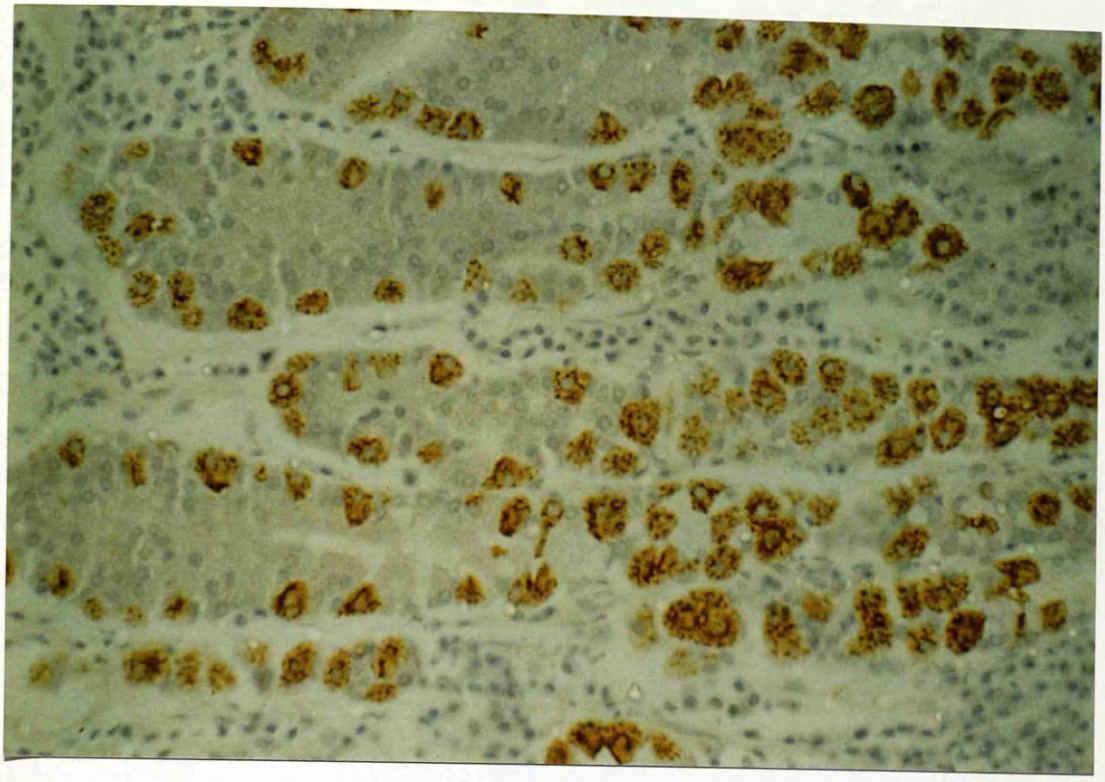
Antral and body type mucosa with no evidence of inflammation displayed no immunoreactivity for CEA. In areas with significant gastritis (ie. gastritis score = 3-5) both antral and body mucosa displayed positive CEA staining in the columnar mucus cells mainly at the base of the crypts. Absorption of the CEA antibody with normal stomach did not abolish this staining. Immuno-peroxidase staining for CEA was found both in the gastric mucosa of the benign and the malignant stomachs.

Intestinal metaplasia

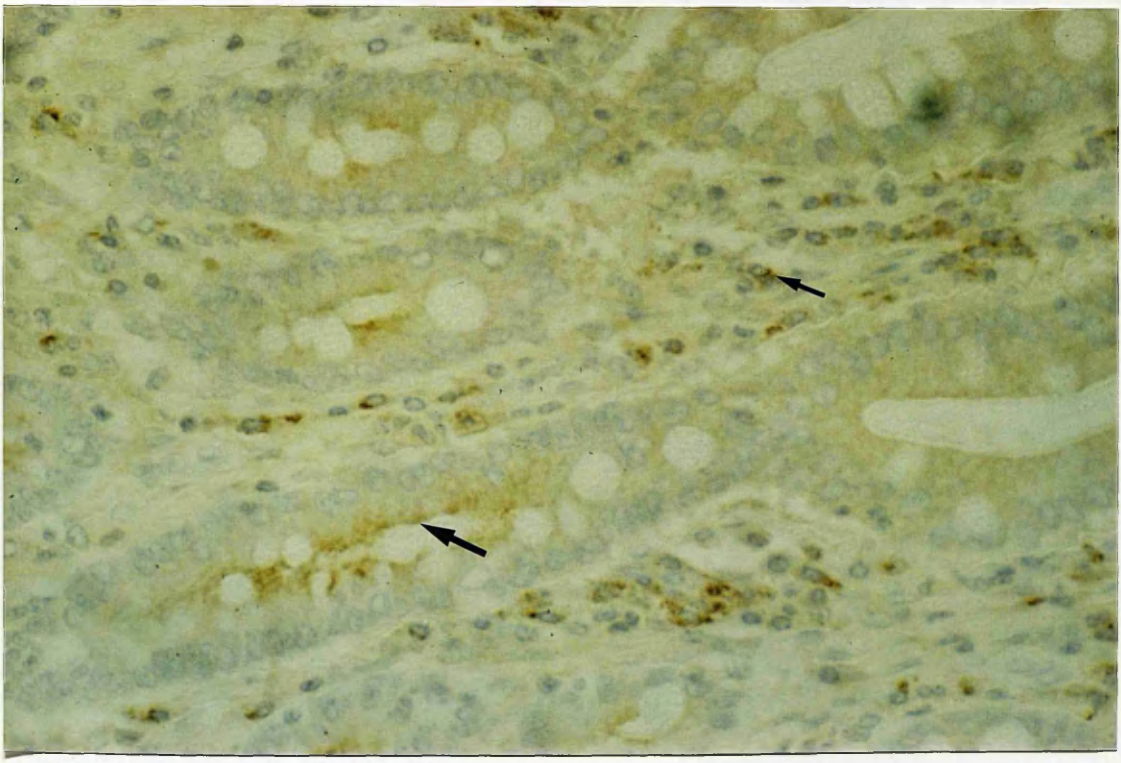
Foci of metaplasia of all three types showed CEA immunoreactivity but not in a consistent pattern. The presence or absence of CEA immunoreactivity is shown in table 33. The columnar cells displayed immunoreactivity in the apical cytoplasm, cells in

Antibody	Positive	Negative
SC	58	6
IgA	54	10
CEA	53	11

Table 34. Frequency of positive and negative staining in 64 gastric tumours with antibodies to secretory component, IgA and carcino-embryonic antigen.



Photomicrograph 7. Parietal cells positive for intrinsic factor. PAP method x 350.



Photomicrograph 8. Type I intestinal metaplasia. Epithelial cells (large arrow) and plasma cells (small arrow) positive for IgA.

all areas of the crypts in each type showing staining. The determining factor in producing CEA immunoreactivity appeared to be the level of inflammation in the surrounding lamina propria. Absorption of the CEA antibody did not alter this pattern of staining.

Carcinoma

Cellular distribution: Two distinct patterns were noted; large intra cytoplasmic granules staining positively for CEA, and a linear band-like immunoreactivity along the apical surface of the cells. Both patterns often co-existed within the same tumour.

Regenerative hyperplasia

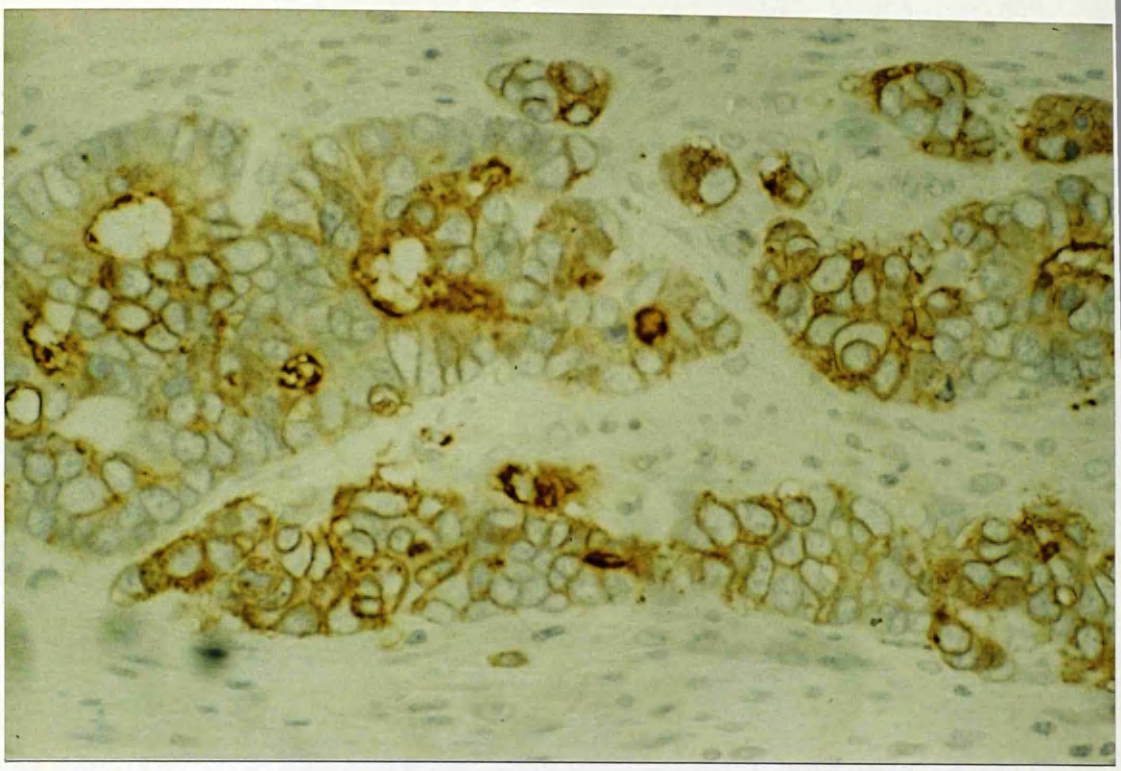
The constituent cells of regenerative hyperplasia displayed staining for CEA in all foci examined. Absorption of the anti CEA antibody with normal stomach did not alter this staining pattern.

Atypical hyperplasia and dysplasia

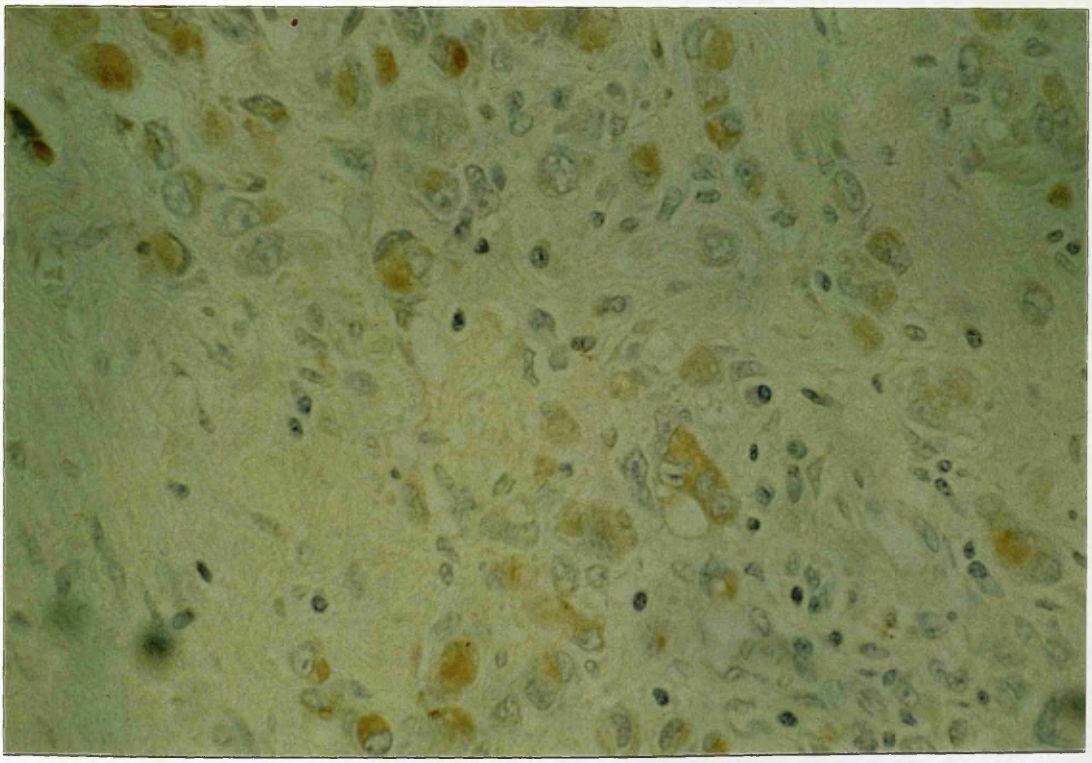
The immuno-peroxidase staining for CEA was present in columnar cells distributed throughout the crypts in areas of atypical hyperplasia, Type I and Type II dysplasia. Absorption of the anti-CEA antibody with normal stomach did not change this staining pattern.

The intracytoplasmic staining was seen in signet ring cells and large anaplastic cells, the apical surface staining in cells forming malignant acini.

The SC, IgA and CEA expression of the tumours in relation to the Lauren classification and the degree of differentiation are shown in tables 35 and 36.



Photomicrograph 9. Well differentiated gastric carcinoma with positive staining for CEA. PAP method x 300.



Photomicrograph 10. Poorly differentiated gastric carcinoma with positive staining for CEA. PAP method x 280.

	Type of Tumour	
Antibody	Intestinal n = 36 positive	Diffuse n = 28 positive
SC	35	23
IgA	33	21
CEA	31	22

Table 35. Frequency of positive staining. 36 intestinal and 28 diffuse tumours (Lauren) with antibodies to secretory component, IgA and carcino-embryonic antigen.

Antibody	Degree of differentiation	
	Well n = 39	Poor n = 25
SC	38	20
IgA	37	17
CEA	37	16

Table 36. Frequency of positive staining in 39 well differentiated and 25 poorly differentiated gastric tumours with antibodies to secretory component, IgA and carcino-embryonic antigen.

Neuro-endocrine cells

The results of the neuro-endocrine cell content as identified by immunoperoxidase staining are shown in table 37.

DISCUSSION

Epithelial and Oncofetal Antigens

The results presented above are based on a single observer. The nature of the sub-types of metaplasia, degree of inflammation and types of tumour are all evident when examining the histological material. This makes the objective interpretation of the immunoperoxidase material difficult as the observer is aware of which particular type of tissue he is studying. The panel of antibodies were selected for two reasons. Firstly, they are all available either commercially or through the auspices of S.A.P.U. The presentation and publication of data using antibodies not available to other centres although of biological interest prevents the validation of such data and limits the use of the antibodies in diagnostic histopathology. Secondly, the panel of antibodies provide information on the expression of antigen of functional and histogenetic interest in gastric carcinogenesis.

On the basis of the preliminary study of the IF antibody (Brown 1982) with its differential pattern of staining appeared to show great promise. The failure in this study to reproduce these results and in fact to demonstrate any staining whatsoever in the gastric carcinomas illustrates the limitations and inadvertent results that can be achieved using immunocytochemical techniques. The initial immunocytochemical studies were performed using non-purified antibody which had not been adequately characterised.

Tissue	No of crypts	Chromogranin positive cells per unit	Neuropeptides present
Normal Antrum	85	17.2	Gastrin Somatostatin
Antrum gastritis grade 3	625	20	Gastrin Somatostatin
Antrum gastritis grade 5	715	22.1	Gastrin Somatostatin Glucagon CCK CGRP GIP
Intestinal metaplasia			
Type I	235	16.7	Somatostatin
Type IIa	60	18.9	Glucagon
Type IIb	85	17.5	Gastrin Bombesin GIP CCK

Table 37. Number of chromogranin positive cells per morphological unit and presence of specific peptide containing cells in normal and inflamed antral crypts and sub-types of intestinal metaplasia.

The positive staining described appears to have resulted from inadequate removal of endogenous peroxidase. In this study endogenous peroxidase activity was abolished using incubation with hydrogen peroxide. Examination of controls ie. normal rabbit serum instead of test IF serum without removal of peroxidase revealed strong positive staining with DAB in approximately 70% of the carcinomas.

The absence of secretory component and IgA staining by normal antral and body mucosa has been well documented (Isaacson, 1982), often, however, this has been taken to imply that gastric mucosa never expresses these antigens (Nagura et al., 1983). Isaacson 1982, in his elegant study demonstrated that in stomachs resected for malignant disease the expression of secretory component appeared to be related to the presence of gastritis. The results of this study confirm those of Isaacson (1982) and in addition demonstrate that a similar phenomenon occurs in benign stomachs.

The staining pattern in intestinal metaplasia has also previously been described (Isaacson, 1982) but these authors did not differentiate the sub-types of metaplasia.

This study illustrates that the variants of intestinal metaplasia all retain the ability to express SC and IgA. The cellular localisation is similar to that of small bowel, ie the columnar cells. Type IIb metaplasia did show different pattern of SC expression. The restriction of SC expression to the base of such crypts is perhaps a reflection of the columnar cells at the higher levels of the crypts synthesising mucus as suggested by (Jass et al., 1984). The ability of such cells to produce mucus

is paralleled by the loss of the brush border and this may be an equally valid reason for the loss of SC expression. The immunocytochemical studies might be interpreted as suggesting that the surface columnar cells are less differentiated but this is not true of the crypts as a whole, as the base retains this ability.

The absence of IgA expression in these crypts suggests that although the cells in the crypt bases express SC the functional ability to transport IgA is lost. The loss of IgA translocation will reduce the bactericidal potential of the mucosal secretion and it has been suggested that this may allow bacteria to convert nitrates to carcinogenic nitrosamines and deconjugate bile acids (Jass et al., 1984). Whilst this can neither be confirmed nor refuted, the small proportion of type IIb crypts in the stomach with intestinal metaplasia as demonstrated in Chapter 3, would seem unlikely to influence significantly the general bactericidal activity.

Commercial CEA antibodies cross-react with NCA, BGP biliary glycoprotein and NCA_2 (Nap et al., 1983). Absorption of anti CEA (Dakopatts) with normal human stomach abolishes NCA_2 immunoreactivity on immnunoperoxidase staining in the normal stomach (Nap et al., 1983). Nagura et al., 1983, examined only normal antral and fundic mucosa and the anti CEA serum was absorbed with ABO blood group antigens and human spleen. This group of workers detected no CEA immunoreactivity in normal stomach but did not examine inflamed mucosa. Borch et al., (1987) studied inflamed antral and body mucosa using anti CEA serum non-reactive with NCA and BGP_1 and absorbed with human AB erthrocytes showed a linear CEA

staining in the glycocalyx of the surface and foveolar columnar cells. The absorption of the Dako anti CEA antibody in this study with spleen , ABO blood groups and normal human stomach resulted in an absence of staining in normal antral and body mucosa but positive staining in gastritis and intestinal metaplasia indicating that the CEA immunoreactivity is related to the presence of "true" CEA rather than NCA_2. CEA has been described in intestinal metaplasia (Nagura et al., 1983; Nielsen and Teglbjaerg, 1984) but the systematic staining of the sub types has not been widely examined. Neilsen and Teglbjaerg, 1984, describe CEA immunoreactivity in "colonic" type metaplasia positive for O-acetyl mucin and the absence of CEA immunoreactivity in "small intestinal" type netaplasia with absence of O-acetyl mucin. Jass et al., 1984, describes CEA staining in both type I and type IIb metaplasia. The results of this study confirm Jass's (Jass et al., 1984) findings insofar as both sub-types show CEA positivity. Type 11b showed a higher percentage of crypts staining and the staining pattern was distributed throughout the cytoplasm rather than just confined to the luminal surface. The significance of these findings is difficult to determine, certainly the expression of CEA cannot be regarded as a marker for neoplasia as it is present on inflamed gastric epithelium.

The immunocytochemical studies do not appear to have shown any distinct pattern. The histogenetic sequence described by Correa et al., 1982, of chronic atrophic gastritis, intestinal metaplasia leading to intestinal type of tumour would fit the expression of SC, IgA and CEA by these mucosal abnormalities. The

confounding feature, however, is the expression of SC, IgA, CEA by both types of tumour. Isaacson (1982) pointed out that as the mucous neck cells of the gastric crypt synthesise SC (and in this study CEA): the finding of CEA, SC in both types of tumour neither supports nor contradicts the hypothesis that intestinal type carcinomas arise from intestinal metaplasia while the diffuse tumours arise from the mucous neck.

The staining pattern of the gastric carcinomas proved to be variable within areas of the same tumour and between tumours. There appeared to be no relationship between the classification into intestinal or diffuse type of the Lauren classification (1965) and the presence of SC, IgA or CEA staining. There did appear to be a trend towards diminishing SC, IgA and CEA staining and the poorer degree of differentiation of the tumour.

Several studies have attempted to use the staining pattern of gastric mucosal alterations to support or refute the concept of two types of gastric carcinoma with differing histogenesis (Hirsch-Marie et al., 1976; Nielsen and Teglbjaerg, 1982; Reid et al., 1982,; Nardelli et al., 1983; Nardelli et al., 1984). Such studies have, for instance, equated the presence of a particular antigen detected by immunocytochemical techniques on intestinal metaplasia and the presence of the same antigen in gastric tumours of intestinal type as being evidence that the tumour is derived from intestinal metaplasia

To draw such inferences from immunocytochemical studies is unjustified without first considering the following factors regarding gastric carcinogenesis. Firstly that intestinal

metaplasia itself is derived originally from gastric epithelium. Secondly that (as shown in Chapter 3) a large proportion of gastric tumours on light microscopy show histological features of both the intestinal and diffuse types as described by Lauren (1965). Thirdly that multidirectional differentiation can occur in epithelial tumours and the change in differentiation is associated with alterations in phenotypic expression (Gould et al., 1981). Multidirectional differentiation has been well described in gastric tumours with metastatic deposits of tumour showing different histological patterns from the primary tumour in a third of cases (Stalsberg, 1972).

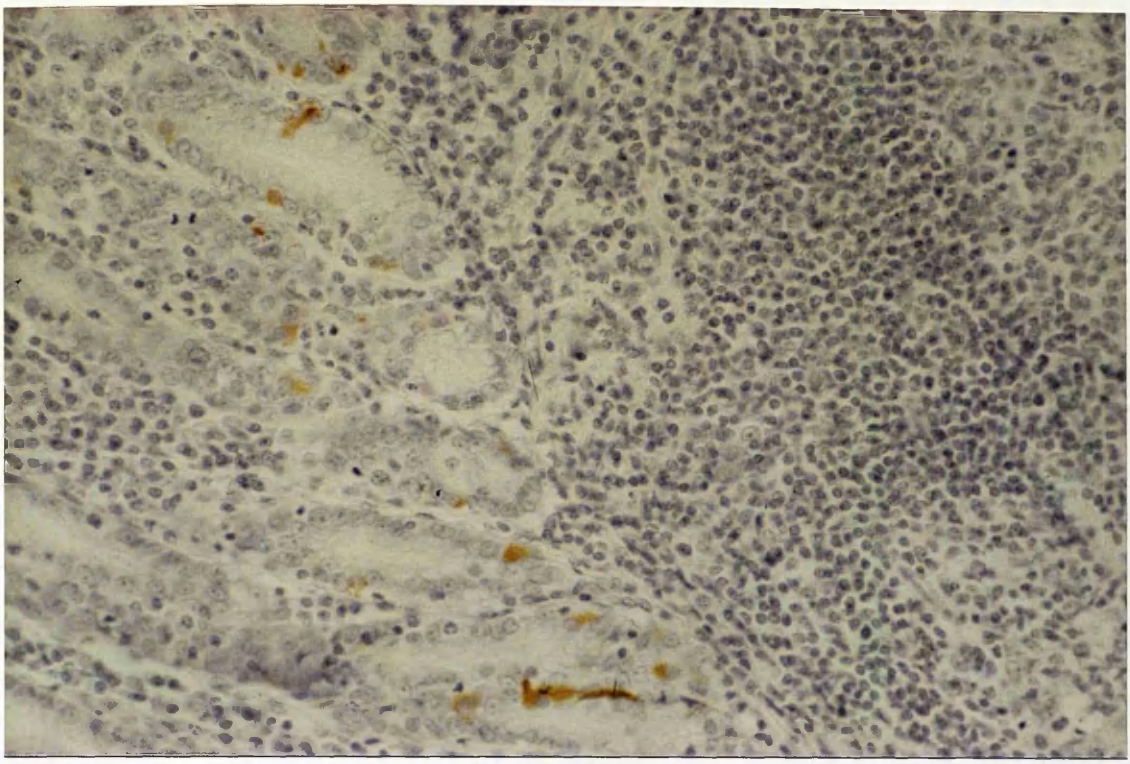
Following consideration of the above-mentioned factors it is perhaps not surprising that the results of the present immunocytochemical studies show no clear pattern in the distribution of epithelial and oncofetal antigens in the gastric mucosal abnormalities and neoplasia studied. It is clear from the present study that the change from normal healthy gastric mucosa to inflamed gastric mucosa is accompanied by alterations in phenotypic expression of the epithelial cells as identified by immunocytochemical techniques. The changes in phenotypic expression that occur in the transition from normal to inflamed mucosa are also seen in varying degrees in the metaplastic, dysplastic and neoplastic epithelium. The present study has not identified any specific "immunocytochemical marker" peculiar to any putative premalignant precursor.

Neuroendocrine Cells

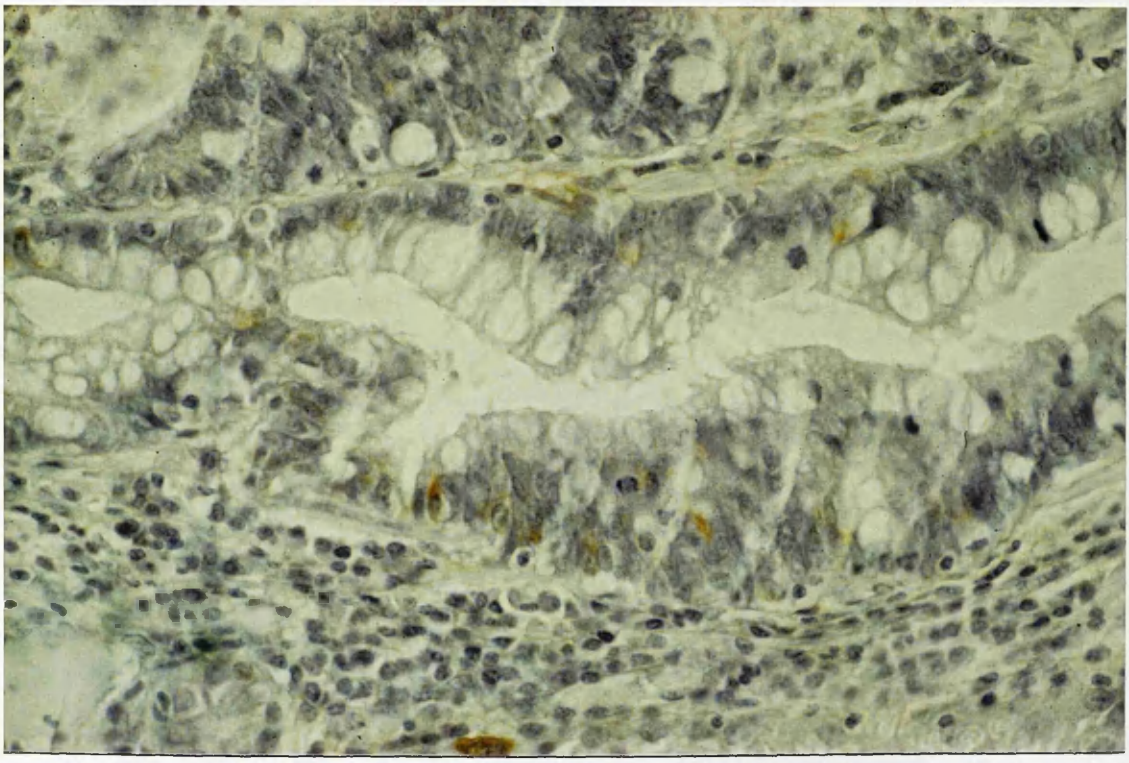
Chromogranin is present in all the identifiable endocrine

cell types in the human gut, thus the number of chromogranin positive cells gives an indication of the endocrine cell population as a whole (O'Connor et al., 1983; Lloyd and Wilson, 1983; Facer et al., 1985). The advantage of chromogranin in the present study has been to attempt to quantify the changes that occur with gastritis and metaplasia. The expression of the data in terms of number of cells per morphological unit has been shown to be more suitable than cells/unit area as oedema and atrophy can modify the dimensions of the tissue substantially (Marsh, 1980; Buchan et al., 1984).

The chromogranin cell quantification demonstrates a stepwise increase in the number of neuro-endocrine cells with increasing severity of inflammation. Similar elevations in neuro-endocrine cells have been detected in the small intestine in active coeliac disease compared to normal controls (Pietroletti et al., 1986). In grade 5 gastritis equivalent to active chronic gastritis not only is there a marked elevation in neuro-endocrine cell numbers but there is also the appearance of peptides not seen in the normal antral mucosa. Glucagon positive cells were found in large numbers and the sporadic appearance of other peptides GIP, CCK, and CGRP. Large numbers of glucagon positive cells were found in antral crypts adjacent to lymphoid follicles often with foci of intestinal metaplasia in the nearby epithelium. The significance and role of the glucagon - immunoreactive cells in this situation is unclear although this finding has been noted in another study (Ito et al., 1984). Glucagon immunoreactive cells can be detected in small numbers at nine weeks gestation in the fetal stomach



Photomicrograph 11. Inflamed antral mucosa with lymphoid aggregate with Chromogranin positive cells in antral glands. PAP method x 100.



Photomicrograph 12. Type IIb intestinal metaplasia with somatostatin positive cells. PAP method x 350.

although they are not detected in the normal adult stomach but are located predominantly in the ileum (Bloom, 1978; Facer et al., 1989). Glucagon has been shown to have a trophic action on intestinal epithelium (Bloom 1981) and the hypothesis that glucagon cells in the situation described in the present study may promote metaplasia via a paracrine route can be advanced.

In the fetal stomach gut activity is preceded by a fully differentiated neuro-endocrine component (Facer, 1989). This embryological form of development is perhaps being seen in the inflamed stomach. The appearance of different neuro-endocrine cells in areas of severe gastritis adjacent to foci of intestinal metaplasia may herald the development of intestinal metaplasia in these crypts. Thus the changes in the neuro-endocrine component are preceding the epithelial changes in a similar fashion to the fetal gut.

In intestinal metaplasia there is an overall decrease in the number of neuro-endocrine cells compared with antral gastritis. The decrease in numbers is accompanied by a change in the type of neuroendocrine cells with somatostatin replacing gastrin as the most frequent peptide and glucagon immunoreactive cells are less frequent compared with antral gastritis. In addition sporadic immunoreactive cells for the peptides CCK, CGRP and GIP appear. The function of these neuro-endocrine cells cannot be assessed by the present histological study. Somatostatin and GIP have both been shown to be potent inhibitors of gastric acid secretion (Pederson and brown, 1972; Bloom et al., 1974; Barros et al., 1975). The inhibition of gastric acid secretion by somatostatin

is partly mediated via inhibition of gastrin release (Bloom et al., 1974). It is interesting to speculate that the release of peptides by areas of intestinal metaplasia may modify the acid environment of the stomach.

No appreciable difference has been shown in the number of chromogranin positive cells per unit length in the various sub-types of intestinal metaplasia. No difference was apparent in the specific peptides contained in the neuro-endocrine cells in the various sub-types. These findings are in concordance with a previous study which examined sulphated versus non-sulphated intestinal crypts (Mingazinni et al., 1984). One previous study has identified quantitative and qualitative differences in the neuro-endocrine cell population in different sub-types of intestinal metaplasia (Tsutsumi et al., 1983). However in this study a combination of silver methods and immunohistochemistry was used and the counting technique was ill defined. The results of the present study suggest that despite the differences in epithelial cells and mucin histochemistry which exist between the various sub-types the neuro-endocrine cell population is similar in number and nature of peptides contained within the cells.

CHAPTER 6

CELL KINETIC STUDIES

INTRODUCTION

General Considerations

The technique of in vitro thymidine labelling is ideally suited for the analysis of cell kinetics in endoscopic gastric biopsy material. The technique of counting cross sections of gastric crypts as discussed in Chapter 1 described by Hansen et al., 1975, allows the Labelling Index to be determined. The Labelling Index is a measure of the proliferative rate of a given tissue. Although the Labelling Index is not as sophisticated as stathmokinetic analysis, it is more suitable within the ethical constraints imposed when dealing with human material. Another advantage of the thymidine technique is that the biopsy material can be re-orientated and important information determined regarding the site of the proliferative zone within gastric and intestinal metaplastic crypts. As the counting of thymidine labelled cells was performed by one observer a statistical analysis was performed to determine the reproducibility of the counting technique.

Area of Study

The effect of alkaline reflux gastritis on gastric cell kinetics in a group of patients who had undergone gastric surgery for benign disease is the main area of study in this chapter. The importance of such patients has been discussed in Chapter 1. There is considerable but disputed evidence that such patients represent an iatrogenic risk group for the development of gastric carcinoma. In addition the histological features of alkaline reflux gastritis have only recently been reported (Dixon et al.,

1986).

The main difficulty in interpreting the results of the cell kinetic studies in the post-surgical patients is the identification of a suitable control group with which to compare and controlling other variables which might affect the labelling index. For this reason preliminary studies were required on biopsy material from patients medically treated for peptic ulcer disease to assess the effects of gastritis, Helicobacter pylori and type of epithelium on the Labelling Index and the site of the proliferative zone.

In addition to the endoscopic biopsy material tissue was also obtained from a proportion of the gastrectomy specimens discussed in Chapter 3. Tissue was taken from tumour, the gastric mucosa immediately adjacent to the tumour or ulcer and from a site distant from any mucosal lesion in the specimen.

The study of the material from the gastrectomy specimens was designed to address several questions. Firstly the labelling indices and the site of the proliferative zone in the intestinal metaplasia sub-types was examined. Although tritiated thymidine studies have been reported on intestinal metaplasia as discussed in the literature review, these have not taken into account the recent description of the variants of intestinal metaplasia.

The study of gastric mucosa adjacent to tumour or ulcer and at a distance from the lesion is the second aspect of the gastrectomy material examined. The intention behind this was to determine if abnormalities of cell kinetics in the gastric mucosa existed in stomachs resected for malignant disease compared with

benign disease.

The third aspect of the gastrectomy material studies is the thymidine labelling of the tumours themselves. The distribution of labelled cells within tumours is of biological interest as it indicates the areas of proliferation. The Labelling Index of tumours particularly comparing intestinal and diffuse tumours warrants study because of the differences between these tumour sub-types discussed in Chapter 1. The effect of the degree of differentiation of the tumours on the cell kinetics is the final aspect studied.

AIMS

1. To determine if the counting of thymidine labelled cells was reproducible by one observer.
2. To determine the Labelling Index in antral and body mucosa in a series of endoscopic biopsy specimens in patients undergoing medical treatment for peptic ulcer disease.
3. To analyse the effect of gastritis, Helicobacter pylori and diagnosis on the Labelling Index.
4. To determine the site of the proliferative zone within antral crypts in patients treated medically for peptic ulcer disease and the effect of gastritis on this parameter.
5. To determine the Labelling Index and site of proliferative zone in endoscopic biopsy material in a series of patients surgically treated for peptic ulcer disease.
6. To compare the Labelling Index and site of proliferative zone between a matched group of medically and surgically treated

patients with peptic ulcer disease and assess the relationship to the the Reflux Score.

7. To establish the Labelling Index and site of proliferative zone in Type I, IIa and IIb intestinal metaplasia.

8. To establish and compare the Labelling Index of the gastric mucosa adjacent to and distant from intestinal and diffuse type tumours and benign lesions.

9. To assess the distribution of labelled cells within gastric tumours and compare the Labelling Index of intestinal and diffuse type and the effect of the degree of differentiation of the tumours on the Labelling Index.

RESULTS

VALIDATION OF COUNTING TECHNIQUES

The statistical analysis on the observer error in the counting techniques demonstrated that the hypothesis that there was no difference between counts from different areas of mucosa or tumour or when the counts were repeated on the same area could not be rejected. The deviances and degrees of freedom associated with the model and each hypothesis are shown in figure 26.

The statistical analysis confirms that the counting techniques used to generate the Labelling Index are reproducible if the count is performed on the same area or another area within the same sample. This finding allows for statistical comparisons to be made between cases.

MEDICAL TREATMENT GROUP

Labelling Index

The labelling index in the antral mucosa was significantly higher ($p < 0.01$) than the body mucosa (see figure 27). There was no significant difference in the LI in both types of mucosa in the three main diagnostic groups of gastric ulcer, duodenal ulcer and normal (see table 38).

Relationship between Gastritis Score and Labelling Index

The Labelling Index showed a stepwise progression with the increase in the gastritis score in the antral mucosa. There were insufficient numbers of cases with high gastritis scores in the body mucosa to make a valid comparison (see figure 28).

Relationship between Labelling Index and Helicobacter pylori

The organism, Helicobacter pylori was detected in the antral

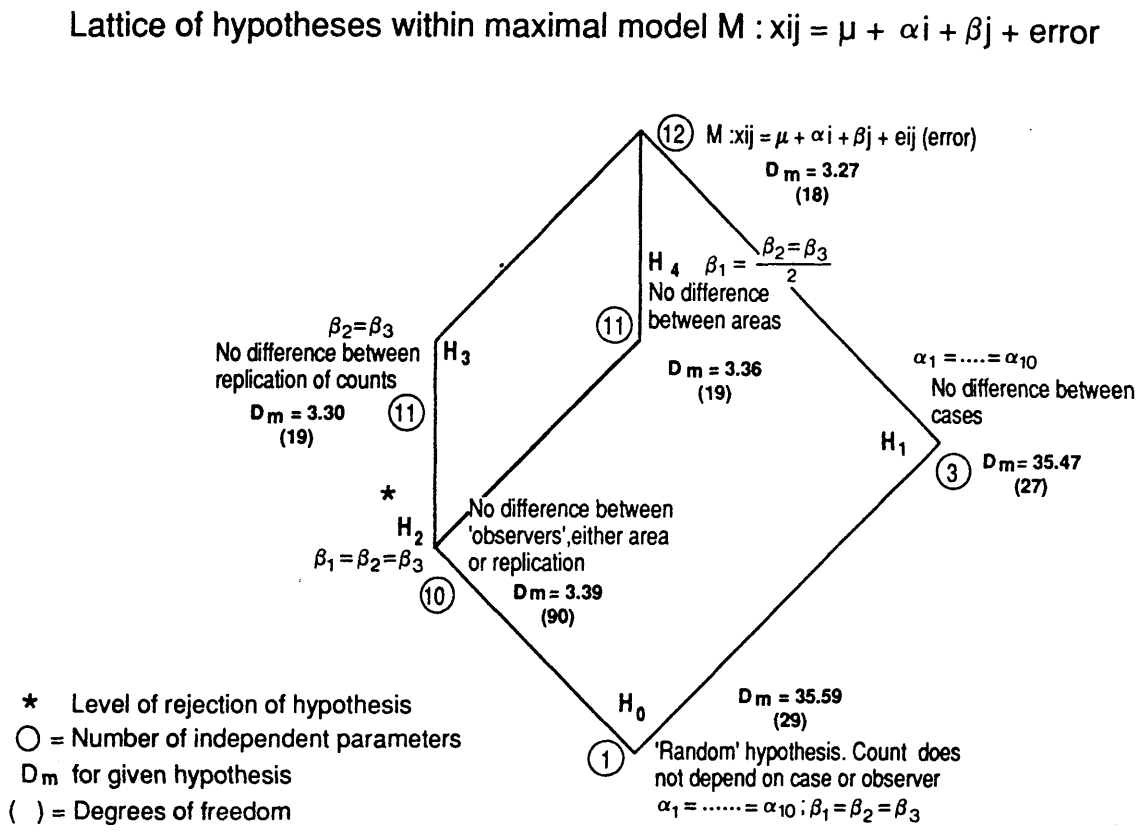


Figure 26. Lattice of hypothesis for observer error of thymidine Labelling Index from different areas of mucosa or when counts were repeated on the same area. The hypothesis (H_2) that there was no difference between observers when the same area was counted twice or different areas within the same specimen could not be rejected (D_M = 3.39, DF = 90) indicating that counts could be replicated in both these circumstances.

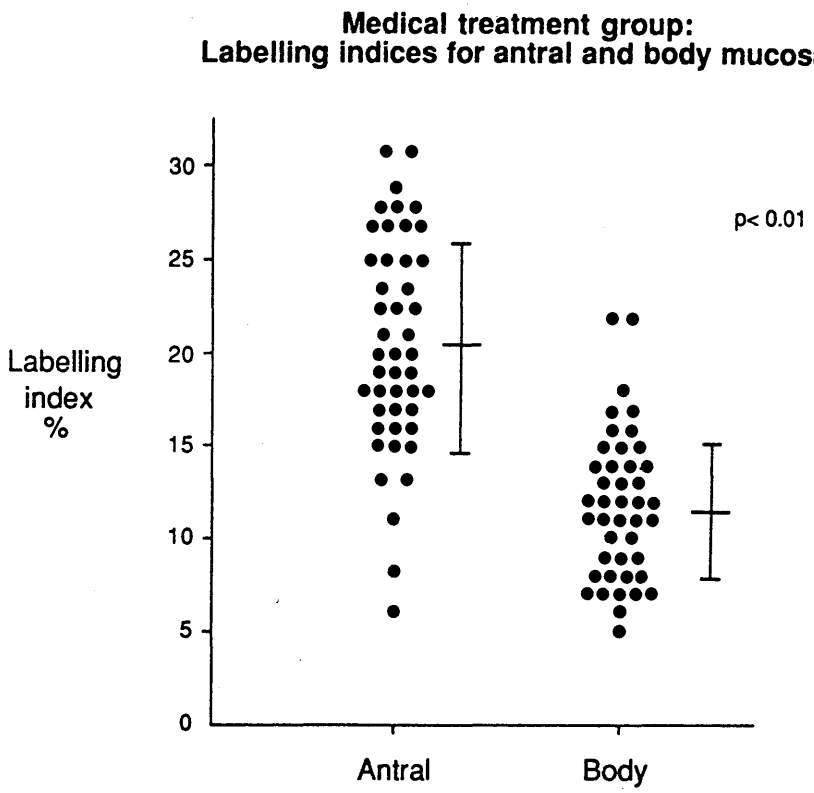


Figure 27. Labelling Indices of antral and body mucosal biopsies from patients in Medical Treatment Group. Labelling Indices of antral mucosa are significantly higher ($p < 0.01$) than body mucosa. Bar indicates mean $\pm$ 1 Standard Deviation.

		Non Ulcer dyspepsia	Duodenal Ulcer	Gastric Ulcer
Body mucosa	(mean)	10.7	12.1	12.7
	(range)	(5-22)	(6-19)	(5-18)
Antral mucosa	(mean)	17.3	21.3	21
	(range)	(8-28)	(13-31)	(17-27)

(No significant difference between groups)

Table 38. Mean value and range of Labelling Indices in the Medical Treatment Group for antral and body mucosa. Medical Treatment Group divided by diagnosis into Non-Ulcer Dyspepsia, Duodenal Ulcer and Gastric Ulcer.

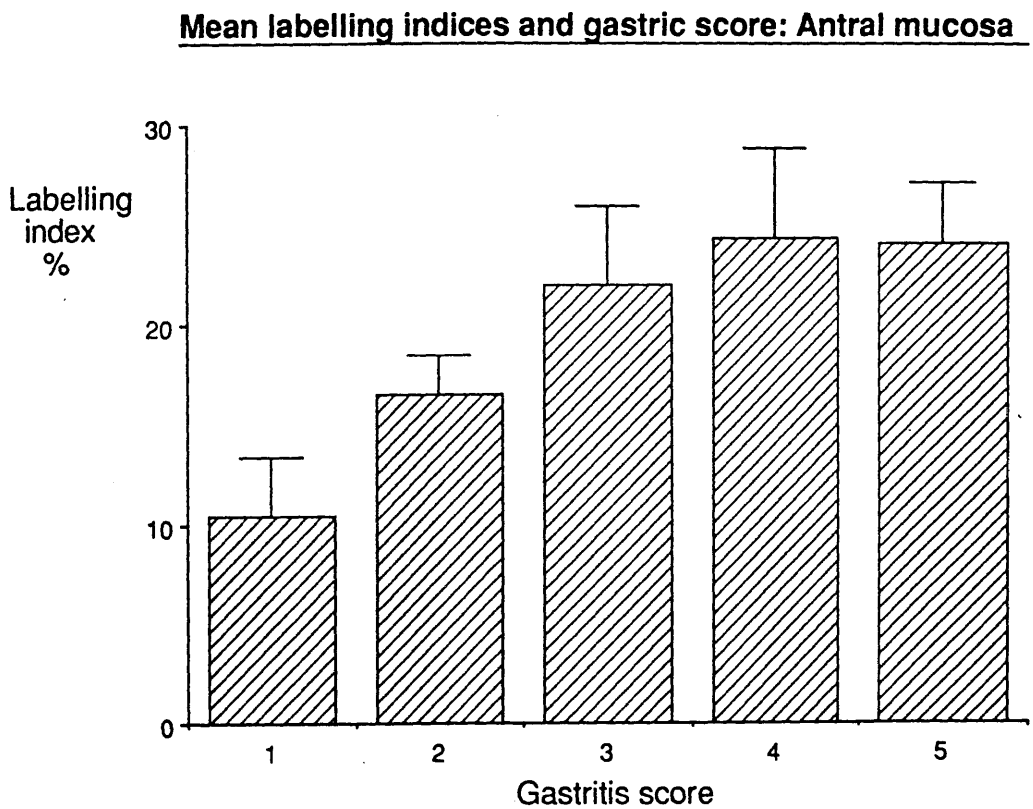


Figure 28. Mean Labelling Indices of antral mucosal biopsies from Medical Treatment Group at different gastritis scores. Bar indicates one Standard Deviation.

biopsies of 27 patients (56%). The presence of the organism was associated with a significantly higher gastritis score and consequently a higher labelling index ($p < 0.05$) but only in antral mucosa (see table 39).

Crypt column length and position of proliferative zone

The crypt column lengths obtained by counting the number of cells from luminal surface to base of the crypts decreased slightly with increasing grades of gastritis (see table 40). The position of the proliferative zone relative to the luminal surface and base did not change. The position of the labelled cells within the crypt at different grades of gastritis is shown in figure 29.

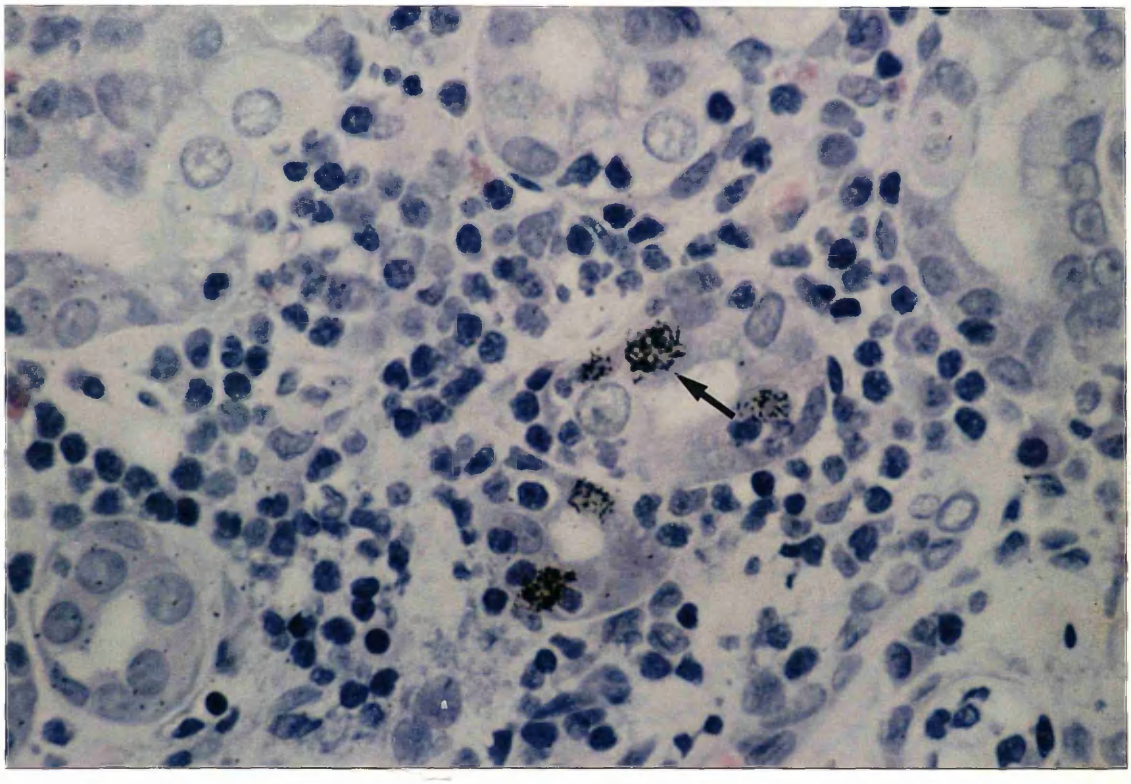
SURGICAL TREATMENT GROUP

Labelling Indices

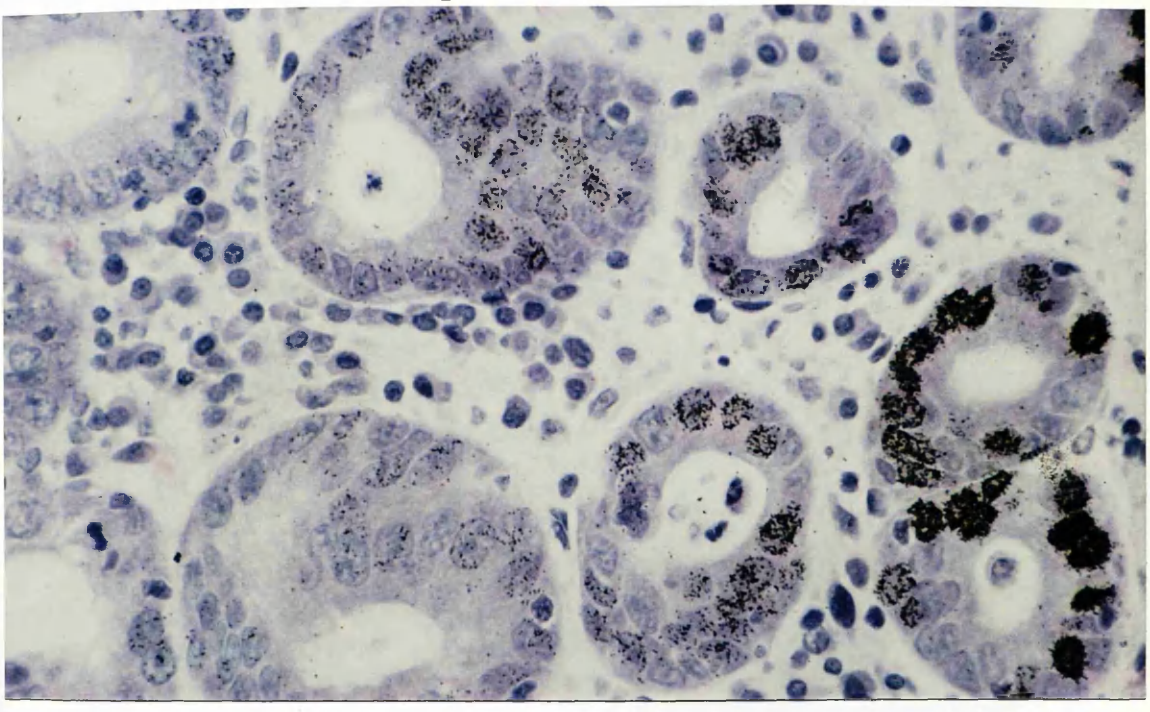
The labelling index from the three sites, 1, 3 and 5 cm from the stoma showed no significant difference (table 41). None of the biopsies from the Surgical Treatment Group showed any evidence of Helicobacter pylori.

COMPARISON BETWEEN SURGICAL AND MEDICAL TREATMENT GROUP

The labelling indices were consistently high in the Surgical Treatment Group. Antral biopsies with the same degree of gastritis and no evidence of Helicobacter pylori were selected from fifteen patients in the Medical Treatment Group who were age matched with the Surgical Treatment Group. The Labelling Index reflux scores and crypt column length of the two groups were compared. The Labelling Index was significantly higher in the post-surgical group compared with the Medical Treatment Group ($p < 0.001$) (see table 42).



Photomicrograph 13. Inflamed gastric mucosal biopsy from body. Arrow indicates thymidine labelling on epithelial cells. Note absence of labelling on parietal cells. Autoradiograph/ haematoxylin and eosin counterstain x 450.



Photomicrograph 14. Biopsy of antral mucosa from patient with alkaline reflux gastritis. Note the large numbers of labelled cells in the cross sections of the crypts. Autoradiograph/ haematoxylin and eosin counterstain x 450.

	H. pylori present	H. pylori absent
Mean gastritis score	4.1*	2
Mean labelling index	23.3*	16.4

* $p < 0.05$

Table 39. Relationship between the presence or absence of Helicobacter pylori in the antral biopsies of the Medically Treated Group and mean gastritis score and mean Labelling Index.

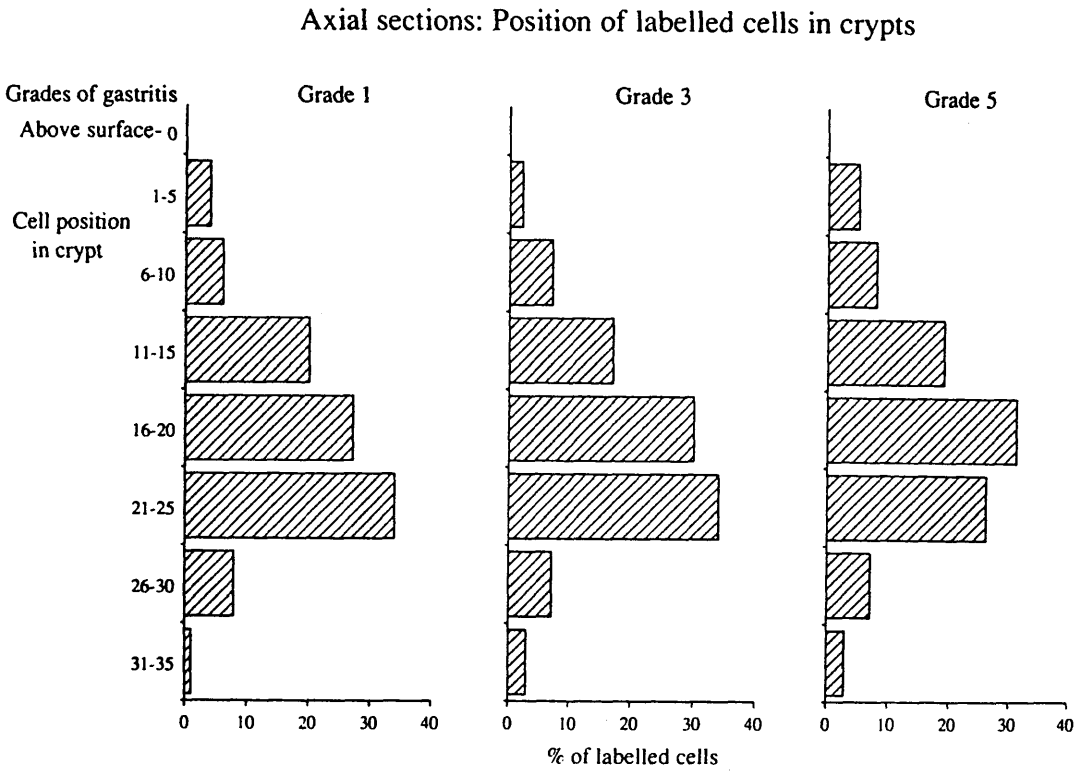


Figure 29. Position of labelled cells in axially sectioned antral crypts from Medical Treatment Group at three grades of gastritis 1, 3 and 5. One hundred crypts analysed for each level of gastritis. Bar represents percentage of total number of labelled cells present with entire crypt at each defined cell position.

Column Length. Medical Treatment Group

Mean Column Length (range)	Grade of Gastritis
38.2 (33-43)	1
36.9 (28-40)	3
34.8 (21-40)	5

Table 40. **Mean value and range in crypt column length in the antral crypts of the Medical Treatment Group at three grades of gastritis 1, 3 and 5.**

Distance (cm)	1	3	5
Mean Labelling Index	32	30.9	31.4

p NS

Table 41. Mean labelling indices of biopsies taken at 1, 3 and 5 cm from stoma in the Surgical Treatment Group.

		Medical Treatment Group n = 15	Surgical Treatment Group n = 16
Age	(mean)	54.1	51.2
	(range)	(26-87)	(32-74)
Gastritis score	(mean)	3	3
	(range)	(1-5)	(1-5)
Labelling index	(mean)	20.6	31.4 *
	(range)	(12-28)	(23-37)
Reflux score	(mean)	7.4	11.2 +
	(range)	(6-11)	(8-15)

* $p < 0.001$ + $p < 0.01$

Table 42. **Mean value and range of age, gastritis score, labelling index and reflux score of age matched patients in Medical Treatment Group compared with Surgical Treatment Group.**

The reflux scores of the age matched Surgical and Medical Treatment groups showed a significant difference $p < 0.01$ (table 42). The reflux score was positively correlated with the labelling index (see figure 30) ($R = 0.618$ $p < 0.001$).

Histological examination of the axially sectioned crypts revealed a striking difference between the surgical treatment and medical treatment group. In conjunction with the foveolar hyperplasia characteristic of reflux gastritis there was an increase in the length of the proliferative zone and its position within the crypt. The column length in cells increased significantly compared with the control group ($p < 0.001$). The proliferative zone extended from the neck of the crypts on to the surface resulting in the distinct feature of cells on the luminal surface displaying labelling with thymidine (table 43, figure 31).

INTESTINAL METAPLASIA

Gastric mucosa containing metaplastic crypts were studied from both the endoscopic biopsy and the gastrectomy material. In the endoscopic biopsy group 18 patients (37.5%) had some degree of intestinal metaplasia present in the endoscopic biopsy material. Eighty-three per cent of the intestinal metaplasia was type I, 17% type IIa. No foci of type IIb were identified. The small amount of tissue contained in the biopsy material made analysis of the labelling index of the metaplastic crypts technically difficult.

The larger amount of material from the gastrectomy group allowed for all three types of metaplasia to be identified in the

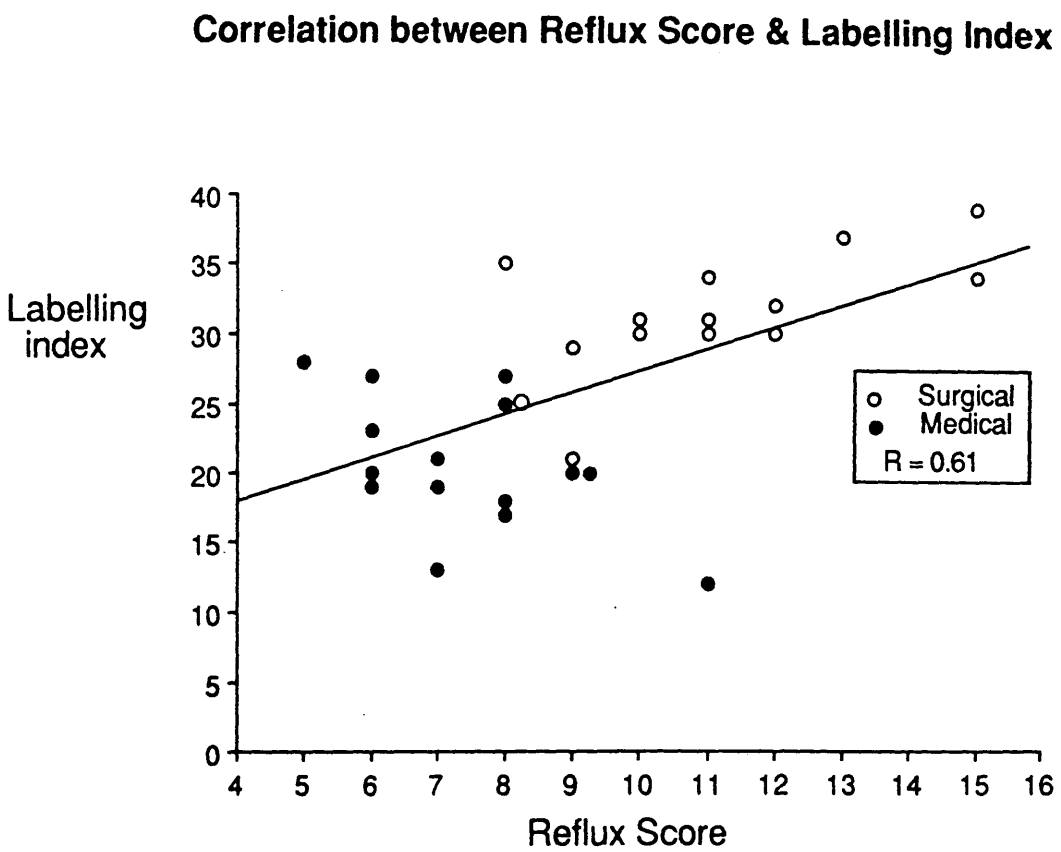


Figure 30. Correlation between reflux score and Labelling Index for Medical and Surgical Treatment Groups.

	Medical treatment Group	Surgical Treatment Group
Crypt column length (mean)	36.7	44.06 *
(cells) (range)	(25-45)	(38-51)
Number of crypts examined	33	25

* $p < 0.001$

Table 43. **Comparison between the Crypt Column Length in antral mucosa between the Medical Treatment and Surgical Treatment Groups.**

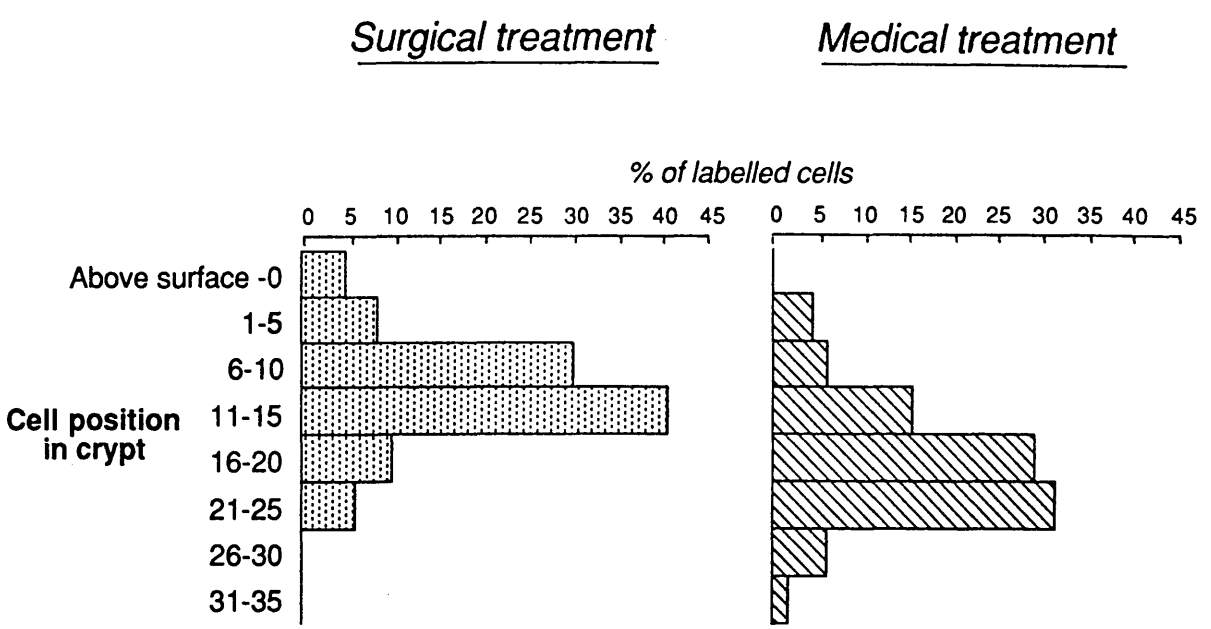


Figure 31. Percentage distribution of labelled cells at defined cell positions within antral crypts: comparison between Medical Treatment and Surgical Treatment Groups.

samples from B and C areas. The focal nature and low proportion of type IIb metaplasia resulted in a low yield of type IIb crypts available for complete examination.

The labelling index for all three types of crypts showed a wide range; no statistically significant difference was demonstrated between the three groups (figure 32). The labelled cell zone was located at the base of the crypts in the type I and type IIb crypts. In the type IIa crypts the labelled zone occupied an intermediate position at the neck of the crypts, similar to the position seen in the gastric crypts.

GASTRECTOMY MATERIAL

Thirty-seven gastrectomy specimens were examined. In four specimens due to technical error the specimens were unavailable for analysis. Thirty-three specimens, 26 for malignant and 7 for benign disease were available for study.

Mucosa adjacent to tumour/ulcer area B

Histological examination showed a wide range of features from mild superficial gastritis to severe gastritis and ulceration with almost complete loss of gastric crypts, the luminal surface being lined by a layer of regenerative epithelium. In four cases there were no discernible gastric crypts on which to perform counting.

The tumours were classified into diffuse and intestinal type and the degree of gastritis scored from 1-5 according to the scoring system previous described. The results are shown in tabulated form (table 44).

A matched comparison between mucosa of the same type (ie

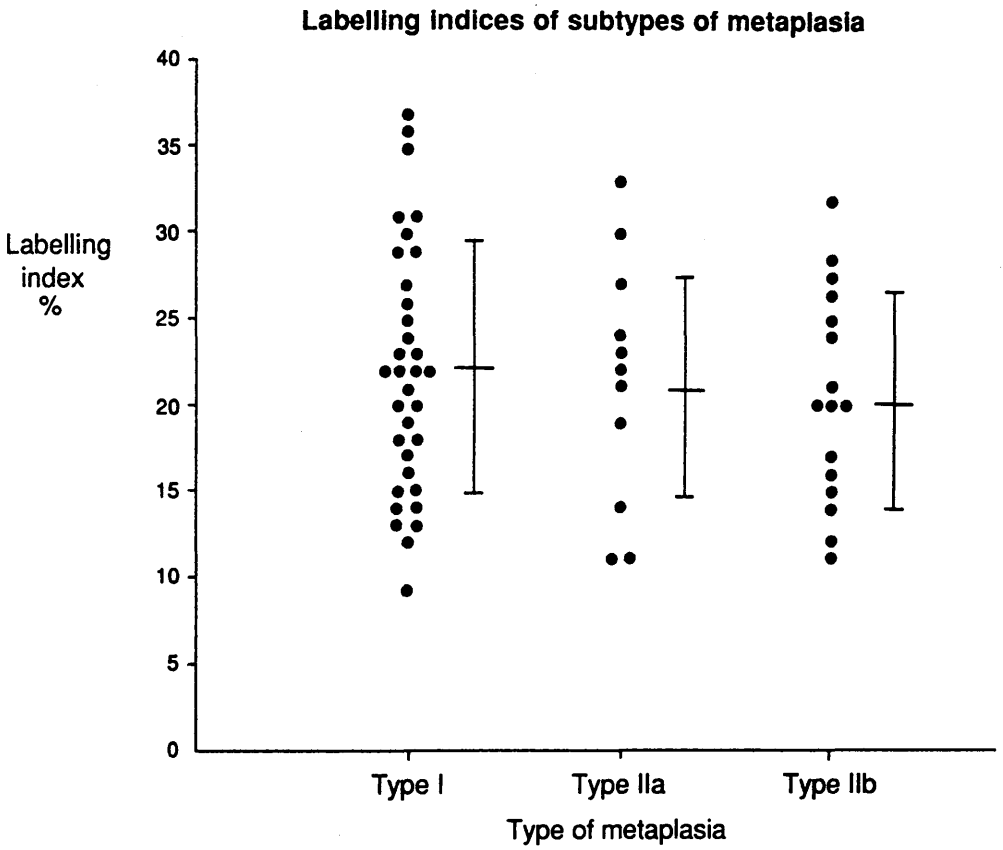
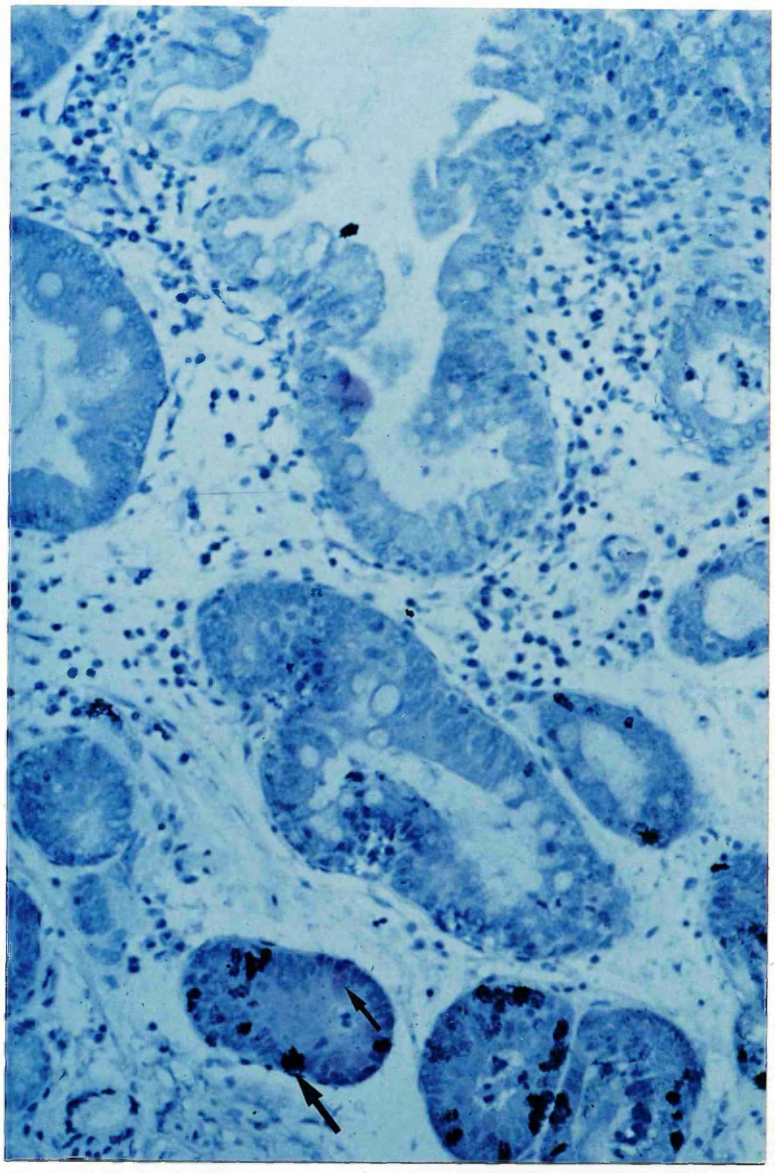


Figure 32. Labelling Indices of intestinal metaplasia sub-types I, IIa and IIb.



Photomicrograph 15. Axial section of Type I intestinal metaplasia crypt. Note paneth cells at base of crypt (small arrow) and thymidine labelled cells at base of crypt (large arrow). Autoradiography/haematoxylin and eosin counterstain x 80.

Nature of Gastrectomy		Area B - Adjacent to tumour		Area C - Distant	
	No of cases	Gastritis Score (mean)	Labelling Index (mean)	Gastritis Score (mean)	Labelling Index (mean)
Benign	7	4.7	24.8	3.9	18
Intestinal	13	4.8	24.3	3.2	18.3
Diffuse	9	5	22.1	3.5	17.4

Table 44. Gastritis score and Labelling Index of gastric mucosa adjacent to (area B) and distant from (area C) tumour or ulcer in the three groups benign, intestinal and diffuse type tumours.

antral or body) and grade of gastritis was not possible because of the small numbers in the study. There was, however, no significant difference between the Labelling Index of the gastric mucosa adjacent to intestinal type tumour, diffuse tumour and benign ulcers using the unmatched data. The regenerative epithelium in both benign and malignant cases showed labelling on the luminal surface.

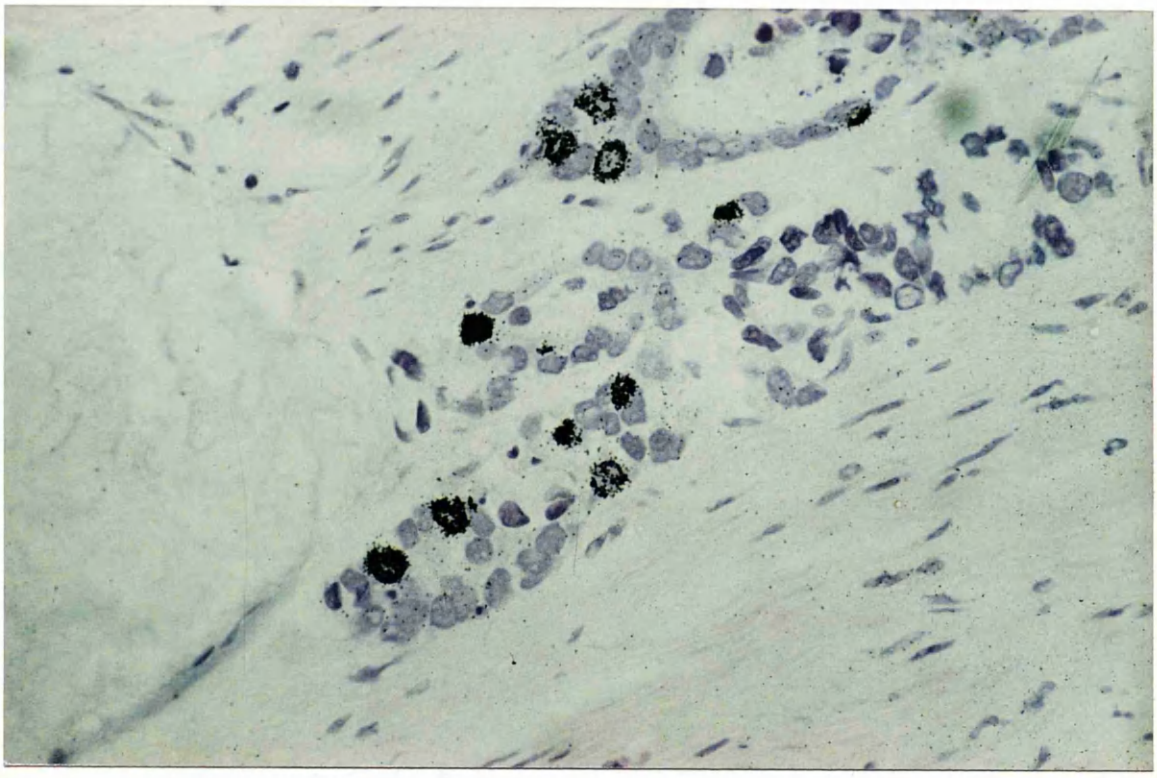
Mucosa at a distance from tumour area C

There was no significant difference between the labelling index of the mucosa at a distance from tumour/ulcer when malignant stomachs were compared with benign, nor was there any difference between the two tumour types. The gastritis scores in the distant mucosa were lower than in the mucosa immediately adjacent to the tumour (table 44).

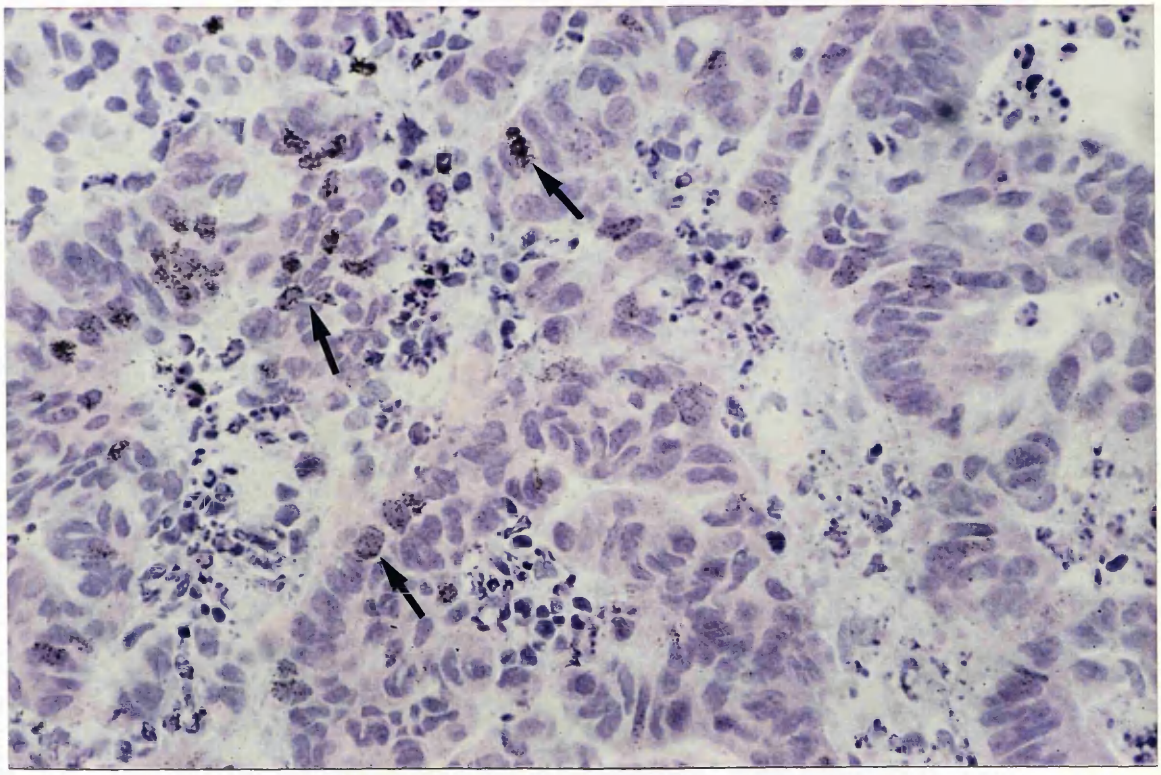
Tumour Material

Histological appearances

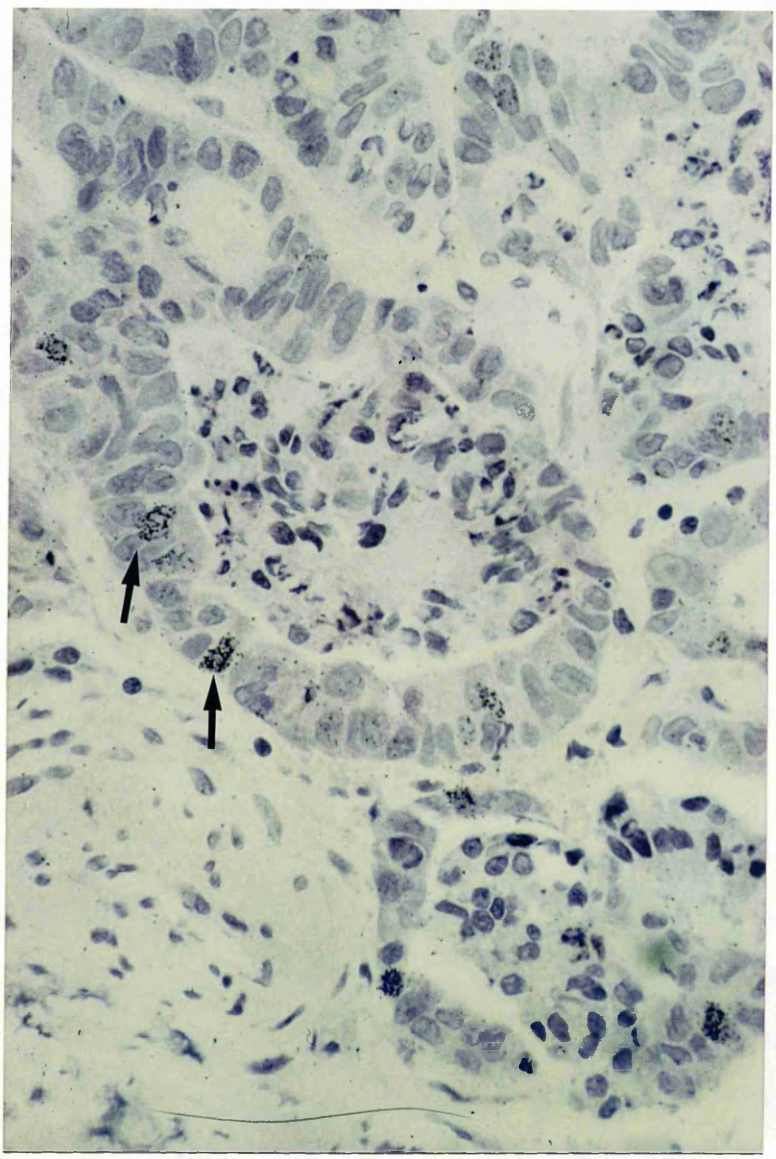
The distribution and frequency of labelled cells varied greatly between tumours and within different areas of the tumour. Acini, either well developed in intestinal type tumours or the rudimentary glandular structure in diffuse tumours showed labelling in cells forming the acini. The number of labelled cells in the acini was greatest if these structures were adjacent to or in some cases within blood vessels or lymphatic channels. The acini which formed the periphery of the tumour did not appear to have more labelled cells than similar structures within the centre of the tumour. Another feature which was associated with a high number of



Photomicrograph 16. Infiltrating gastric tumour cells labelled with thymidine adjacent to vein. Autoradiograph/haematoxylin eosin counterstain x 350.



Photomicrograph 17. Partially necrotic gastric tumour with cells labelled with thymidine (arrows). Autoradiograph/haematoxylin and eosin counterstain x 350.



Photomicrograph 18. Moderately well differentiated gastric carcinoma with thymidine labelled cells in tumour acini (arrows). Autoradiograph/ haematoxylin and eosin counterstain x 400.

labelled cells was the presence of polymorphonuclear leucocytes within the acini.

Sheets of tumour cells which were characteristic of diffuse type carcinoma showed no particular pattern on initial examination. However closer scrutiny demonstrated that the classic signet ring cells did not appear to label with thymidine. The cells which labelled in this type of tumour were those with large nuclei and absence of intracellular mucin. Tumours which provoked a desmoplastic host response (predominantly diffuse type) resulted in the host stromal cells labelling with thymidine.

Labelling Index

The Labelling Index of the tumours varied considerably with a tenfold difference between the lowest and highest values.

There was no significant difference between the LI of the intestinal and diffuse tumours classified according to the Lauren system. There was no significant difference in LI between well and poorly differentiated tumours (see figures 33 and 34).

DISCUSSION

Medical Treatment Group

These results confirm that antral and body mucosa have differing cell kinetics as has been demonstrated by other workers (Hansen et al., 1975; Hansen et al., 1977). In view of the histological differences between these two types of gastric mucosa this finding is not entirely unexpected. The importance of this finding is to ensure that a histological assessment of the types of mucosa examined is taken into consideration in any study comparing

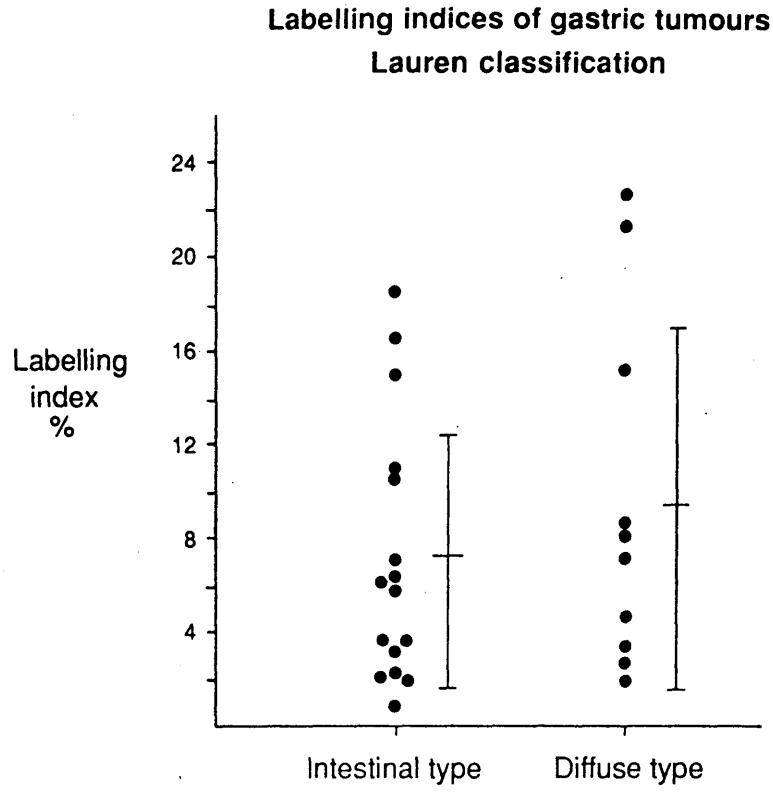


Figure 33. **Labelling Indices of intestinal and diffuse type tumours (Lauren Classification).**

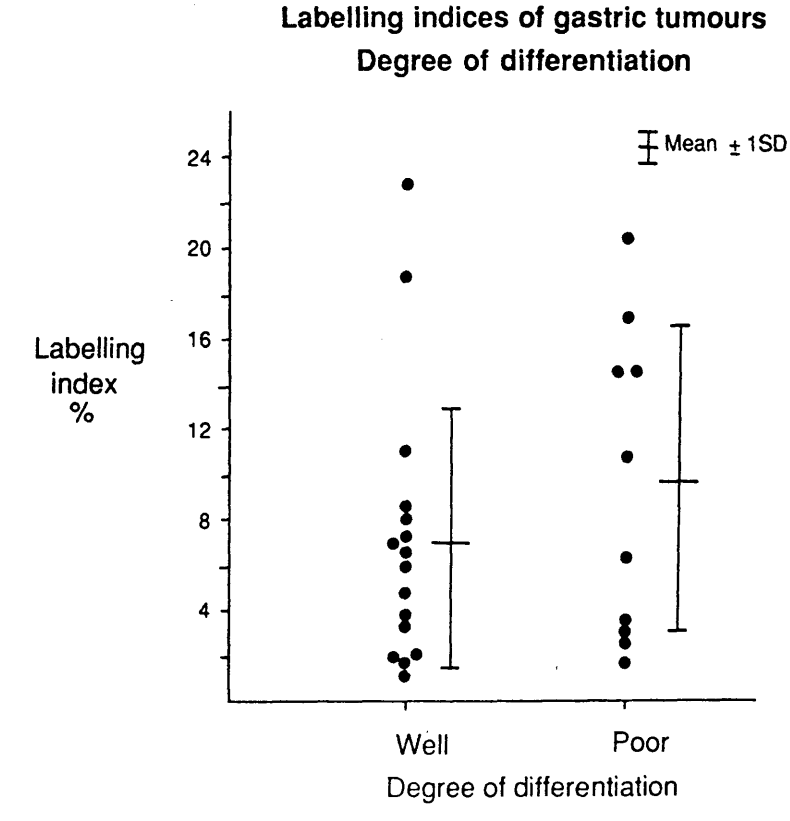


Figure 34. **Labelling Indices of gastric tumours divided into well and poorly differentiated groups.**

groups of patients. In the Surgical Treatment Group the two patients who had undergone a Billroth II resection by definition did not have antral mucosa and the biopsies were of body type epithelium. The biopsies were included in the comparison with the Medical Treatment Group in order to achieve sufficient numbers for statistical analysis. As body type has a lower labelling index than antral this factor would have tended to reduce the mean labelling index of the Surgical Treatment Group.

The positive correlation between the gastritis score and labelling index has also been noted by earlier workers (Hansen et al., 1977). The aetiology of the inflammatory change has been ill defined until the recent identification of campylobacter-like organisms in gastric biopsy specimens (Marshall and Warren, 1984). The presence of the organism has been strongly associated in some studies with both gastritis and peptic ulceration (Marshall and Warren, 1984; Jones et al., 1984). Although the organism does appear to be strongly associated with gastritis the relationship with peptic ulceration has been disputed by some authors (Rollason et al., 1984; Forrest et al., 1984).

Fifty six per cent of the biopsies contained Helicobacter pylori which is similar to the findings in other endoscopic surveys (Marshall and Warren, 1984; Rollason et al., 1984; Forrest et al., 1984). The significantly higher LI in patients with the organism is a reflection of the similar elevation in gastritis score in this group. No other reports have appeared in the literature regarding the relationship of Helicobacter pylori to

cell kinetics.

The decrease in Crypt Column Length that accompanies increasing severity of gastritis is simply a numerical expression of the gastric atrophy that results from gastritis. Despite severe inflammation, however, the relative position of the proliferative zone does not alter. This is in marked contrast to the situation in the post-surgical stomach.

Surgical Treatment Group

The Labelling Index of the mucosal biopsies from this group were notably higher than the Medical Treatment Group. The LI of the biopsies from all distances from the stoma were elevated suggesting that this feature was widespread throughout the stomach. The absence of Helicobacter pylori in all the biopsy material from the post-surgical patients has been noted in similar groups of patients in another study (O'Connor et al., 1986). The absence of the organism has been ascribed to the post-surgical alteration in the gastric milieu due to altered pH and bile acid content.

Comparison between Medical and Surgical Treatment Groups

Age matched patients medically treated for peptic ulcer disease and dyspepsia represent the best available control group for comparison with the post-surgical group. Both groups of patients share the same original disease process in peptic ulceration.

The results of the Medical Treatment Group study demonstrates a significant difference in LI between antral and body mucosa, differing grades of gastritis and the presence of Helicobacter

pylori. For this reason the comparison between Medical and Surgical Treatment Groups used cases which were matched for these variables. The finding of a highly significant elevation in LI in the post-surgical patients with matched medically treated patients is of considerable interest as it suggests that reflux in some manner increases the LI. Unfortunately no assessment was made of the degree of enterogastric reflux or of bile and alkali concentrations within the surgically treated group. All of these patients had, however, endoscopic evidence of bile reflux and were subsequently treated for this condition.

Although the Medical and Surgical Group were matched for gastritis score the surgical patients had a significantly higher reflux score. This apparent discrepancy can be explained by examining the scoring systems. The gastritis score is based primarily on the quantity and content of the inflammatory infiltrate; the reflux score is dependent on more subtle epithelial changes ie. foveolar hyperplasia. The results of this study suggest that the abnormal histological features identified by Dixon et al., 1986, in reflux gastritis are associated with a significant elevation in the Labelling Index. Not only is there a numerical increase in the LI but the proliferative zone shifts position to involve the luminal surface in the post-surgical group.

The relationship of this abnormal kinetic picture in the post-operative stomach to carcinogenesis remains speculative. Assuming that the S phase is constant the LI is proportional to the rate of cell proliferation then the elevated LI in the post-

surgical group is indicative of a higher rate of cell turnover. This increase in cell proliferation coupled with an expansion in the proliferative zone has been noted in pre-neoplastic lesions in the colon (Bleiberg et al., 1970). The shift of the proliferative zone on to the luminal surface appears to be a feature unique to the post-surgically treated stomach as it was not seen even in mucosa in medically treated patients.

Intestinal Metaplasia

The presence of intestinal metaplasia in over one third of cases from the endoscopy group is comparable to the range of 20-37% found in larger series in benign stomachs (Rothery and Day, 1985; Filipe et al., 1985).

Thymidine autoradiographic studies of intestinal metaplasia have been reported by several authors (Willems and Lehy, 1975; Hattori and Fujita, 1979) but with conflicting results. The sub-types of intestinal metaplasia as described by Jass (1980) have not been studied. Winawer and Lipkin (1969) described incorporation of thymidine into cells at or near the luminal surface in both gastric and intestinal metaplastic glands in two patients with gastric carcinoma. Hattori and Fujita (1979), failed to confirm this finding and demonstrated only a shift of the generative zone from the neck of the gastric crypts to the base of the intestinal crypts.

The findings of this study partially confirm those of Hattori and Fujita (1979), as the labelled zone was located at the base of the crypts in type I and IIb sub-types. A possible explanation

for the discrepancy between the two previous authors might have been that they were examining different sub-types of metaplasia. The nature of the material studied by Hattori and Fujita (1979), and Winawer and Lipkin (1969) was different: Lipkin (1969), examined biopsies taken from the stomach immediately adjacent to an adenocarcinoma and from a patient who had undergone surgery for gastric carcinoma and also had pernicious anaemia. Hattori and Fujita (1979) examined mucosal biopsies taken from non-diseased portions of the mucosa away from any tumour. Given our subsequent knowledge on the distribution of intestinal metaplasia it would be reasonable to suppose that some type IIb metaplasia was included in Winawer and Lipkin's (1969) study, however, as no mucin histochemistry was performed it is impossible to comment definitively on this fact. Winawer and Lipkin's (1969) second patient had several factors which may have led to abnormal gastric mucosal kinetics; previous surgery leading to bile reflux, pernicious anaemia. Hattori and Fujita (1979) study by taking biopsies away from the carcinoma is likely to have missed any foci of type IIb by this sampling technique.

The present study involves a larger number of patients and examination of different sub-types of intestinal metaplasia identified by mucin histochemistry.

Gastrectomy Material

Mucosa adjacent to tumour and mucosa at a distance (<5 cm)

The mucosa immediately adjacent to tumour is of considerable interest as it is closest to the region from which the tumour has

arisen. Cell kinetic changes similar to those found in tumour development might be seen in this area as dysplastic features are commonly found in the mucosa adjacent to tumours. The thymidine Labelling Index is difficult to interpret in the mucosa immediately adjacent to tumours as abnormalities may arise due to the secondary effects of the tumour rather than in association with the evolution of the tumour. The secondary effects of the tumour most apparent are ulceration, necrosis and inflammation and for this reason gastric ulcers have been included as controls.

The high labelling index adjacent to tumours was also demonstrated adjacent to benign ulcers in the present study. This elevation in the labelling index may be explained by the severe gastritis associated with the presence of a tumour rather than a specific relationship to malignant process.

Tumour material

The interpretation of the thymidine labelling index in carcinomas is more complex than in gastric crypts. A relatively constant S phase duration has been demonstrated in gastric epithelium thus the labelling index is indicative of a cell growth fraction; no such relationship has been demonstrated for the S phase in malignant gastric epithelium. In vivo stathmokinetic studies have been performed but ethical and considerable technical problems severely limit the usefulness of this technique (Wright et al., 1977).

The high counting and sampling error pointed out by other authors (Buyse and Bleiberg, 1987) prompted statistical evaluation

of this problem, which indicated that statistical analysis was valid.

The great variability of the LI between different carcinomas has been highlighted by other authors (Smallwood et al., 1983). There is no clear explanation for this phenomenon. As the S phase duration is not known and its constancy unproven in gastric carcinoma, the biological relevance of an LI in isolation is doubtful. No attempt has been made in this study to compare the LI of the gastric carcinomas with gastric or metaplastic mucosae. The LI of the malignant material is an expression of the number of labelled cells in the total population whereas the LI in mucosa is a measurement of the number of labelled cells in the gastric crypts. These are not the same measurement. Previous studies have tried to compare the two but this is not tenable. The present study has not demonstrated any statistically significant differences between the LI of intestinal type and diffuse type tumours or between well and poorly differentiated tumours. Assuming that the S phase duration is constant this can be interpreted as showing that there is no biological difference between intestinal and diffuse tumours or well and poorly differentiated tumours in terms of cell kinetics. However as the S phase duration has not been estimated in the present study tumours with a similar LI may have widely differing S phase duration and cell cycle times. For this reason firm conclusions regarding cell kinetic parameters cannot be drawn from the results of this study.

The peripheral edge of a tumour is often assumed to be the site of growth and invasion. The labelled cells appear to be distributed throughout the tumour and the mode of growth of the tumour would appear to be by cell division amongst cells in all areas of the tumour. This distribution of labelled cells occurred in both intestinal and diffuse types of tumour. Lauren's (1965) description of differing modes of growth (infiltrative for diffuse type, expanding for intestinal type) for his two main types of tumour do not appear to be reflected in the distribution of thymidine labelled cells.

Lymphatic and blood vessel invasion by tumour cells has been shown to be associated with a poor prognosis (Monafo et al., 1962). The high number of labelled cells in tumour acini, in or around these vessels, noted in this study may have at least two possible implications. Firstly that such cells have an inherently high proliferative activity and so tend to be more invasive. Secondly, that some blood or lymphatic factor induces proliferation in these sites.

CHAPTER 7

ANIMAL EXPERIMENTAL MODEL

INTRODUCTION

General Outline

The literature review has discussed the various gastric tumour models that exist in the rat. The experiment described in this chapter has utilised MNNG as the carcinogenic agent in a low dose as this has been shown to induce both intestinal metaplasia and gastric carcinoma in the rat (Tatematsu et al., 1983). The metaphase arrest (or stathmokinetic) technique previously described allows for more dynamic measurements of the cell cycle than tritiated thymidine labelling. In the bowel the number of cells produced by the crypts can be estimated and expressed as Crypt Cell Production Rate per hour.

AIMS

The aim of the animal experiment was to use an established model for the induction of intestinal metaplasia and gastric neoplasia to study the cell kinetic changes that occur in these states. The metaphase arrest technique was used to establish the Crypt Cell Production Rate and tritiated thymidine to determine alterations in the Labelling Index and site of the proliferative zone.

RESULTS

Histological Abnormalities

The histological abnormalities in the carcinogen exposed and control animals at each sacrifice interval are shown in table 45. No foci of intestinal metaplasia and no tumours were identified at any stage in either group.

Metaphase Arrest Data

The metaphase arrest data is displayed graphically in figure 35 in the form of metaphases accumulated over the four hour study period post vincristine injection. The values shown are the corrected values using the Tannock Factor of 0.53 established in the Material and Methods section in Chapter 2. The rates of entry into mitosis calculated from the slope of the regression line is equal to the Crypt Cell Production rate per hour and these values are shown in table 46. There was no significant difference in the Crypt Cell Production rate per hour between the carcinogen exposed and the control animals at any of the sacrifice intervals.

Thymidine Labelling Data

The thymidine labelling index data is shown in table 46. No statistical comparison was made in the LI between the control and experimental groups in view of the small numbers of animals studied with the thymidine technique. There did not appear, however, to be any marked difference between the groups with regard to LI on simple scrutiny of the figures.

The site of the proliferative zone within the crypts did not differ between the groups or throughout the duration of the

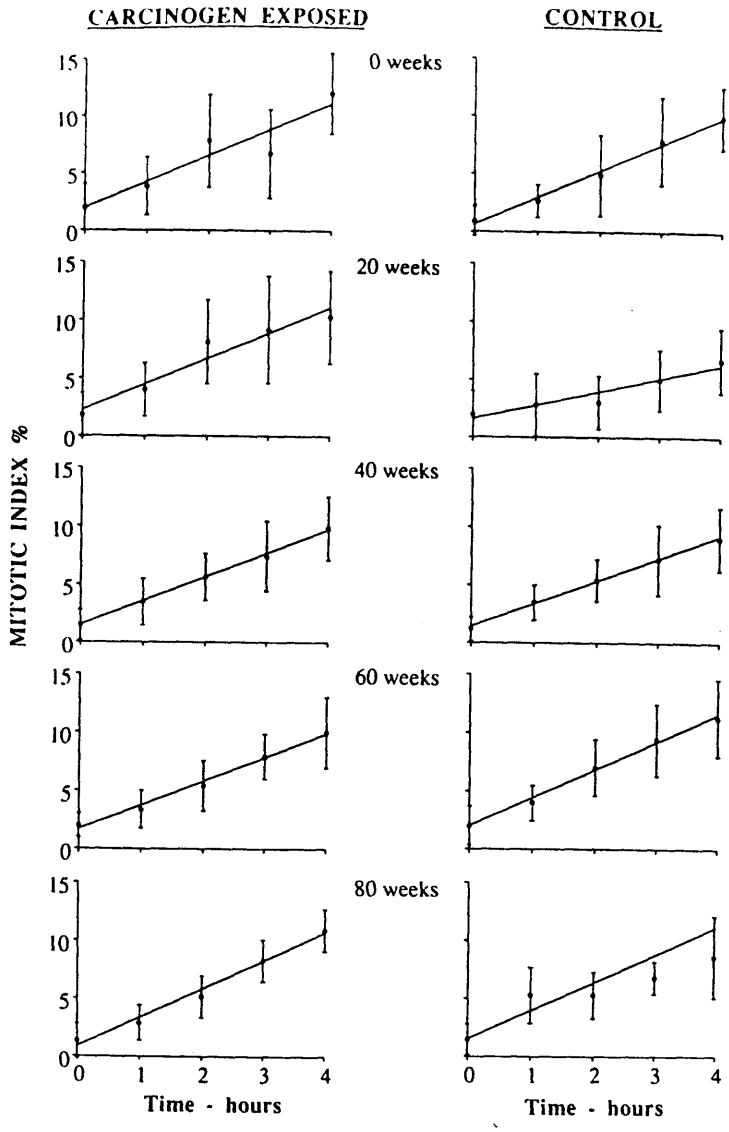


Figure 35. Metaphase accumulation data for control and carcinogen exposed groups at sacrifice intervals from 0 - 80 weeks. Slope of graphs fitted using least squares method. No significant difference in slope identified between carcinogen and control groups at any of the sacrifice intervals.

Histological Abnormalities

Time weeks	Gastritis	Ulceration	Neoplasia	Metaplasia
0	-	-	-	-
20	-	-	-	-
40	2 (1)	(1)	-	-
60	3 (1)	-	-	-
80	1	1 (2)	-	-

Table 45. Histological abnormalities in rat stomachs at sacrifice intervals. Numbers indicate number of animals affected: Controls shown in parentheses.

Sacrifice Interval	Carcinogen Exposed		Control	
	CCPR	LI	CCPR	LI
0	2.2	20.9	2.3	16.2
20	2.3	22.6	1.4	19.3
40	2.0	-	1.9	-
60	2.4	14.9	2.1	17.1
80	2.4	13	2.5	16.6

Table 46. Crypt Cell Production Rate per hour (CCPR) and Labelling Index (LI) for Control and Carcinogen Exposed Animals at Sacrifice Intervals.

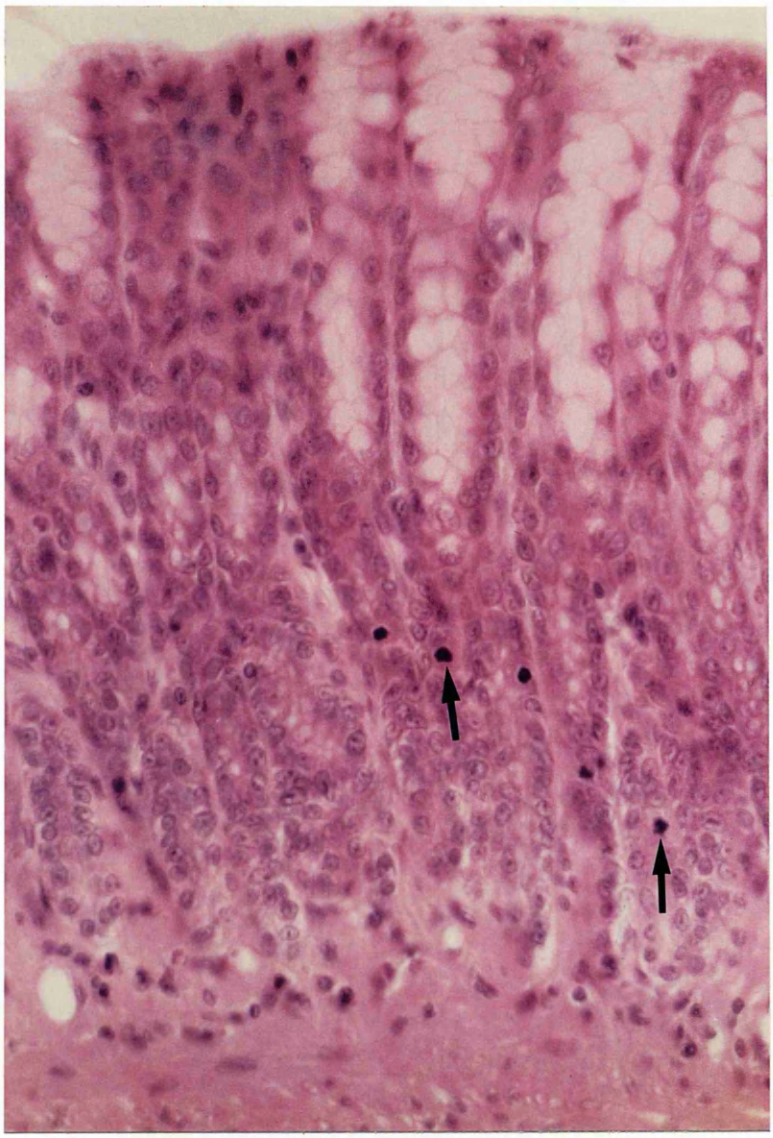
experiment. The labelled cells were distributed in the lower third of the antral crypts in all cases examined. At the 40 week sacrifice interval no thymidine was visualised in any of the sections from the four animals after autoradiographic development.

DISCUSSION

The administration of MNNG ad libitum in the drinking water of male Wistar rats at a concentration of 50 ug for 16 weeks has not resulted in the production of intestinal metaplasia or neoplasia in the present study.

A review of the literature and the experimental design of the present study showed no difference between the present experiment and studies which have reported a 60-90% incidence of intestinal metaplasia and adenocarcinoma in rats treated for 16 weeks with 50 ug MNNG (Matsukura et al., 1978; Tatematsu et al., 1983). The gastric carcinomas and foci of IM produced in the rats are microscopic (Matsukura et al., 1978) and a possible explanation for the absence of tumours in this study was that inadequate amounts of the rat stomachs were examined; to refute this the entire antrum in the experimental animals were cut into blocks and the material ribboned. This procedure, though laborious, discounts the criticism of inadequate sampling resulting in an absence of tumour yield.

The substance MNNG used as the carcinogen may not have been "active" or administered correctly is a second possible reason for the failure of the experiment. The material was stored at -40^{o}C according to the manufacturers instructions (Sigma), and was



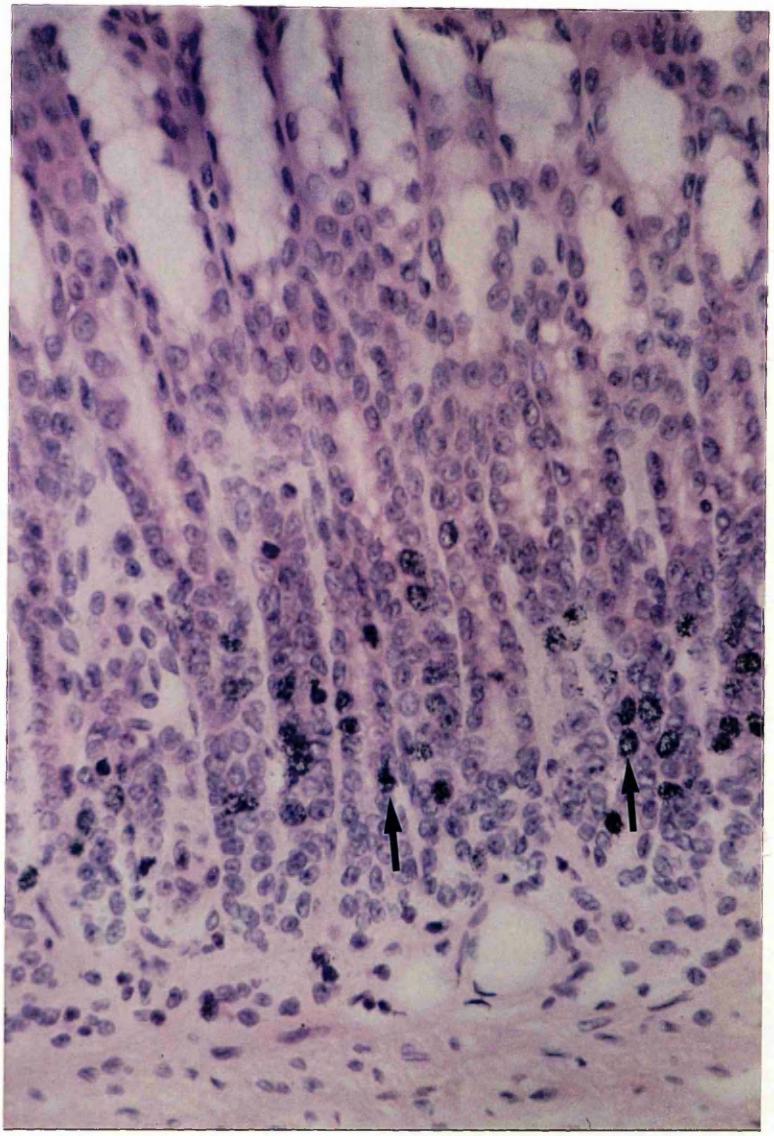
Photomicrograph 19. Longitudinal section of rat gastric mucosa two hours post vincristine injection. Note arrested metaphases within crypts (arrows). Haematoxylin and eosin x 60.

obtained from the same source as the Japanese workers (Matsukura et al., 1978). The carcinogen was diluted in a similar manner and administered ad libitum. Administration by gavage would have ensured a fixed dose received by each animal but this experimental procedure was not employed by the previous workers. Unfortunately no in vitro method is available to test the carcinogenetic activity of the chemical.

The male Wistar strain employed in the present experiment were the experimental animals employed in all the previous published work.

The metaphase arrest data demonstrates that with the Tannock Correction Factor the Crypt Cell Production Rate varies from 1.4 - 2.6 per hour. There did apear to be a trend towards and increasing CCPR in the carcinogen exposed group at 20 weeks which although not statistically significant did suggest that an alteration in cell kinetics was starting to occur at this stage of the experiment. This trend however did not appear to be sustained at subsequent sacrifice intervals. The higher CCPR at 20 weeks in the carcinogen exposed group may indicate that the carcinogen did initiate some abnormality in cellular proliferation but that this was not sustained perhaps due to the lack of some undefined co-factor.

The addition of autoradiographic techniques in the present study was designed primarily to determine the position and changes in position of the generative zone with intestinal metaplasia and neoplasia in view of the abnormalities described in Chapter 6 in



Photomicrograph 20. Longitudinal section of rat gastric mucosa with epithelial cells labelled with thymidine (arrows). Autoradiograph/ haematoxylin and eosin counterstain x 60.

the human stomach. The absence of any neoplasia or metaplasia within the rat stomachs meant that this aim was not achieved. The Labelling Index was calculated and displayed a wide range from 13 - 22.6. As only two rats in each group were injected with thymidine no statistical comparison between the Labelling Index in the two groups was possible.

CHAPTER 8

IMMUNOLOGICAL STUDIES

INTRODUCTION

The review of the literature in Chapter 1 demonstrated that the majority of studies of gastric mucosa in relation to immunology have concentrated on the humoral component. The role of cell mediated immunity in the gastrointestinal tract has only recently been established and knowledge is still incomplete. The cell mediated responses in the small bowel in diseases such as Crohn's and coeliac states have received most attention.

The present study was designed to determine the nature of the T cell infiltrate in gastritis and in response to gastric tumours. Gastritis plays a fundamental role in the development of intestinal metaplasia and the elucidation of the T cell infiltrate is an important component of the inflammatory process. The differing pattern of the immune response to the intestinal and diffuse type tumours embodied in the Lauren classification system is of interest and may be related to differences in prognosis.

The material for the present study was derived from the gastrectomy specimens discussed in Chapter 3. The immunocytochemical techniques using lymphoid markers are difficult to perform and also difficult to interpret. Quantification of the T cell subsets has been performed in small bowel. As the results of the present study were dependent on one observer the critical stage was to validate the counting techniques.

AIMS

1. To assess three techniques of quantifying the T cell subsets in the gastrectomy material by one observer.
2. To determine the pattern of T cell subsets and Class II MHC antigens in normal, (HLA Dr) inflamed and metaplastic gastric mucosa.
3. To assess the effect of Helicobacter pylori on T cell subsets and Class II MHC antigens (HLA Dr).
4. To determine the pattern of T cell subsets and Class II MHC antigens (HLA Dr) in gastric carcinomas of intestinal and diffuse type.

RESULTS

Investigation of Observer Error in the Counting of Lymphoid Markers

The three techniques of counting outlined in the Materials and Methods section, Chapter 2, were assessed.

(1) Number of labelled cells/unlabelled cells per high power field.

(2) Point counting using graticule.

(3) Number of labelled cells/unlabelled cells for a given mucosal length which was selected as four gastric crypts.

Three sets of counts for each counting technique were obtained using the Leu 1 antibody in five cases by the investigator. The first set of counts was on area 1, the second and third counts were on the same area 2. This provided information on the reproducibility of the counts between two different areas of the same case and also on the ability of the investigator to reproduce stable counts in the same area. The data was analysed using log ratios and the GLIM (Generalized Linear Interactive Model) statistical package as described in Chapter 2.

The deviance D_M and degree of freedom d_M for the data on the three counting techniques are shown in table 47. The deviance D_H and degrees of freedom d_H for the hypothesis H_0 there is no difference between cases or observer ie the counts are completely random and are also shown in table 47. Using the F test the H_0 hypothesis cannot be rejected at the $p < 0.10$ level for all of the counting techniques under study. This indicates that counts cannot be reproduced by one observer in the same area or within different areas from the same case.

The statistical analysis demonstrates that counting

Counting Technique	Statistical Results		
High Power Fields	Model M	$D_M = 8.97$	$d_M = 18$
	Hypothesis H_0	$D_H = 14.42$	$d_H = 29$
Point counting	Model M	$D_M = 3.81$	$d_M = 14$
	Hypothesis H_0	$D_H = 6.48$	$d_H = 23$
Mucosal unit length	Model M	$D_M = 3.88$	$d_M = 14$
	Hypothesis H_0	$D_H = 5.28$	$d_H = 23$

Table 47. Results of GLIM analysis for counting techniques of lymphoid markers. Model M represents the data obtained by replicate counts by one observer on the same area and different areas in the same case. Hypothesis H_0 represents the random hypothesis that count results are completely random. D = deviance, d = degrees of freedom. Using the F test the H_0 hypothesis could not be rejected for any of the counting techniques.

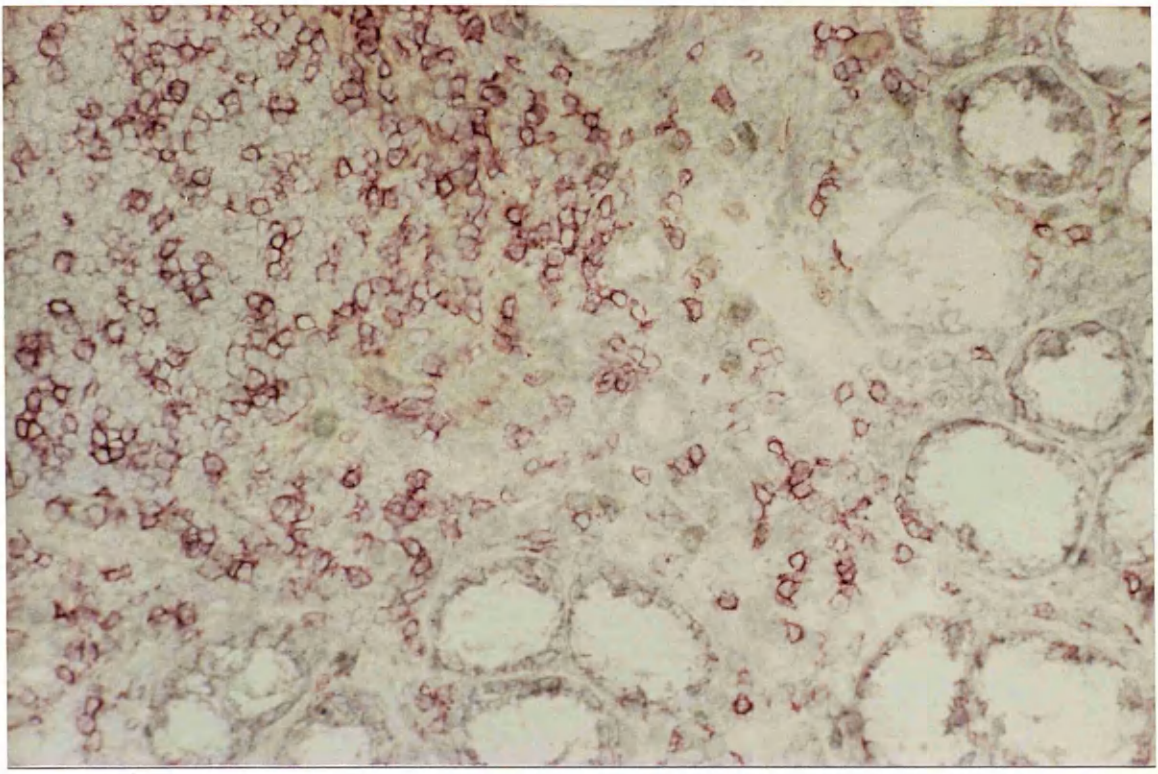
techniques on the lymphoid markers were not reproducible. The results of the study are therefore presented in a subjective descriptive manner.

In the interpretation of the T cell subsets to simplify the results the following assumptions have been made; Leu 1 and Leu 4 positivity indicates Pan T cell origin, Leu 3 positivity indicates T helper/inducer cells, Leu 2 positivity indicates T cytotoxic/suppressor cells, Leu 7 and 11b positivity indicates natural killer cells (NK).

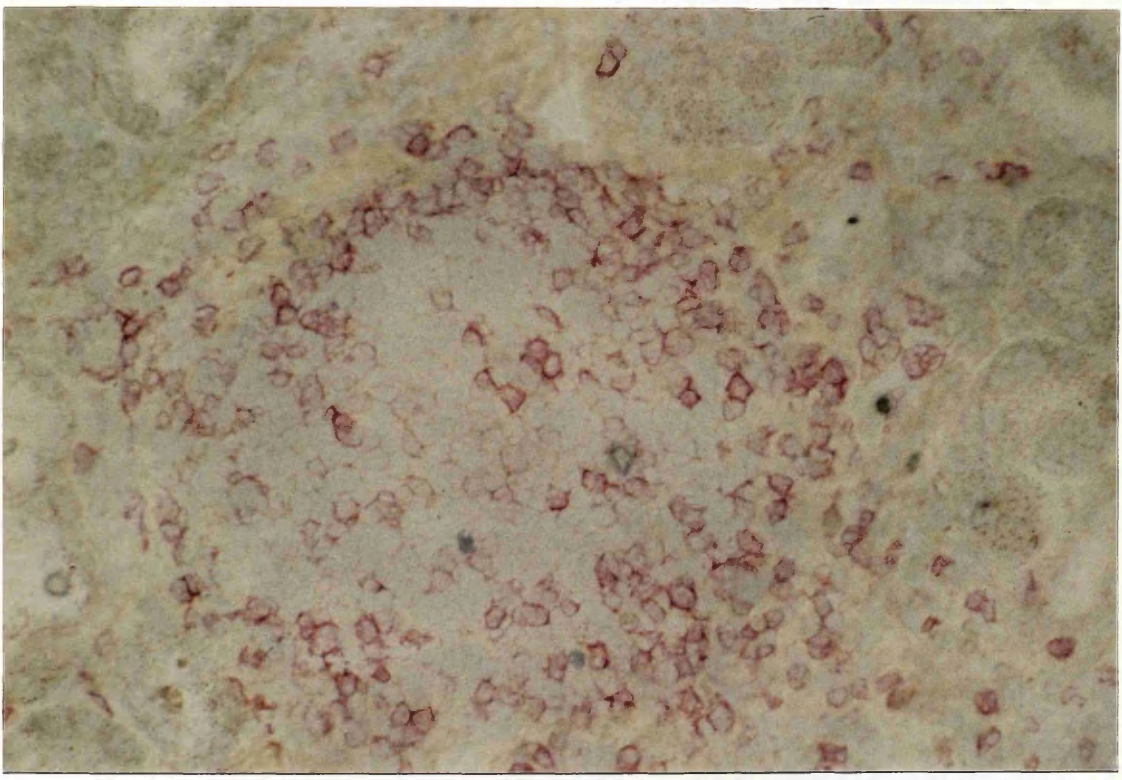
Gastric mucosa

In the mucosa which appeared histologically normal ie no excess of inflammatory infiltrate and normal glandular pattern, a consistent distribution of T cell subsets was seen. The lymphocytes in the lamina propria were predominantly of the T helper/inducer subset. A small number of intra-epithelial lymphocytes were seen mainly in the upper reaches of the glands and these were T cyt/suppressor cells. There was some faint HLA Dr staining within the lamina propria but the epithelium showed no HLA Dr positivity. Very occasional lamina propria and intra-epithelial cells (IEL) were NK positive. These findings were present in both antral and body type mucosa. Staining of myenteric nerve bundles was seen with Leu 7.

With increasing degrees of gastritis antral and body type mucosa from both the benign and malignant stomachs showed increasing numbers of intra-epithelial T cytotoxic/suppressor cells and lamina propria T helper/inducer cells. A higher proportion of



Photomicrograph 21. Inflamed antral mucosa with lymphoid follicle with positive staining for Leu 1. Alkaline phosphatase technique x 300.



Photomicrograph 22. Lymphoid follicle in antral mucosa with positive cells for Leu 1. Alkaline phosphatase technique x 340.

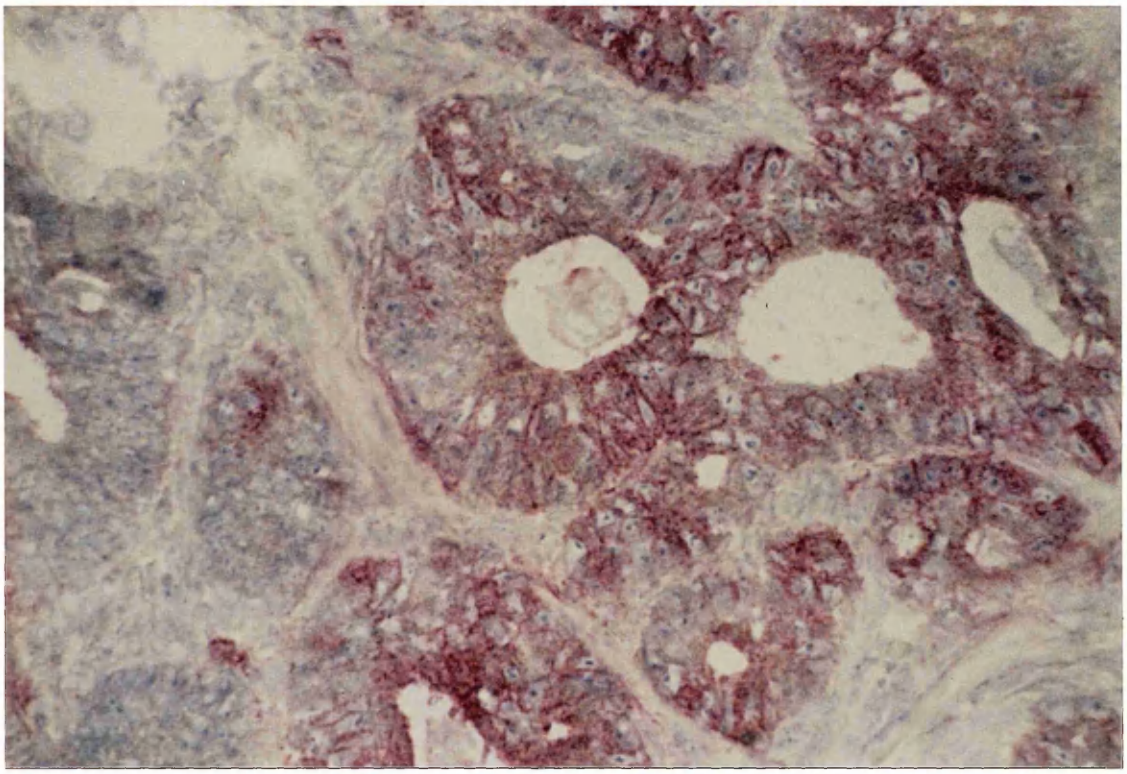
IEL showed an NK phenotype. Lymphoid follicles when present stained in the germinal centre and at the periphery strongly for T helper/inducer cells and T cytotoxic/suppressor cells. HLA-Dr was expressed strongly on the epithelium at the isthmus of the crypt particularly adjacent to lymphoid follicles. With increasing severity of inflammation and with a strong active component ie polymorphonuclear infiltrate, HLA-Dr was expressed widely on all areas of the epithelium throughout the crypt and on all cell types.

Intestinal Metaplasia

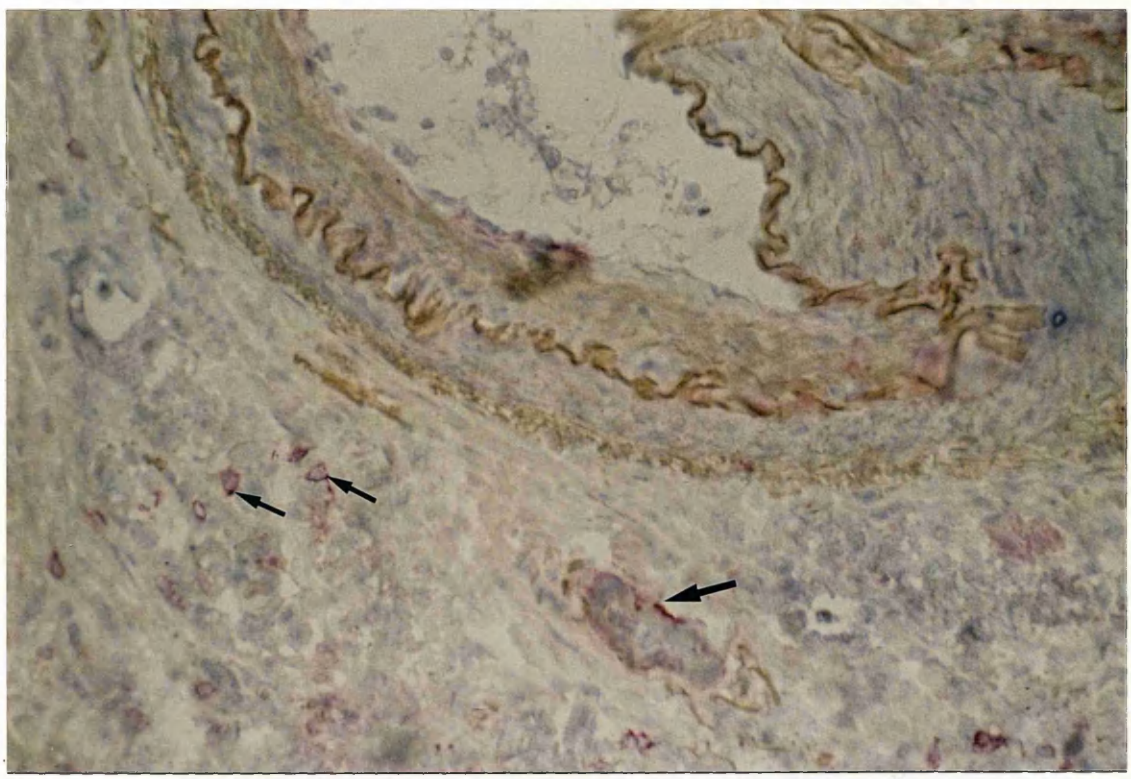
Areas of gastric epithelium immediately adjacent to foci of intestinal metaplasia showed a consistent and characteristic feature; large numbers of intra-epithelial lymphocytes which showed expression of NK markers. Within areas of well developed intestinal metaplasia there was a marked reduction in the number of T helper/inducer lymphocytes within the lamina propria compared with the inflamed gastric mucosa. Unfortunately the alkaline phosphatase enzyme used (despite removal of endogenous alkaline phosphatase) in the immunocytochemical technique stained the surface villi of the intestinal metaplasia making interpretation difficult. It was, however, possible to determine IEL lymphocytes exhibiting NK markers within the intestinal metaplasia. Areas of both type 1, 11a and type 11b metaplasia were studied; no difference was seen between the expression of the T cell subsets in the lamina propria or intra-epithelial lymphocytes.

Mucosa adjacent to tumour/ulcer

The area of mucosa adjacent to the benign gastric ulcers and



Photomicrograph 23. Intestinal type tumour with widespread positive staining for HLA Dr. Alkaline phosphatase method x 360.



Photomicrograph 24. Infiltrating tumour with positive staining for Leu 7 (small arrows) and non specific staining of nerve (large arrow) for Leu 7. Alkaline phosphatase technique x 360.

ulcerated tumours were compared to determine if any difference could be seen in T cell subsets. Both areas showed the distribution of T cell subsets described above for severe inflammation.

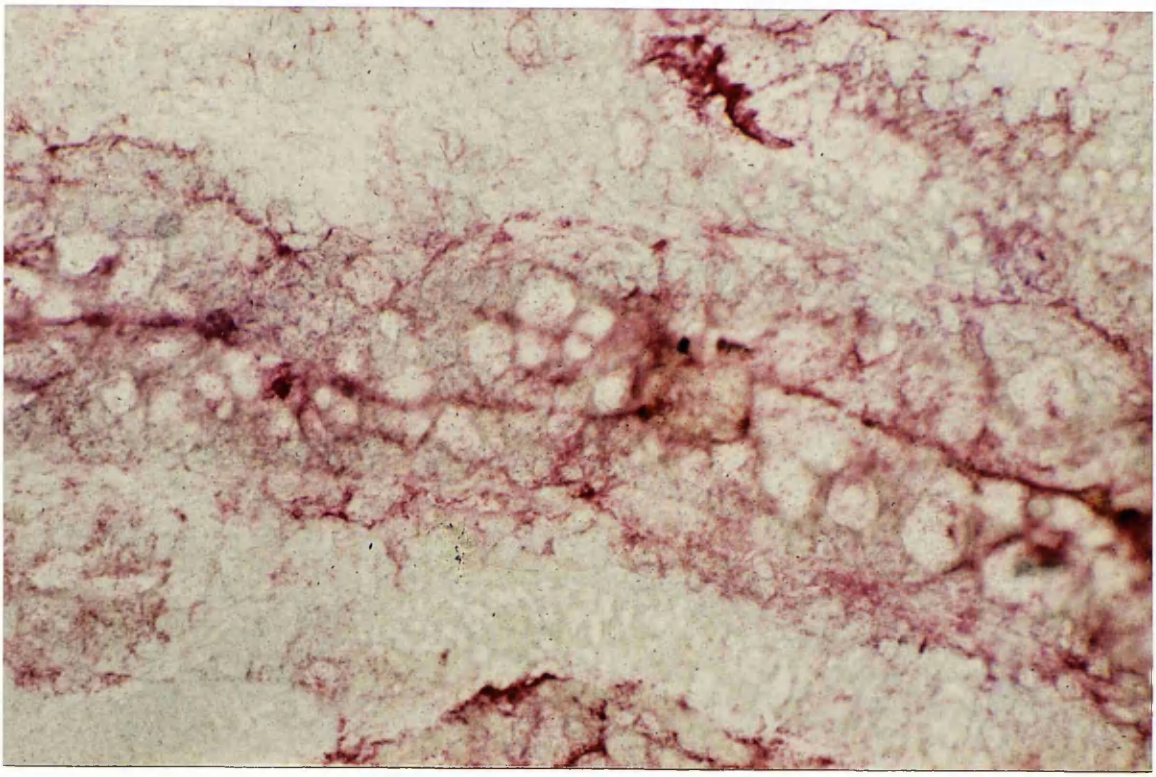
Effect of Helicobacter Pylori

In the series of gastrectomy specimens there were six stomachs in which the organisms had been demonstrated by cresyl violet staining, four benign and two malignant. Although the mucosa within the stomach tended to show severe inflammation with an active component there was no obvious difference in the pattern of distribution of the T cell subsets and HLA-Dr expression when compared to mucosa from other specimens with similar severity of inflammation which did not show the presence of the organism.

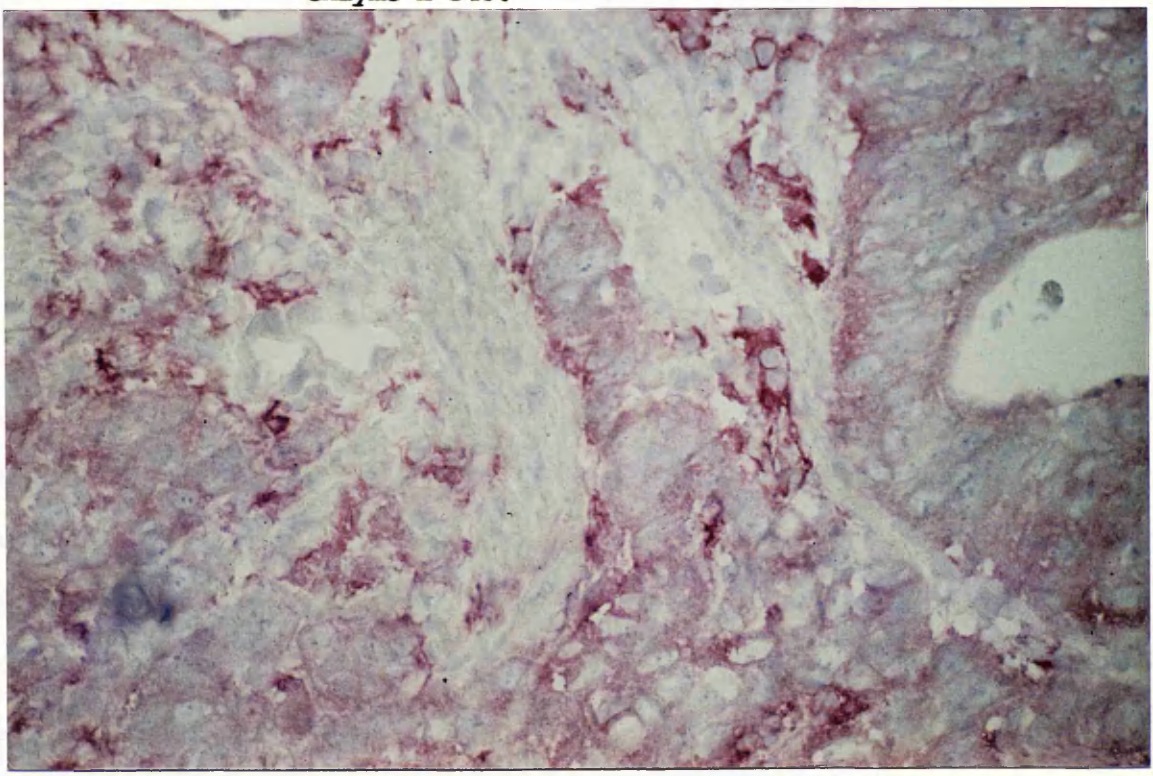
Tumour

Both the intestinal (7 cases) and the diffuse tumours (5 cases) classified by the Lauren criteria (1965) displayed both T helper/inducer and T cytotoxic/suppressor cells in the inflammatory infiltrate within the tumours and in the tissue surrounding the tumours. There was no appreciable difference in the number nor any obvious difference in the histological distribution of the T cell subsets in the inflammatory infiltrate between the two types of tumour. Faint staining with IL2 was seen in scattered lymphocytes in all the tumours.

In four of the seven cases of intestinal type of tumour lymphocytes with the T cyt/suppressor and NK phenotype were identified in between malignant cells forming glandular lumina.



Photomicrograph 25. Negative control for alkaline phosphatase technique in area of intestinal metaplasia showing non-specific staining of metaplastic crypt with alkaline phosphatase enzyme x 340.



Photomicrograph 26. Intestinal type tumour positive for HLA Dr. Alkaline phosphatase technique x 360.

The three remaining cases of intestinal type of tumour did not show this feature.

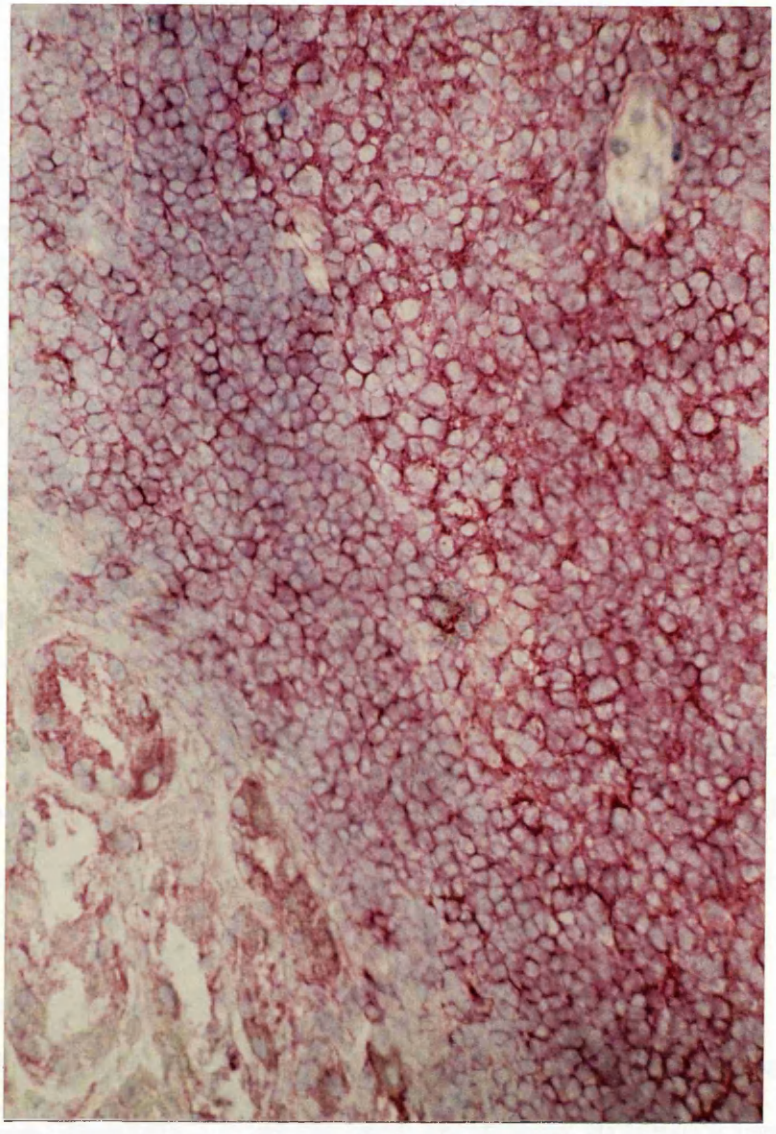
All the tumours examined showed immunoreactivity with the HLA-Dr antibody in lymphocytes in the inflammatory infiltrate. In four of the intestinal cases and all the diffuse cases the tumour cells showed positive staining with the HLA-Dr antibody. In the remaining three intestinal cases there was a complete absence of staining of tumour cels with the HLA-Dr antibody.

DISCUSSION

General Points

Immunocytochemical studies using antibodies to lymphocyte sub-populations allows the micro-anatomical distribution within the tissue and the preponderance of given sub-populations to be determined. Such histological studies however provide no information regarding the functional role of lymphocyte sub-populations in the tissue under study. The optimum approach would be a combination of histological and functional studies of the T cell sub-populations. Functional studies in human tissue lymphocytes are extremely difficult to perform for logistical and technical reasons. The present study is limited to the examination of T cell subsets and their distribution within the gastric mucosa by immunocytochemical methods. In the absence of functional studies no firm conclusions can be drawn about the role played by the T cell subsets in the immune response.

The use of the alkaline phosphatase enzyme instead of peroxidase in the present study has circumvented the need for



Photomicrograph 27. Inflamed antral mucosa with lymphoid follicle with widespread positive staining for HLA Dr on adjacent epithelium. Alkaline phosphatase method x 300.

removal of endogenous peroxidase from the gastric specimens. Positive staining with alkaline phosphatase of foci of intestinal metaplasia however occurred due to the presence of this enzyme within this type of epithelium (Matsukara et al., 1980). The presence of this non-specific staining made interpretation of intra-epithelial lymphocyte staining in foci of intestinal metaplasia difficult to interpret although it did not affect the identification of T cell sub-sets in the surrounding lamina propria in foci of intestinal metaplasia.

Quantification

The value of counts per unit area or in relation to some stable reference feature has been proven to be more useful than a subjective visual impression in determining the differences in the relative numbers of lymphocyte sub-sets in inflammatory conditions in the skin (Beck et al., 1986). In the lamina propria of the bowel several studies have used counts per high power field to compare T cell sub-sets in different disease states (Selby et al., 1981; Selby et al., 1983). The present study was performed by one observer (due to the constraints of the context of an MD thesis). The counting of the immunocytochemical preparations proved to be technically difficult due to several factors. The use of frozen section material made the discrimination of cells harder to perform visually. There was an uneven distribution of lymphocytes within the lamina propria; lymphoid follicles or aggregates with large numbers of densely packed cells being particularly difficult to count. The presence of oedema and

atrophy in the mucosal specimens caused considerable variation in the lamina propria in some cases. In view of the fact that counts were performed by one observer and the technical difficulties encountered a statistical analysis was performed to validate the reproducibility of counts using three different counting techniques. This analysis demonstrated that stable counts could not be reproduced by any of the techniques. The results of the study could therefore be expressed only as a subjective visual impression of the differences in the relative numbers of T cell subsets. Thus the results are constrained by the observations of Beck et al., 1986, who demonstrated this to be less useful than counting techniques.

Normal Mucosa and Intestinal Metaplasia

T Cell Sub-sets

On the basis of a subjective visual impression the present study has demonstrated a predominance of T helper/inducer cells in the lamina propria in gastric mucosa which appears histologically normal. The increased numbers of lamina propria lymphocytes which are recognised by conventional light microscopy as "gastritis" are due to increasing numbers of T helper/inducer cells and a relative increase in the number of T cyt suppressor cells. In addition to these changes in the lamina propria increasing numbers of intra-epithelial lymphocytes expressing mainly T cyt/suppressor phenotype are seen. The findings of the present study for normal mucosa are similar to those reported for the normal small intestine (Selby et al., 1981; Selby et al., 1983). The changes in the lamina

propria and intra-epithelial lymphocyte populations described in the present study are also similar to the changes indentified in patients with coeliac disease receiving a diet containing gluten.

An important finding in the present study is the identification of intra-epithelial lymphocytes expressing NK phenotype. These cells appeared most frequently in areas of gastric epithelium adjacent to foci of intestinal metaplasia. In the small intestine the NK phenotype on intra-epithelial lymphocytes has been identified by some workers (Shioda et al., 1984) but not by others (Selby et al., 1983; Cerf-Bensussan et al., 1983) although ultrastructurally the intra-epithelial lymphocytes appear similar to circulating granular lymphocytes with NK activity (Cerf-Bensussan et al., 1983).

Intra-epithelial lymphocytes are in intimate contact with the antigenic load of the gut and constitute one of the largest lymphocyte populations in the body (Ferguson, 1977). The role of the intra-epithelial lymphocytes in local defence is still unclear. Intra-epithelial lymphocytes have been shown to have NK activity in vitro (Mowat et al., 1983) but not in vivo (Baca et al., 1987). This discrepancy between the in vitro and in vivo properties of intra-epithelial lymphocytes appears to be due to the inability of the cells to recirculate in vivo. Recent work has suggested that the intra-epithelial lymphycytes are already activated in vivo and may play an important role in protecting the epithelium (Baca et al., 1987).

The finding in the present study of NK phenotype in the

intra-epithelial lymphocytes in the gastric crypts in view of the current knowledge regarding intra-epithelial lymphocyte function suggests that this forms part of the immune response of the gastric mucosa to an antigenic stimulus from the lumen. The presence of Helicobacter pylori on the luminal surface of the gastric crypts would appear to be a candidate for the source of this antigenic stimulus. However NK phenotype was identified in the intra-epithelial lymphocytes in cases in which Helicobacter pylori could not be demonstrated.

The identification of an increased frequency of intra-epithelial lymphocytes with NK activity in the gastric crypts adjacent to foci of IM in the present study is of interest. It is possible that the NK positive cells represent a response to a marked antigenic stimulus. The presence of adjacent foci of intestinal metaplasia suggests that the end stage of the gastric epithelia response may be the transformation to intestinal metaplasia.

Although mentioned in the introduction to the discussion it must again be stressed that in the absence of quantification and functional studies of the lymphocyte sub-populations the interpretation of the present study is based solely on the subjective impression of the micro-anatomical distribution of the T cell subsets.

HLA-Dr

Class 11 histocompatibility antigens (HLA-Dr) are genetically encoded molecules that restrict the immune response (Nixon et al.,

1982). In addition to the detection of HLA-Dr antigens on bone marrow derived cells such antigens have been detected on cells in the gut (Scott et al., 1980; Selby et al., 1981; Spencer et al., 1986). Previous studies have identified that HLA-Dr positive epithelial cells appear in the normal small intestine and colon (Scott et al., 1980; Spencer et al., 1985) and in the gastric mucosa affected by gastritis (Spencer et al., 1986). The epithelial cells expressing the HLA-Dr antigen appear in specific sites in the gut; on epithelial cells adjacent to lymphoid follicles in the stomach and the intestine (Scott et al., 1980; Spencer et al., 1986).

The present study has demonstrated (in a larger number of cases than previously reported) that epithelial cells expressing HLA-Dr antigen are absent from normal mucosa but with lymphocytic infiltrate within the lamina propria the gastric epithelial cells adjacent to the lymphoid follicles express HLA-Dr antigen. In severe gastritis with a heavy infiltrate of lymphocytes the crypt epithelium at all levels express HLA-Dr. Previous studies have postulated that the presence of HLA-Dr antigen in specific sites adjacent to lymphoid tissue suggests a relationship between the lymphoid tissue and the HLA-Dr positive epithelium (Spencer et al., 1986). The hypothesis has been advanced that the lymphoid tissue may produce a substance, possibly interferon (Steeg et al., 1982; Hirsch M R et al., 1983) which may recruit epithelial cells into the immune process. The epithelial cells expressing HLA-Dr may be able to present antigens from the lumen underlying lymphocytes in the lymphoid follicle. Although the present study

has confirmed the previous observation that HLA-Dr expression is present in mild gastritis on the epithelium adjacent to lymphoid follicles (Spencer et al., 1986) it has also demonstrated that in severe gastritis the HLA-Dr expression is present on all cell types in the gastric crypts from the luminal surface to the base and is not always associated with organised lymphoid structures. If the hypothesis of antigen presentation by the epithelial cells is correct then it suggests that in severe gastritis this is a widespread phenomenon.

Tumour

T Cell Sub-sets

The inflammatory response to gastric carcinoma has been identified as an important prognostic variable (Monafo et al., 1962). The nature of the inflammatory response is also one of the criteria used by Lauren (1965) to distinguish between intestinal and diffuse type tumours. The difficulty in interpreting the results of any study on T cell sub-sets and tumour material is in determining what represents a response to necrosis within the tumour rather than a specific response to tumour itself. Within the limits of the present study in which objective numerical data on the T cell sub-sets was not available and the number of cases are small, there appeared subjectively to be no difference in the distribution of T cell sub-sets between the intestinal and diffuse type tumours of Lauren.

Lauren (1965) described the inflammatory response to the intestinal type of tumour as predominantly polymorphonuclear and

that to diffuse as mononuclear. The polymorphonuclear response to the intestinal type is frequently associated with partial tumour necrosis (Mehrotra et al., 1978). The present study suggests that if T cell sub-sets are examined independently of the polymorphonuclear response then there is no subjective difference between the immune response in intestinal and diffuse types.

The identification of lymphocytes in the present study within tumour acini in an "intra-epithelial" position is of interest in view of the role of intra-epithelial lymphocytes discussed in the previous section. Are these "tumour intra-epithelial lymphocytes" responding to some antigenic stimulus within the glandular lumina, and if so then what is the nature of the stimulus? The answer to these questions requires further study.

HLA-Dr

The present study has identified a dichotomy in the staining with the anti HLA-Dr antibody on the tumour epithelial ie present in some cases, completely absent in others. A similar pattern of expression of HLA-Dr has been noted in colorectal carcinoma (Daar et al., 1982; Van de Ingh, 1987) and these studies have not identified any relationship between the degree of differentiation of the tumour or tumour stage and the presence of HLA-Dr. The expression of HLA-Dr in the present study on inflamed gastric epithelium suggests that the expression of HLA-Dr by the tumour cells is not a feature of overt malignant transformation only.

CHAPTER 9

FETAL STOMACH

INTRODUCTION

Several aspects of the developing human fetal stomach are of relevance to the histogenesis of gastric carcinoma. The concept of intestinal metaplasia as a congenital heterotopia although abandoned by Magnus (1937) has been rekindled by the description of intestinal type cells in fetal stomachs by Salinius (1962). The second aspect of relevance to the adult is the expression of mucins and carcino-embryonic antigen by fetal gastric epithelia. To what extent the mucin histochemical and immunocytochemical changes in the adult stomach described in Chapter 4 and Chapter 5 represent a return to an embryonic pattern requires investigation. A further related feature is the appearance of specialised cells in the gastric mucosa during fetal development and the intrinsic factor antibody developed in Chapter 5 has been used to examine parietal cell development.

AIMS

1. To determine if intestinal type epithelium is present in the fetal stomach in the various stages of gestation.
2. To examine the mucin expression of fetal gastric epithelial throughout gestation.
3. To examine the expression of carcino-embryonic antigen by fetal gastric epithelial throughout gestation.
4. To study the development and distribution of the parietal cell using an intrinsic factor antibody in fetal gastric epithelium throughout gestation.

RESULTS

GESTATIONAL AGES

The number of cases and gestational age of the fetal stomachs calculated using the crown rump length is shown in table 48.

HISTOLOGY

The preservation of the fetal and neonatal gastric mucosa was excellent in most cases. However in four specimens the columnar mucus cells which constituted the cells of the gastric crypts were disrupted and lay free in the lumen of the stomach.

Changes throughout development

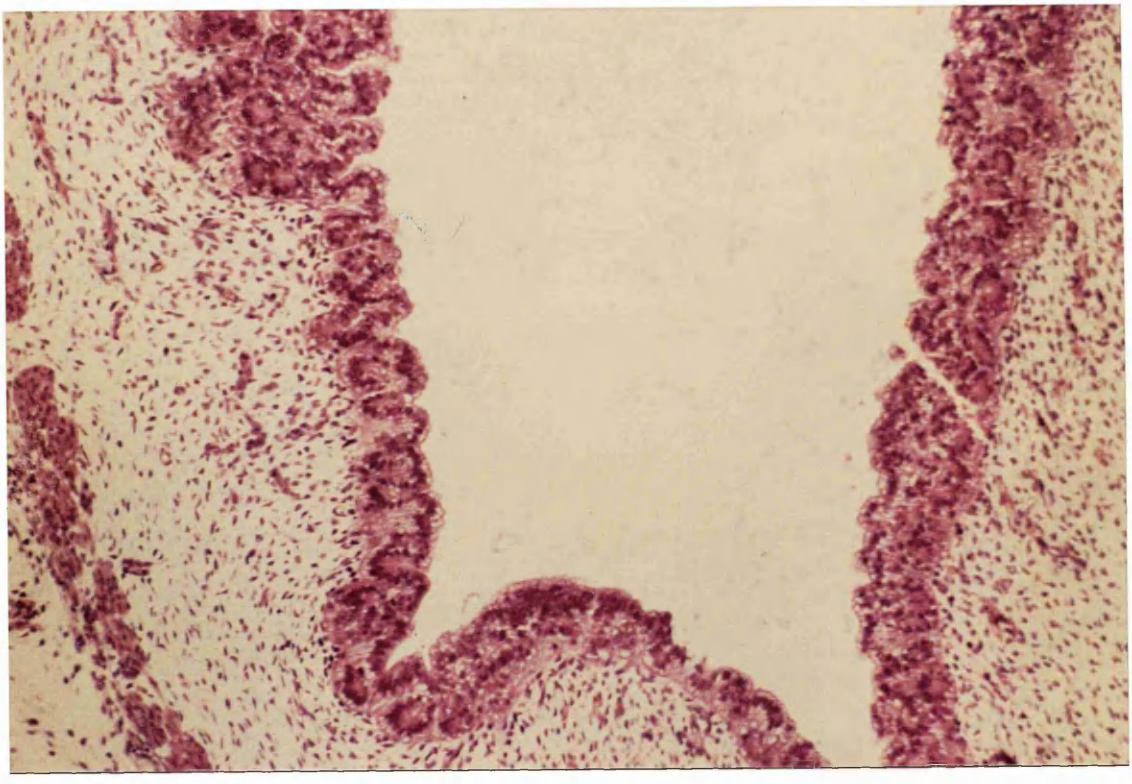
In the earliest fetus (gestational age 8 weeks) examined the gastric mucosa was formed by a pseudostratified columnar epithelium containing numerous mitotic figures. The mucosal cells had a uniform appearance and none showed any distinct morphological features.

By 11 weeks rudimentary gastric crypts were easily identified with an average three to four larger eosinophilic cells lying at the base of the glands in all areas of the stomach examined. These cells closely resembled parietal cells on morphological grounds: they were roughly conical in outline with the broad base protruding out from the wall of the gland. At the 11 week stage the epithelial changes were accompanied by the development of a well defined circular muscle layer.

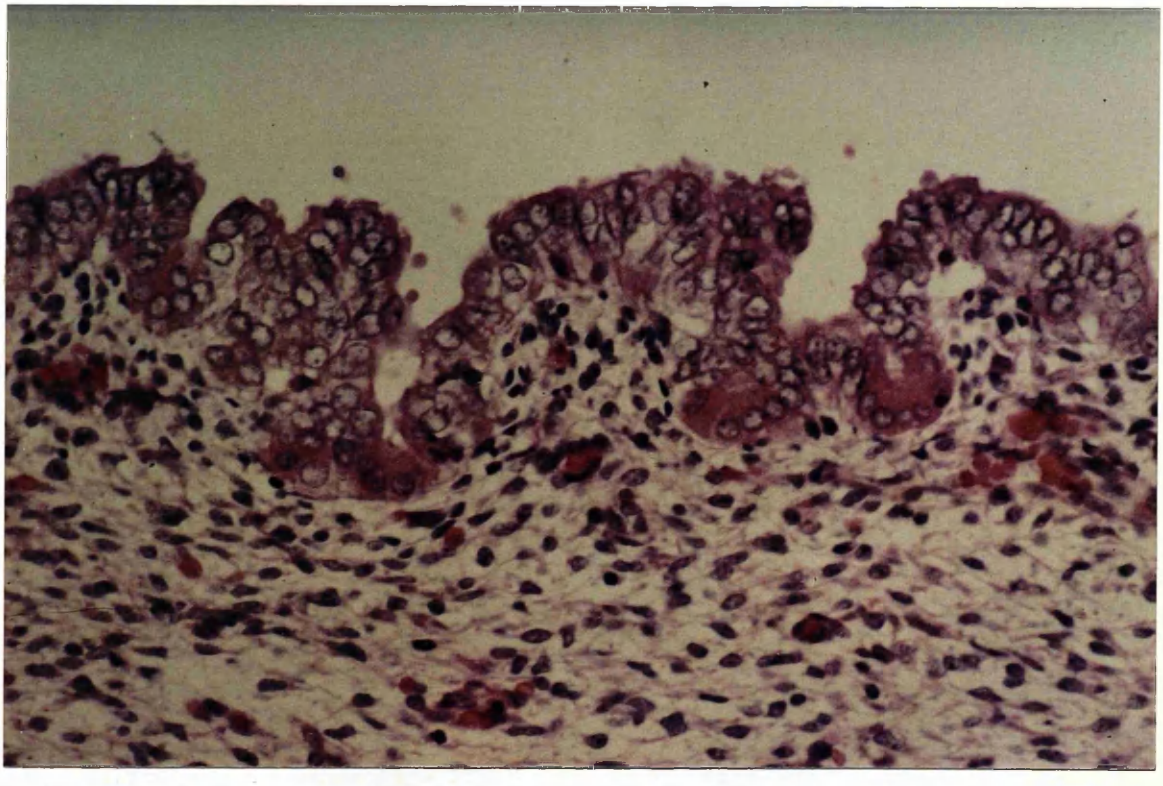
In fetuses from 13-17 weeks the gastric crypts still appeared rudimentary but had increased in height. In both the antrum and body of the stomach groups of 4-5 cells with the morphological

	Fetus													Neonate			
	Gestational age (weeks)													Term + days			
	8	11	13	14	15	17	18	20	21	24	25	26	28	+1	+3	+2	1+6
No of cases	1	1	4	3	1	1	2	5	3	4	2	6	8	1	1	1	1

Table 48. Gestational age of fetuses and age of neonates.



Photomicrograph 28. Stomach of 11 week gestation fetus with primitive gastric crypts. Haematoxylin and eosin x 100.



Photomicrograph 29. Fetal stomach at 11 weeks gestation showing primitive crypts with eosinophilic cells at base. Haematoxylin and eosin x 420.

features of parietal cells at the base of the glands.

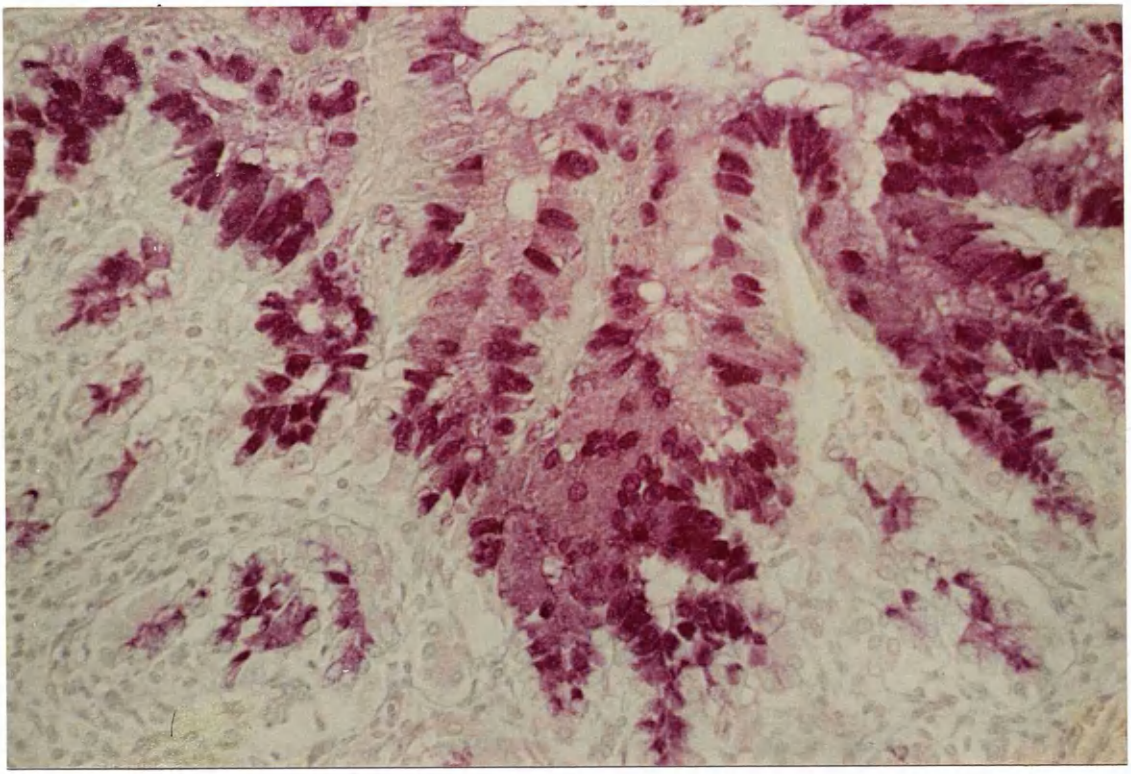
In fetuses of 18-2 weeks the mucosa had increased in thickness with the gastric glands in the body taking on the characteristic adult pattern of simple tubular glands. The surface was lined by tall mucous secreting columnar cells that extended down to the isthmus of the crypts. Below the level of the isthmus the tubular gastric glands were cut in cross section. The antral region at the 18-28 week stage was formed by glands of branched tubular type containing a large proportion of mucous columnar cells.

Neonatal Stomachs

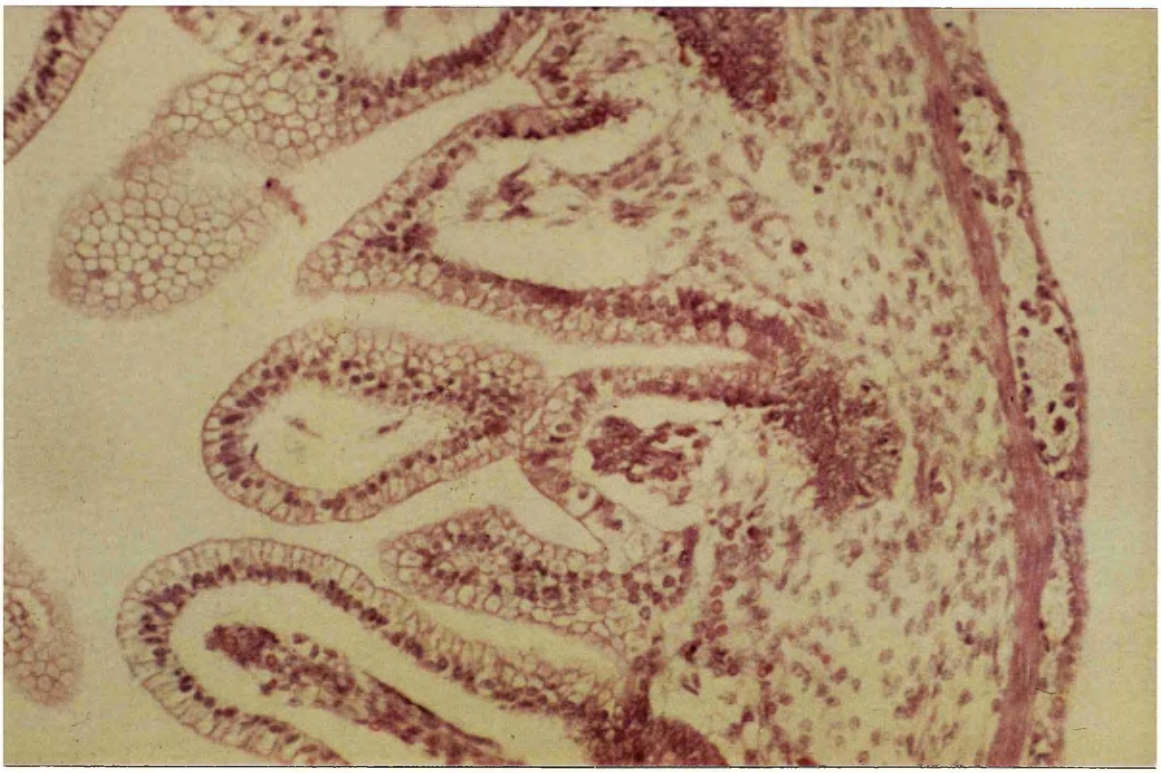
In the five neonatal stomachs examined the body and antral mucosa had a similar appearance to adult gastric mucosa although the thickness was less. Morphologically distinct parietal and peptic cells were present in large numbers in the body mucosa. However in all the cases examined small numbers of parietal cells were identifiable histologically in the antral mucosa situated mainly at the base of the glands just above the muscularis mucosae.

Observations regarding intestinal metaplasia in the fetal and neonatal stomach

All the fetal stomachs were carefully examined histologically for the presence of foci of intestinal metaplasia. In the early gestation period when the stomachs were embedded and cut as a single sample small intestinal crypts were seen on the sections but these crypts were clearly situated within the duodenum. An area of transition between the gastric epithelium and duodenal



Photomicrograph 30. Fetal stomach at 14 weeks gestation. Neutral mucin in epithelial cells AB/PAS x 300.



Photomicrograph 31. Fetal duodenum at 18 weeks gestation. Haematoxylin and eosin x 100.

epithelium was identified in a few cases. In this transitional zone occasional goblet cells and columnar absorptive cells were present in the gastric epithelium immediately adjacent to the duodenal mucosa. In two cases in the transitional zone between the gastric and oesophageal mucosa goblet like cells were identified.

MUCIN HISTOCHEMISTRY

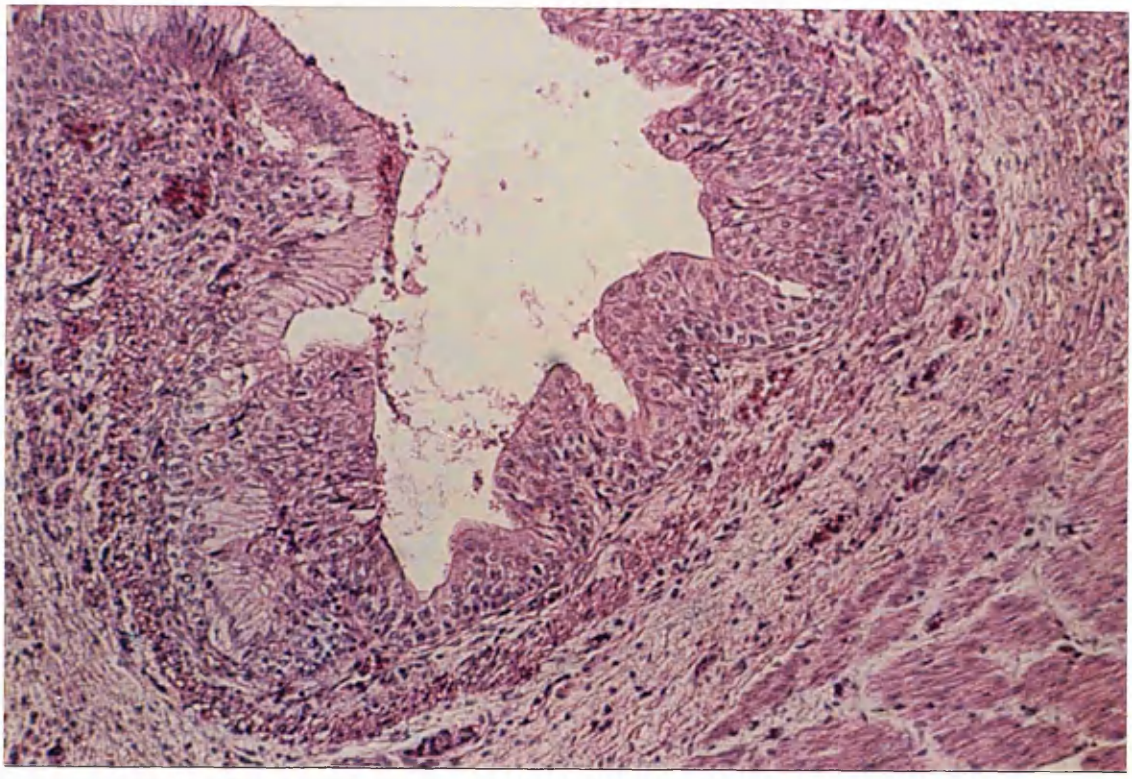
At eight week gestation in the earliest fetus examined mucin stains did not reveal the presence of any type of mucin. By 11 weeks of gestation the columnar cells were seen to contain neutral mucins only with no evidence of sialo or sulphomucins within these cells. In the 13-17 week fetuses the columnar cells both at the base and surface of the glands contained neutral mucin. In fetuses of 18-28 weeks the body mucosa contained neutral mucins in the columnar cells on the surface and upper half of the glands, the columnar epithelial cells at the base did not display any positive staining for mucin. In the antral glands of fetuses from 18-28 weeks columnar cells containing mucin were distributed throughout the entire length of the crypts.

Sialo and sulphomucins were identified in only two fetuses and were restricted to the oesophago-gastric junction as described in the histological section. N acetyl sialo-mucin and sulphomucin were present in both columnar and goblet cells in this area.

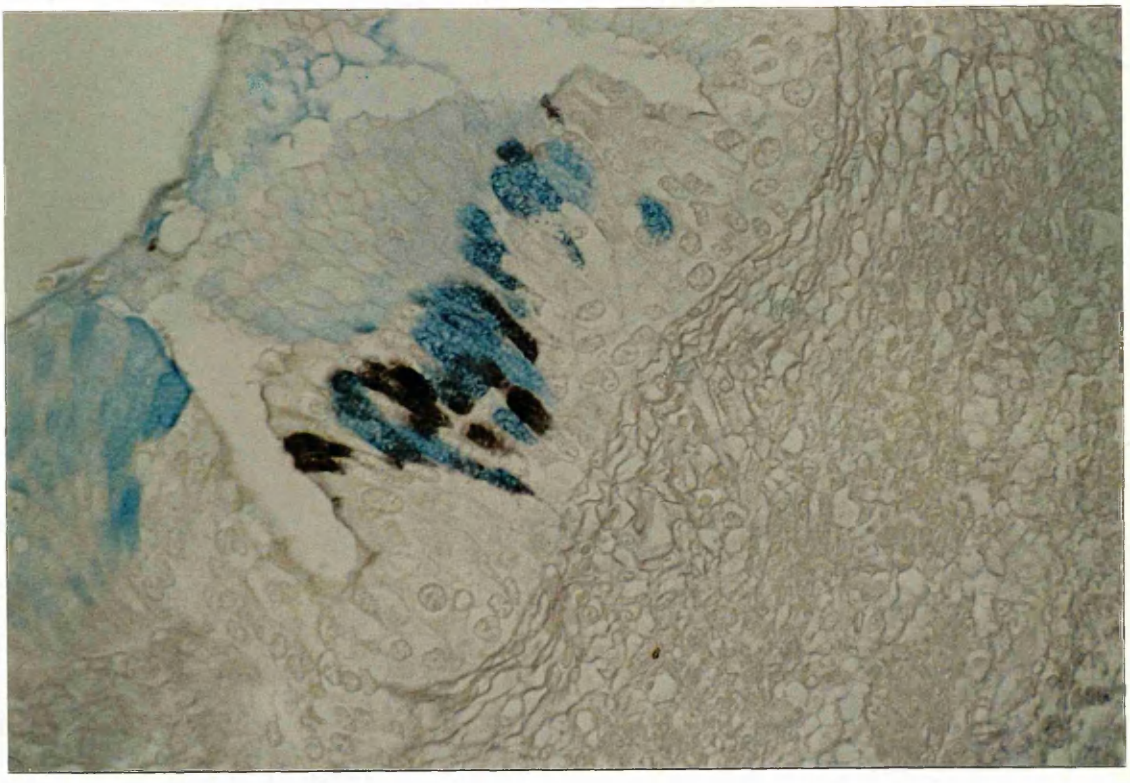
IMMUNOCYTOCHEMISTRY

Intrinsic Factor

In the earliest fetus (8 weeks) no immunoreactive cells were identified with the intrinsic factor antibody. In the 11 week fetus the larger eosinophilic cells lying at the base of the glands



Photomicrograph 32. Oesophago-gastric junction at 18 weeks gestation. Haematoxylin and eosin x 100.



Photomicrograph 33. High iron diamine/Alcian blue stain of oesophago-gastric junction at 18 weeks gestation showing sulphomucin positive material in columnar cells x 360.

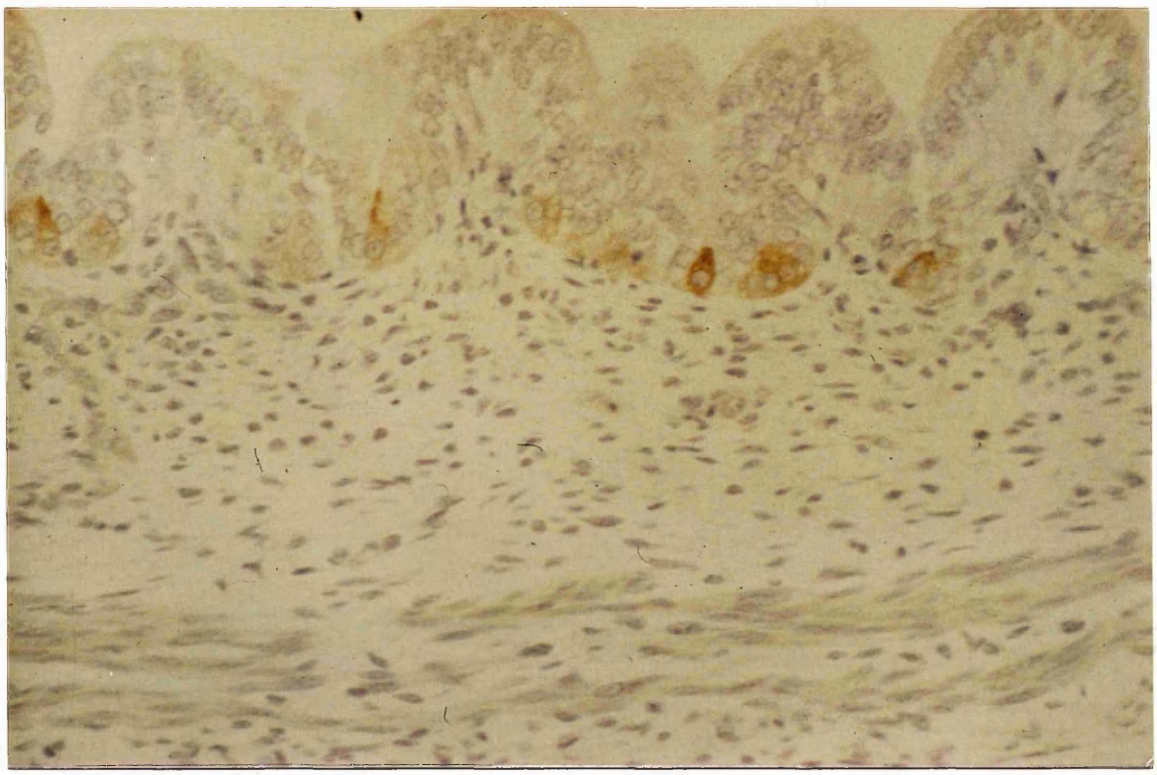
were strongly immunoreactive for intrinsic factor, the luminal and supranuclear cytoplasm showing intense staining in a bandlike configuration. In fetuses from 13-17 weeks the cells with morphological features of parietal cells in both the antrum and body were strongly immunoreactive for intrinsic factor.

In fetuses of 18-28 weeks in the body mucosa the glands lying below the level of the isthmus of the gland which were cut in cross sections contained numerous cells strongly immunoreactive for intrinsic factor. Occasional cells with eosinophilic cytoplasm and a centrally placed nucleus were identified at the isthmus of the glands, or above this level, between the mucous columnar cells and the surface mucosa. These cells were also strongly immunoreactive for intrinsic factor.

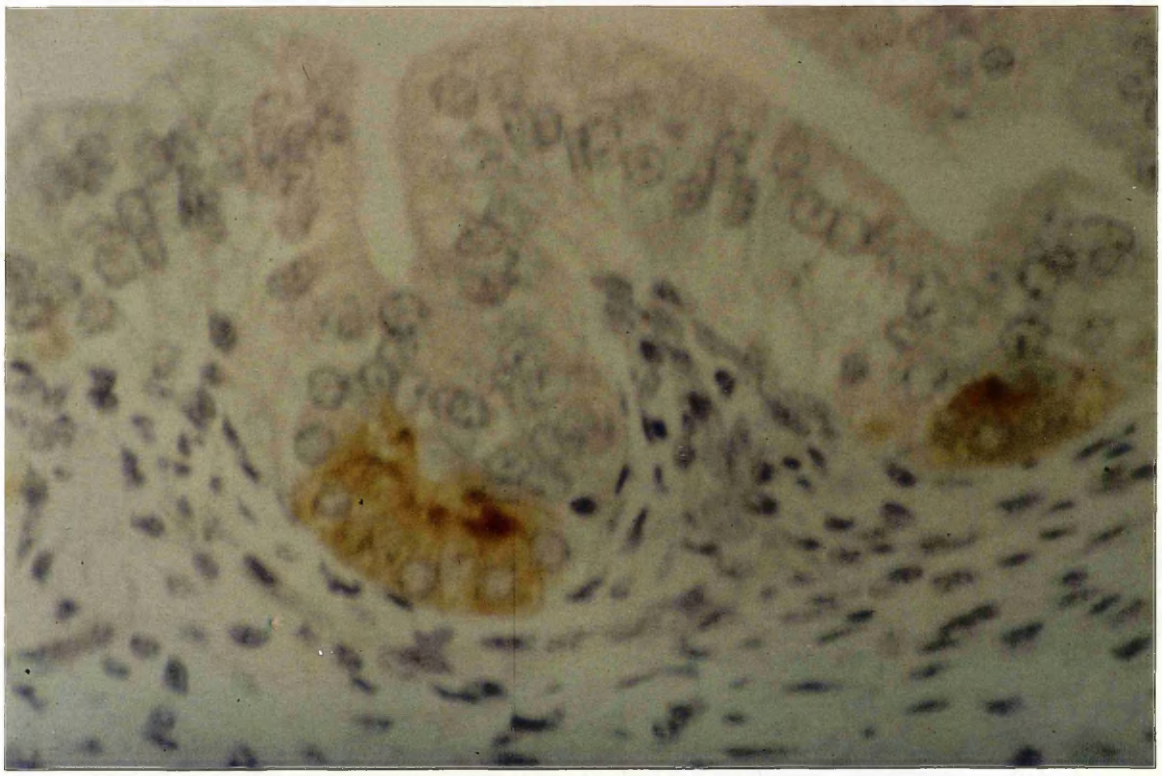
In the antral crypts of fetuses 18-28 weeks parietal cells were present in the basal part of the glands in small numbers and these were immunoreactive for intrinsic factor.

Sections of duodenum and oesophagus from fetuses of all gestational ages studied showed no positive staining with the intrinsic factor antibody.

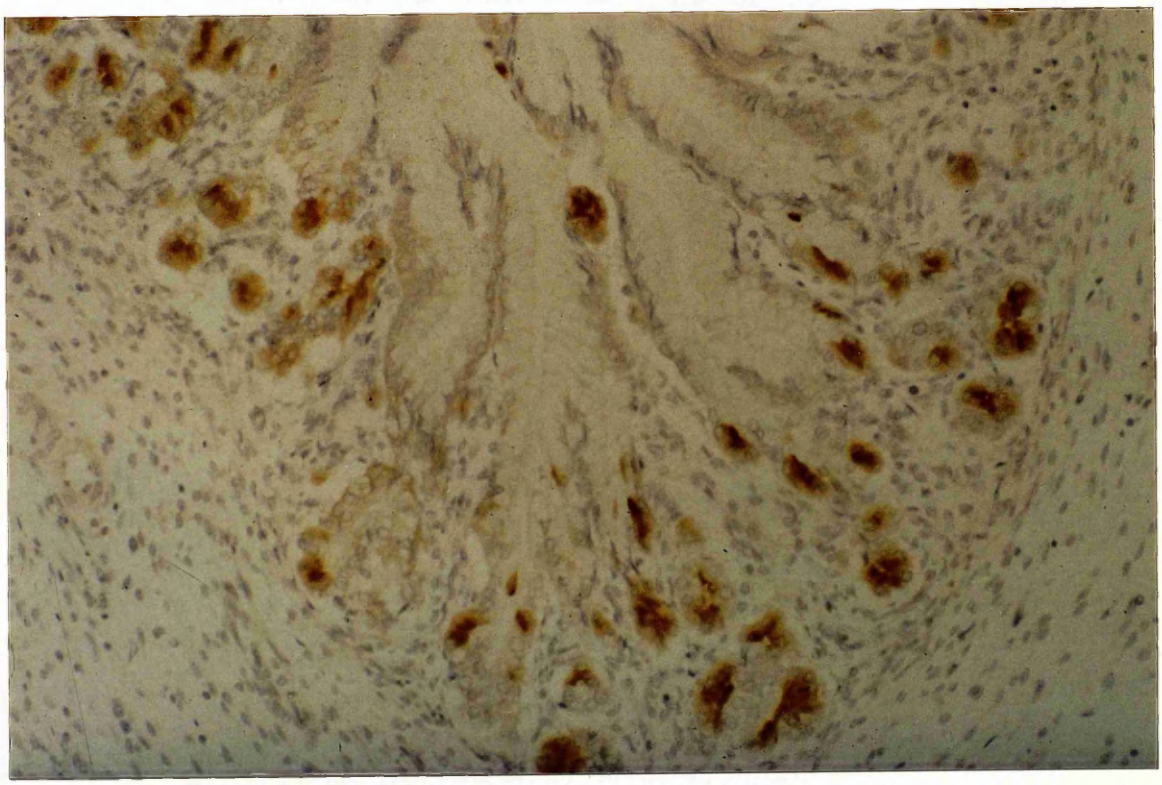
In the neonatal stomachs parietal cells could be clearly identified in the body mucosa on histological grounds and these were immunoreactive for intrinsic factor and were distributed below the isthmus of the gland. The parietal cells sporadically identified in the neonatal antral mucosa by histology were also immunoreactive for intrinsic factor.



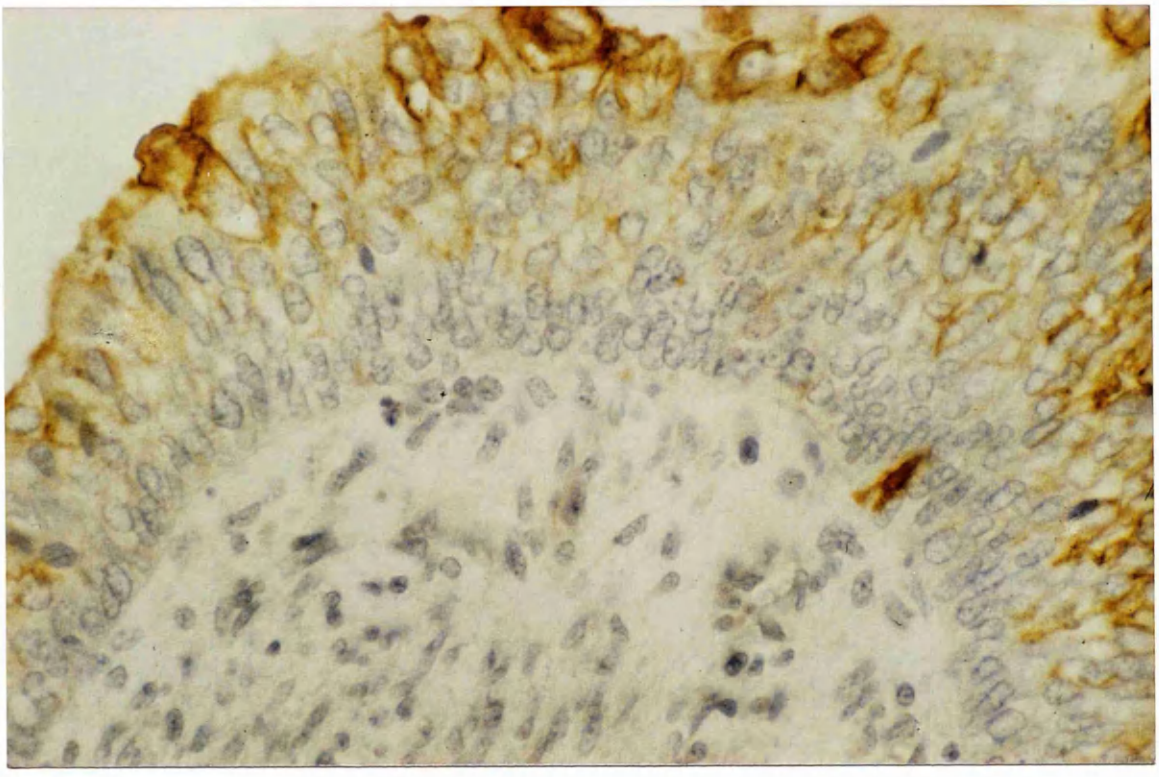
Photomicrograph 34. Fetal stomach at 11 weeks gestation with parietal cells positive for intrinsic factor at the base of the crypts. PAP method x 360.



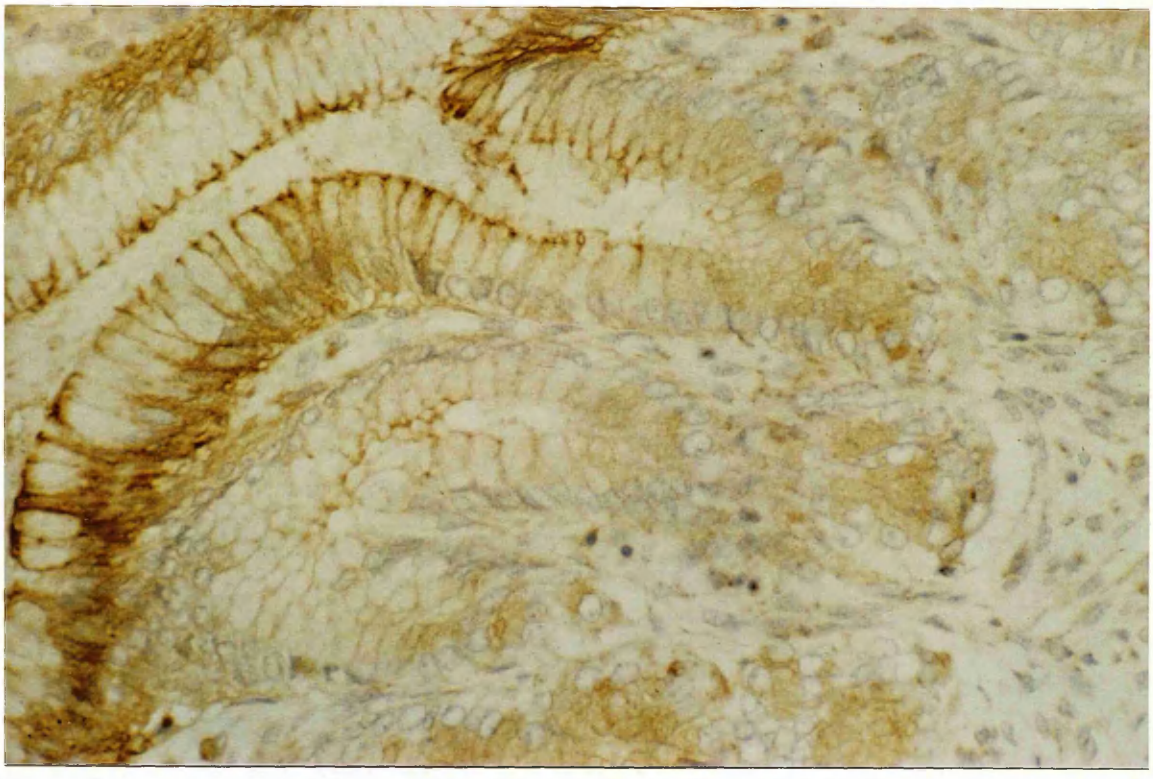
Photomicrograph 35. Fetal stomach at 11 weeks gestation with parietal cells positive for intrinsic factor at the base of the crypts. PAP method x 420.



Photomicrograph 36. Fetal stomach at 24 weeks gestation with parietal cells with positive staining for intrinsic factor in the lower part of the glands.



Photomicrograph 37. Fetal stomach at 8 weeks gestation. Gastric epithelium formed by pseudo-stratified cells positive for CEA. PAP method x 400.



Photomicrograph 38. Fetal stomach at 18 weeks gestation with cells throughout the gastric crypts positive for CEA. PAP method x 360.

eosinophilic cells at the base of the primitive pits at 11 weeks confirms the finding of Salenius (1962). The identification of these cells as parietal cells and the presence of these cells in the antral region are discussed later in relation to the intrinsic factor antibody.

Observations on the presence of intestinal epithelium

Earlier this century the presence of intestinal epithelium in the gastric mucosa was regarded as a congenital heterotopia (Taylor, 1927). The basis for this view appears to have been the histological findings of intestinal epithelium in stomachs resected for gastric carcinoma or ulcer in areas of the stomach with "no signs whatever of an inflammatory condition". This finding in Taylor's view resulted in the conclusion that their (aberrant glands)"only abnormality is one of position and their congenital and their nature is beyond doubt" (Taylor, 1927). The concept of the presence of intestinal epithelium in the gastric mucosa as an example of congenital heterotopia was challenged by Magnus (1937) who regarded the presence of intestinal islets in the gastric mucosa as a metaplasia as the result of faulty regeneration of the gastric mucosa as a consequence of gastritis. To emphasise the acquired rather than congenital nature of intestinal metaplasia Taylor (1927) examined 12 fetal stomachs of gestational age six to nine months and found no histological evidence of intestinal epithelium.

The congenital origin of intestinal heterotopia was disregarded following the work of Magnus (1937) until the recent

description of intestinal type cells in fetal stomachs by Salenius (1962). This finding has led to the suggestion that intestinal metaplasia seen in the adult with atrophic gastritis could be associated with a return to this more primitive cell population (Deren, 1971). The apparent discrepancy between the work of Magnus (1937) and Salenius (1962) regarding the presence of intestinal cells in the fetal stomachs can be explained by the different gestational ages examined by the two authors. Salenius (1962) describes intestinal type cells being numerous in fetuses up to the age of five months, whereas Magnus (1937) studied only fetuses of six months gestation onwards.

Although Salenius (1962) describes "intestinal type cells" being "encountered abundantly" up to 20 weeks gestation, two aspects of his work require to be considered. First his description is not of mature foci of intestinal epithelium within the fetal gastric mucosa but is of isolated goblet cells and absorptive type columnar cells. Secondly that the occurrence of such cells is restricted to two sites; the pyloro-duodenal junction and the cardio-oesophageal junction. The presence of intestinal type cells in the antrum is not recorded in the present study as the junction between stomach and duodenum was regarded as a transitional zone, neither truly stomach nor truly small intestinal type epithelium. The presence of goblet cells and columnar absorptive cells with gastric epithelium in this small area of transition was not regarded as a heterotopia but rather as gradual change from one type of mucosa to another.

The present study has also detected goblet cells at the cardio-oesophageal junction as described by Salenius (1962). It is difficult in the histological material to determine if these cells are oesophageal or gastric in origin. The mucin histochemistry of this area shows a pattern similar to that found in Type IIb metaplasia (Jass, 1980). Goblet and columnar cells in the adult at the gastro-oesophageal junction may originate from the cardiac glands or from the development of columnar epithelium lined oesophagus (Barret's oesophagus) (Peuchmaur et al., 1984). Columnar epithelium lined oesophagus is generally regarded as an acquired condition (Bremner et al., 1970) of adult life so the origin of the goblet cells in the present study is likely to be from the cardiac glands. The goblet cells in the present study were present only in two cases and unfortunately disappeared on serial sectioning. The site of such cells at the gastro-oesophageal junction and their likely origin in the cardiac glands cannot be construed as evidence that heterotopic intestinal epithelium is present in the gastric mucosa.

Mucin histochemistry

Sialomucins have been identified in the total gastric epithelial cells using Alcian blue pH 2.5 stain in two previously reported studies (Stemmerman, 1967; Lev 1968). Small numbers of cases were examined in these studies and the exact stage of gestation during which the sialomucins were identified is not clear (Stemmerman, 1967; Lev 1968). The present study identified only neutral mucins in the columnar cells of the fetal gastric

epithelium at all stages of gestation. The reason for the discrepancy between the results of the present study in which neutral mucins only were identified and previous studies in which sialomucins (Lev, 1968; Stemmerman, 1967) were identified is not clear. The results of the present study do not support the hypothesis that the expression of sialomucin and sulphomucin in the diseased adult stomach represents a reversion to a fetal pattern as suggested by some authorities (Filipe, 1979).

Immunocytochemistry

Intrinsic factor antibody

In the adult gastric mucosa, parietal cells are easily recognised by their typical histological appearance as described by Ito (1967); the cells are located on the periphery of the wall of the gastric gland - hence "parietal" - and this name is preferred to the alternative "oxyntic" (acid forming) cell. They are easily distinguished from the chief cells, mucous neck and surface mucous cells and neuro-endocrine cells by their characteristic position and histological appearance.

The first differentiated gastric cells appear at the base of the evolving gastric crypts at 11 weeks gestation. Ultrastructural studies (Nomura, 1966) and enzyme histochemical studies (succinic dehydrogenase)(Salenius, 1962) have suggested that these cells are parietal cells. The present study using a polyclonal antibody has localised intrinsic factor within these cells, presumptive evidence that these large eosinophilic cells at the base of the crypts have already differentiated into functional

parietal cells by 11 weeks of gestation. The significance of the finding of intrinsic factor within parietal cells at such an early gestational age is not clear and requires further study. Recent work (Facer et al., 1989) has demonstrated that the development of the fetal gastric epithelium may be preceded by the development of a fully differentiated endocrine cell component as endocrine cells are identifiable by 8 weeks gestation in the human fetal stomach. The relationship between the endocrine component and the development of the fetal stomach has still to be determined.

There is considerable variation of opinion as to the distribution of parietal cells within the developed stomach, a point which is of importance in the interpretation of gastric biopsies. Textbooks of histology such as Ham (1974) suggest that parietal cells may be seen in the region of the pyloric sphincter. Ito (1967) agrees with this but some clinical texts, for example, Morson and Dawson (1979), say there are no "acid secreting" cells in the region. The intrinsic factor-containing cells identified in the present study in the antral region of the fetal stomach are according to standard morphological criteria parietal cells. Schwartz & Weber (1971) demonstrated intrinsic factor to be present in homogenates of the antral region of the fetal stomach at 11 weeks gestation. Salenius (1962) described the presence of parietal cells in the antrum in his study of the ontogeny of human gastric epithelial cells. Tominaga (1975) also noted parietal cells in the antral region of both adult and neonatal stomachs in 98% of cases. The present study using the polyclonal antibody has

detected parietal cells at all stages of gestation in the antral region. Although fewer in number than in the body of the stomach the present study suggests that in the fetal stomach the antrum and body do not show a sharp demarcation with regard to the distribution of the parietal cells.

Carcino-embryonic antigen

The investigation of the concept that human embryonic tissues and adult tumours might share similar antigens led to the discovery of carcino-embryonic antigen discovered by Gold and Freeman, 1965, as present in fetal gut in the second and third trimester and was also present in a variety of adult epithelial tumours of gut origin. Since its initial discovery it is now clear that CEA may occur in benign disease of the adult gastro-intestinal tract (Huitric et al., 1976; Isaacson and Judd, 1977). The significance of CEA is further complicated by the cross reactions with normal antigens that can occur with anti CEA sera (Nap et al., 1983). Non specific cross reacting antigen (NCA) and non specific cross reacting antigen 2 (NCA_2) have been shown to occur in normal stomachs (Nap et al., 1983).

Although CEA was first extracted from fetal gut (Gold and Freeman, 1965) little is known about the cellular localisation of CEA within the fetal stomach. The present study has demonstrated that, using immunoperoxidase techniques and the commercially available (DAKO) with CEA antibody, CEA can be demonstrated in the gastric epithelium of first and second trimester fetal stomachs. Unfortunately third trimester stomachs were not available for study

but CEA could not be demonstrated in the neonatal stomachs studied. After absorption of the DAKO antibody with normal adult stomach to remove the NCA_2 activity (Nap et al., 1983) the immunoperoxidase staining pattern was unchanged, thus indicating that the immunoreactivity was due to CEA rather than "CEA like material".

Characteristic patterns of CEA localisation have been described in gastric metaplasia and normal and neoplastic colonic epithelia (Nagura et al., 1982; Neilsen et al., 1982; Ahnen et al., 1982). In intestinal metaplasia and normal colon the CEA was found on the apical surface of the cells and in the cytoplasm adjacent to the nucleus whereas in malignancy the CEA was distributed over the entire cell. The present study has demonstrated that CEA in fetal stomachs is located on the luminal surface and the basal cytoplasm adjacent to the nucleus in a similar manner as that described in intestinal metaplasia and normal adult colon. The CEA was present in cells at all levels of developing gastric crypts. The biological significance of the expression of the CEA glycoprotein in the fetal stomach is unclear. Studies on adult gastric epithelium presented in this thesis indicate that in the presence of gastritis the mucus neck cells of the gastric crypts express CEA. The expression of CEA may be a primary or secondary event in cell transformation both in the fetal and adult stomach.

CHAPTER 10

Summary and Conclusions

The aim of this thesis was to investigate aspects of the sequence outlined in figure 11 with particular reference to the role of intestinal metaplasia in the histogenesis of gastric carcinoma. To summarise and discuss the conclusions of the thesis it is appropriate to start at the origin of the sequence - the fetal stomach, and end with the intestinal and diffuse type tumour.

The histological study of the forty fetal stomachs illustrated the development of the gastric epithelium from a primitive pseudostratified structure to the specialised glandular formation similar to that in the adult. No histological evidence was seen to support the concept of intestinal metaplasia as a congenital heterotopia. Intestinal type cells were seen but in two distinct sites - the oesophago-gastric and duodeno-gastric junction. Both these sites represent a transition from one type of epithelium to another and with an oblique section the presence of goblet or columnar cells in this region in the gastric mucosa was not considered to represent a true heterotopia.

The immunocytochemical studies on the fetal tissue demonstrated two important findings. First that as early as 11 weeks gestation cells were immunoreactive with intrinsic factor antibody. Secondly that in the first and second trimester the fetal stomach is immunoreactive with CEA antibody. The first finding is of biological interest but further studies are required to assess the functional significance. The second finding illustrates that in the evolving stomach CEA is expressed by the epithelium as it is in the adult stomach in response to gastritis,

metaplasia, dysplasia and neoplasia.

Several aspects of the change from a healthy to an inflamed gastric mucosa have been addressed in this thesis. This change is accompanied by alterations in phenotypic expression of the epithelial cells. Abnormal mucins have been detected and the epithelial cells express secretory component, IgA and carcino-embryonic antigen. In addition to these changes in the epithelial cells there is an increase in number of neuro-endocrine cells within the gastric crypts. An increased cell turnover has been demonstrated in the inflamed mucosa by the thymidine labelling studies. The immunological studies have illustrated increasing numbers of intra-epithelial lymphocytes with T cytotoxic/suppressor and NK phenotype in gastritis and widespread induction of Class II MHC antigens (HLA-Dr) on the inflamed epithelium.

To try to integrate all the individual items of information obtained on the alterations that occur in the gastric crypts and lamina propria with inflammation into a cohesive whole is extremely difficult. The study of histological material although providing micro-anatomical detail does not provide information regarding the functional significance of these changes. The finding of large numbers of neuro-endocrine cells adjacent to lymphoid follicles suggests that there may be some link between these components of the gastric mucosa and that in turn the neuro-endocrine cells may play a role in controlling via a paracrine route the increased cell turnover of the crypts identified in the present study.

Two specific elements amongst the many aetiological factors

proposed for gastritis have been studied, namely alkaline reflux and Helicobacter pylori. Alkaline reflux gastritis is of particular surgical interest because of the possible role of surgery for benign peptic ulceration on the development of gastric cancer. The study of patients in this thesis who had undergone gastric surgery for peptic ulceration has confirmed the work by Dixon et al., 1986, in identifying the specific histological features characteristic of reflux gastritis. The kinetic studies in the present work have shown an elevation in labelling index in such patients correlated to the reflux score when compared with controls and changes in the distribution of the proliferative zone within the gastric crypts. In view of the reported increased incidence of gastric cancer in these patients these kinetic abnormalities may be direct relevance to the carcinogenetic process. The exact role played by vagal sectioning, bile constituents and alteration in pH in the genesis of the kinetic changes in the post-operative stomach require to be addressed. The most suitable vehicle for this would be an animal model.

The identification of Campylobacter-like organisms within the stomach by Warren and Marshall in 1984 coincided with the start of the collection of data for this thesis. The incidence of Helicobacter pylori in the gastrectomy study and in the biopsy material from the Medical Treatment Group is similar to that of previous studies. The low incidence of the organism in the post-operative stomach and the absence of the organism in foci of intestinal metaplasia reported by other authors, have been

confirmed. The presence of the organism has been shown in the present study to be significantly associated with a higher gastritis score and this in turn results in a significantly higher labelling index when compared with mucosal biopsies in which the organism could not be demonstrated. The relationship of the organism to the genesis of intestinal metaplasia was examined by comparing the amount of intestinal metaplasia in the gastrectomy specimens with and without the organism. The comparison showed no significant difference in the amount of metaplasia between the groups. The natural history of Helicobacter pylori remains unclear and if gastritis with subsequent intestinal metaplasia formation results in eradication of the organism then a comparison of the amount of intestinal metaplasia in gastrectomy specimens might not be expected to show any difference as the stomach with extensive IM may have eradicated the organism. The study of T cell subsets revealed no difference in the pattern of T cell distribution in inflamed mucosa with or without the organism. The presence of intra-epithelial lymphocytes in inflamed mucosa suggests that these cells in this position are a response to an antigen within the gastric lumen. Helicobacter pylori may represent the antigenic stimulus in some cases but the migration of lymphocytes demonstrated in this thesis into the intra-epithelial position is not specific to stomachs with Helicobacter pylori present.

The role of intestinal metaplasia in the histogenesis of gastric carcinoma was the initial central area of investigation of

this thesis. The present study has identified a significant association between the presence of intestinal metaplasia in stomachs resected for malignant disease compared with benign disease and a significantly increased amount of intestinal metaplasia in stomachs resected for malignant disease compared with benign disease. The benign and malignant groups however are not comparable as there were differences in age and the secondary effects of the tumours ie. inflammation and ulceration were more marked than the benign lesions. For this reason a multivariate analysis was performed and this demonstrated that age, inflammation and ulceration provided at least an alternative (and at the extremes of age, inflammation and ulceration are a more likely) explanation for the presence of intestinal metaplasia than the presence of malignancy per se. A similar pattern was seen when Type IIb intestinal metaplasia was assessed. The significant association of Type IIb intestinal metaplasia demonstrated by Jass 1980 with the intestinal type of tumour was again demonstrated in this thesis. However the results of this thesis suggest the reason for the association is not a histogenetic link between Type IIb and the intestinal type of tumour but due to effect of a higher age, gastritis score and frequency of ulceration in the intestinal tumour group.

The interpretation and identification of dysplasia is extremely difficult and observer dependent. The sub-types of dysplasia described by Jass 1983 were identified in the study but no association between any particular sub-type and specific types of tumour could be identified. Studies are required to clarify the natural history of gastric dysplasia and its clinical

significance.

The classification of the gastric tumours into the major classification systems and a comparison with the Lauren system resulted in data which was difficult to interpret but did give some insight into the problems in dealing with tumours with such a heterogenous histological picture. In an attempt to examine the Lauren classification system in a more objective manner an artificial scoring system was created. This demonstrated the considerable histological overlap between the intestinal and diffuse types of tumour. This finding raises serious misgivings about the validity of the Lauren system in dividing the tumours into intestinal and diffuse types. The question mark placed by the work of this thesis on the separate existence of tumours of intestinal and diffuse types is a further reason for doubting the role of intestinal metaplasia in the histogenesis of gastric carcinoma.

The mucin and immunocytochemical studies presented have identified a series of phenotypic changes that occur in inflamed gastric mucosa. Metaplasia, dysplasia and neoplasia have also been shown in this study to result in phenotypic changes which do not appear to be significantly different from those occurring in inflamed mucosa. The results of these studies have been interpreted as objectively as possible and no attempt has been made to prove or disprove any particular preconceived theory of histogenesis using the changes in phenotypic expression. When the results are interpreted from this viewpoint there does not

appear to be any discernible sequence of changes in phenotypic expression which are specifically associated with malignancy or any tumour sub-group.

The most important facet of any study in the field of medicine is the benefit to patients. The clinico-pathological section of the thesis has illustrated that the majority of patients present with an advanced tumour and that the prognosis is extremely poor. The clinical and pathological staging of the tumours is poorly performed. The encouraging results from Japan quoted in this thesis even in the treatment of advanced lesions may hopefully alter the therapeutic approach to these patients.

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APPENDIX

Statistical Methods

Mucin Histochemical Techniques

Statistical Methods

1. Statistical tests

The two sample t test, Chi square analysis with Yates' correction, method of least squares and correlation coefficient analysis were performed using the methodology outlined in "Interpretation and Uses of Medical Statistics" by Bourke, G.J., Daly, L.E., McGilvry, J., Blackwell Scientific Publications, Oxford, 1985.

2. GLIM analysis

A. Estimates of probability for the presence of intestinal metaplasia of any type are given below for single variables and for combinations of variables.

For any particular hypothesis the probability of the presence of metaplasia in a patient with a particular set of variable values takes the form:

$$\frac{\exp(t)}{\exp(t) + 1}$$

where t depends on the hypothesis and the variable values.

Ulceration

$t = c + u_r$ $\quad u_1$ = non ulcerated = 0

u_2 = ulcerated = -0.2301

c = 1.186

Inflammation

$t = c + i_s$

i_1 = grade 1 gastritis = 0

i_2 = grade 2 gastritis = 0.1335

i_3 = grade 3 gastritis = 1.281

i_4 = grade 4 gastritis = 10.92

c = -0.6931

Age

$t = c \times age$

c = -2.751 b = 0.06155

Diagnostic Group

$t = c + gr$

g_1 = benign = 0

g_2 = intestinal type = 9.681

g_3 = diffuse type = 8.929

c = -9.496

Inflammation and age (i + a)

$t = c + i_r + b \times age$

i_o = 0

i_2 = 0.8485

i_3 = 1.972

i_4 = 11.85

i_5 = 11.67

c = -6.363

b = 0.0808

Ulceration and age

$t = c + u_r + a$

u_1 = no ulceration = 0

u_2 = ulceration = -0.2482

b = 0.06178

c = -2.650

Ulceration and inflammation

$t = c + u_r + i_s$

i = 0

i_2 = 0.1643

i_3 = 1.487

i_4 = 11.27

i_5 = 11.35

u_1 = no ulceration = 0

u_2 = ulceration = 0.8811

c = -1.312

Ulceration, inflammation and age

$t = c + u_r + i_s + b$ x age

i_1 = 0

i_2 = 0.9052

i_3 = 2.358

i_4 = 12.36

i_5 = 12.56

b = 0.09068

u_1 = 0 = no ulceration

u_2 = 1.172 = ulceration

c = -7.940

B. Estimates of probability for the presence of Type IIb intestinal metaplasia for single variables and combinations of variables are given below.

Inflammation

$t = c + i_s$

i_1 = grade 1 gastritis = 0

i_2 = grade 2 gastritis = -0.693

i_3 = grade 3 gastritis = 0.5109

i_4 = grade 4 gastritis = 2.120

i_5 = grade 5 gastritis = 1.341

c = -1.609

Age

$t = c + b \times age$

c = -3.923

b = 0.04853

Ulceration

$t = c + u_r$

u_1 = no ulceration = 0

u_2 = ulceration = 0.3875

Diagnostic group

$t = c + g_r$

g_1 = benign = 0

g_2 = diffuse type tumour = 0.575

g_3 = intestinal type tumour = 3.526

c = -2.079

Inflammation and age

$$t = c + i_s + b \text{ age}$$

$$i_1 = 0$$

$$i_2 = -0.2527$$

$$i_3 = 0.7906$$

$$i_4 = 2.527$$

$$i_5 = 1.527$$

$$b = 0.04946$$

$$c = -5.124$$

Ulceration and age

$$t = c + u_r + b \times \text{age}$$

$$u_1 = 0$$

$$u_2 = -0.0403$$

$$i_1 = 0$$

$$i_2 = 0.6944$$

$$i_3 = 0.502$$

$$i_4 = 2.109$$

$$i_5 = 1.325$$

$$c = -1.583$$

Ulceration, inflammation and age

$$t = c\ u_r + i_s\ b \times \text{age}$$

$u_1 = 0$

$u_2 = 0.0603$

$i_1 = 0$

$i_2 = -0.2547$

$i_3 = 0.8009$

$i_4 = 2.545$

$i_5 = 1.551$

$b = 0.04978$

$c = -5.185$

3. Validation of Counting Techniques

A. The repeated counts of the number of gastric crypts and intestinal metaplatic crypts in 10 cases from the human gastrectomy study are shown in table 49.

B. The repeated counts between two different areas and on the same area in 10 cases from the Thymidine Labelling Study are shown in table 50.

C. The repeated counts between two different areas and on the same area in 10 cases from the Immunological Study for the three counting techniques, high power fields, point counting and mucosal unit length are shown in tables 51, 52 and 53.

Case	First Count				Second Count				Coefficient
	gastric	I	IIa	IIb	gastric	I	IIa	IIb	of variation
1	1317	32	0	0	1341	32	0	0	1.27
2	164	821	0	0	175	837	0	0	3.2
3	707	221	8	23	725	208	11	24	4.3
4	342	17	0	9	353	19	0	8	0.43
5	1417	36	0	9	1417	36	0	9	0
6	1457	48	7	0	1506	53	6	1	3.8
7	1011	52	11	13	1001	56	8	14	2.5
8	1628	57	0	0	1639	63	0	0	4.6
9	2178	0	0	0	2206	0	0	0	0.9
10	1439	210	0	0	1480	26	0	0	9.2

Table 49. Repeated counts of the number of gastric crypts and intestinal metaplastic crypt sub-types (I, IIa and IIb) in 10 cases from the Human Gastrectomy Study. Coefficient of variation for ratio of gastric to metaplastic crypts between counts is shown.

	Area 1		Area 2			
			First count		Second count	
Case No	L	U	L	U	L	U
1	15	762	4	465	9	891
2	48	1011	27	843	76	1733
3	51	758	91	1046	87	1209
4	173	1937	159	1221	182	1226
5	11	2263	17	866	23	798
6	47	1174	23	897	27	809
7	118	2181	60	1607	49	1584
8	253	1712	167	1022	152	933
9	65	1304	84	1212	79	1331
10	3	1198	5	643	8	1554

Table 50. Repeated counts in 10 cases from Thymidine Labelling Study. Two areas counted - area 1 and area 2, count repeated in area 2.
L = labelled cells; U = unlabelled cells.

Case	Count 1, Area 1			Count 2, Area 1			Count 3, Area 2		
	L	U	T	L	U	T	L	U	T
1	51	162	213	24	185	209	17	120	137
2	17	89	106	35	90	125	53	65	118
3	22	97	119	41	97	138	29	84	113
4	37	113	150	60	78	138	18	113	131
5	11	51	52	21	54	75	37	57	94
6	61	76	137	53	85	139	23	72	95
7	33	143	176	15	129	144	48	135	183
8	12	80	92	70	36	106	18	77	95
9	9	124	133	34	99	133	48	102	150
10	23	163	186	41	158	191	30	163	193

Table 51. Repeated counts in 10 cases using High Power Fields x 5 for Leu 1 labelled cells. Three counts performed: two counts on same area and third count on different area (area 2).

L = labelled, U = unlabelled, T = total number of cells counted.

Case	Count 1, Area 1			Count 2, Area 1			Count 3, Area 2		
	L	U	T	L	U	T	L	U	T
1	21	41	62	13	26	39	23	28	51
2	17	24	41	9	41	50	17	31	48
3	30	60	90	22	37	59	30	19	49
4	7	43	50	15	20	35	33	42	75
5	45	29	74	16	33	49	15	15	30
6	11	19	30	25	40	65	19	26	45
7	26	15	41	28	51	79	21	28	49
8	14	26	40	10	19	39	35	39	74

Table 52. Repeated counts in 10 cases using Point Counting Technique for Leu 1 labelled cells. Three counts performed: two counts on same area and third count on different area (area 2).
L = labelled, U = unlabelled, T = total number of cells counted.

Case	Count 1, Area 1			Count 2, Area 1			Count 3, Area 2		
	L	U	T	L	U	T	L	U	T
1	27	65	92	37	41	78	33	81	114
2	33	81	114	47	80	127	41	69	110
3	40	73	113	15	72	87	26	42	68
4	19	49	68	29	65	94	43	67	110
5	45	60	105	34	51	85	27	72	99
6	16	73	89	37	66	103	39	83	122
7	13	87	90	44	90	134	25	56	81
8	43	93	136	18	84	102	49	41	90
9	17	50	67	24	26	50	36	59	95
10	42	63	85	18	41	59	23	38	61

Table 53. Repeated counts in 10 cases using Mucosal Unit Length for Leu 1 labelled cells. Three counts performed: two counts on same area and third count on different area (area 2).
L = labelled, U = unlabelled, T = total number of cells counted.

4. Correction Factor for counting thymidine labelled cells in cross sections (after Hansen et al., 1975).

When cross sections containing labelled cells are counted, the labelling index will be overestimated because cross sections through the progenitor region which do not have labelled cells will not be included in the estimation. This error can be reduced by the use of the equation:

$$(1 - p)^n = 1 - \frac{p}{LI}$$

where p represents the corrected labelling index, n equals the mean number of cells in the cross sections and LI the actual estimated labelling index.

MUCIN HISTOCHEMISTRY

Alcian Blue/PAS technique (pH 2.5)

Method

(1) Bring sections to water.

(2) Stain in freshly filtered 1% Alcian blue 8GX in 3% acetic acid for 30 minutes.

(3) Wash in water.

(4) Oxidize for 5 minutes in 1% aqueous periodic acid.

(5) Wash in running water for 5 minutes and rinse in distilled water.

(6) Treat with Schiff reagent for 15 minutes.

(7) Wash for 10 minutes in running water.

(8) Counterstain with haematoxylin.

(9) Dehydrate, clear and mount in synthetic resin.

Diastase-periodic acid Schiff (ref 133)

Method

(1) Bring sections to water.

(2) Digest with diastase (1:1000 malt diastase in distilled water) for 30 minutes at 37°C.

(3) Wash in water for 5-10 minutes.

(4) Wash with 90% alcohol, then absolute alcohol for half a minute.

(5) Transfer to stoppered container of 1% celloidin in equal parts

alcohol and ether for 2 minutes.

(6) Drain off the excess of celloidin and transfer to 80% alcohol for 5 minutes.

(7) Wash in running tap water for 2 minutes.

(8) Oxidize for 5 minutes in 1% aqueous periodic acid.

(9) Wash in running water for 5 minutes, and rinse in distilled water.

(10) Treat with Schiff reagent for 15 minutes.

(11) Wash for 10 minutes in running water.

(12) Counterstain with haematoxylin.

PB/KOH/PAS (REF 134)

Method

(1) Bring sections to water

(2) Oxidize in 1% periodic acid at room temperature for 1 hour.

(3) Wash in water for 10 minutes.

(4) Treat with 0.1% sodium borohydride in 1% disodium hydrogen phosphate for 30 minutes.

(5) Wash in water.

(6) Treat with 0.5% potassium hydroxide in 70% alcohol for 30 mins.

(7) Wash in 70% alcohol.

(8) Wash in water.

(9) Stain by PAS technique.

(10) Dehydrate, clear and mount in synthetic resin.

PB/PAS Method

Used as a control to ensure that all PAS reactivity has been

abolished by the periodic acid.

Method

(1) Bring sections to water

(2) Treat as above, omitting steps (6)-(8).

(3) Dehydrate, clear and mount in synthetic resin.

High Iron Diamine/Alcian Blue

Reagent - diamine solution.

N,N-dimethyl-m-phenylenediamine dihydrochloride 120 mg

N,N-dimethyl-p-phenylenediamine hydrochloride 20 mg

Diamines dissolved in 50 ml of distilled water and 1.4 ml of 40% ferric chloride. The pH of prepared solution should be between 1.5-1.6. The solution is used immediately.

Method

(1) Bring sections to water.

(2) Stain in diamine solution in Coplin jar for 24 hours.

(3) Stain in freshly filtered 1% Alcian Blue 8GX in 3% acetic acid (pH 2.5) for 30 minutes.

(4) Wash in water.

(5) Dehydrate, clear and mount in synthetic resin.

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