STUDIES ON PROSTAGLANDINS, NON-STEROIDAL ANTI-INFLAMMATORY DRUGS, AND HELICOBACTER PYLORI IN PEPTIC ULCER DISEASE

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PREFACE.

This Thesis describes a group of studies carried out during my appointment as a Research Fellow at the Gastro-enterology Unit, Royal Infirmary, Glasgow. Parts of this work were published and others are being considered for publication. Reprints, where available, are submitted with the Thesis. Some of the work described was also presented to learned societies.

The help and contribution of a number of colleagues and co-workers are formally acknowledged. Except where indicated, the work and writing of this Thesis has been personally carried out by me.

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SUMMARY.

Gastric acid has always been recognized as a major factor in the pathogenesis of peptic ulcer disease. Recently, it has been suggested that three more factors are likely to influence the natural history of peptic ulceration: prostaglandins (PGs), non-steroidal-anti-inflammatory drugs (NSAIDs), and Helicobacter pylori (H. pylori).

Despite the widespread recognition of the potentially useful properties of PGs, the methodology used in their research has often given rise to conflicting and irreproducible results. Apart from the type of assay used (e.g. bioassays, radioimmunoassays, gas chromatography - mass spectroscopy), the method of handling the tissues prior to the assay is likely to affect their potential to synthesise PGs. Evidence is presented in this Thesis, that freezing gastric and duodenal biopsies alters their ultrastructure and capacity to synthesise PGs. When paired gastric and duodenal biopsies were studied (each pair consisted of one fresh and one frozen biopsy), it was found that freezing resulted in disintegration of the cell membranes of all the cells within the individual biopsies. Specimens incubated after having been frozen, produced more PGE_2 and PGI_2 than their fresh counterparts. This could be due to the availability of more PG precursors derived from the cell membranes which have been damaged by the effect of freezing. Compared to 67% cytolysis produced by some ordinary homogenizers, freezing seems to be a more

convenient and effective method of homogenization, since it causes damage of 100% cells in biopsies destined for PG measurements. In the absence of general guidelines as to whether biopsies should be processed fresh or after freezing them in the context of PG measurements, the above findings would help understand the consequences of both of these options.

In recent years diet has emerged as being able to affect gastric mucosal PG synthesis, and as a result, the capability of the mucosa to defend itself against injurious agents. Most experiments have used individual polyunsaturated fatty acids like arachidonic acid and linoleic acid. It was not clear if these acids would still stimulate gastric PG synthesis if given as components of oil diets that also contain other fatty acids capable of inhibiting the cyclo-oxygenase pathway, such as eicosapentaenoic acid. This issue is addressed in this Thesis by studying gastric tissue PG synthesis in four groups of male Lewis rats which were fed for six weeks on fat-free diet (control group), 7% fish oil diet, 7% evening primrose oil diet, and 7% olive oil diet. Gastric PGE_2 was higher in rats taking any of the oil diets studied as compared to the control group. Olive oil diet induced the production of more PGE_2 than fish oil, which was found to contain a significant proportion of the inhibitory acids, eicosapentaenoic acid and clupanodonic acid (docosahexaenoic acid). Given the cytoprotective characteristics of PGs, these results

indicate that diets rich in the above oils would help the gastric mucosa maintain its integrity. If such findings could be confirmed in man, they might be relevant to the understanding of the incidence of peptic ulceration in those areas in the world where olive or fish oils constitute part of the local staple diet.

The relationship between duodenal mucosal PG synthesis and duodenal ulcer formation remains controversial since some studies showed deficiency in PG synthesis, while others did not. The situation has been further complicated by the failure of PG analogues to make a significant impact in the treatment of duodenal ulcers when compared to H_2-antagonists. The state of duodenal PG synthesis is re-examined in this Thesis in a group of patients with duodenal ulcers. The results show no evidence of any deficiency in PGE_2, PGI_2, or thromboxane B_2 in duodenal biopsies taken from patients with duodenal ulcers, as compared to a group of control subjects without ulcers. The failure of duodenal mucosal PGs, in duodenal ulcer patients, to rise in response to the challenge with an acid load shown by a previous study, is probably more important than their basal PG values.

There is a continuous demand for an alternative anti-inflammatory therapy with fewer gastric side effects, especially in patients with chronic diseases like rheumatoid arthritis. Endoscopy is probably one of the most, if not the most, reliable means of monitoring the side effects of NSAIDs on the gastric and duodenal

mucosa. Such procedure will have to be used at one stage or another in the course of the proper assessment of the gastro-duodenal side effects of NSAIDs, especially the newer preparations. The efficacy of one of the new NSAIDs, etodolac, and its effect on the gastro-duodenal mucosa as judged by endoscopy, are studied in this Thesis in a group of patients with active rheumatoid arthritis, as compared to naproxen which has an established record in the treatment of arthritic diseases. After four weeks of treatment both agents appeared to have a comparable efficacy. Endoscopic abnormalities developed in a greater number of patients taking naproxen, who also had significantly worse endoscopic scores. Most lesions were asymptomatic, and the gastric antrum was mainly affected especially in smokers.

Another study in this Thesis investigates the possibility that the differences between naproxen and etodolac on the gastric and duodenal mucosa are related to their effects on mucosal PG synthesis. PG values were measured at baseline and after four weeks of taking either agent in therapeutic doses by patients with active rheumatoid arthritis. Naproxen suppressed gastric PGE_2, duodenal PGE_2 and PGI_2, while etodolac did not. The fact that naproxen caused a greater number of endoscopic abnormalities might therefore be related to its effect on gastric and duodenal PG production, but the study found no correlation between PG values and endoscopic scores. Also, patients taking etodolac who developed evidence of

gastric damage, showed no significant change in their mucosal PG synthesis. This study, which is unique in being the first to assess the effect of individual NSAIDs on gastric and duodenal tissue PGs in rheumatoid patients, shows that not all NSAIDs suppress gastric or duodenal PG production. Other mechanisms of mucosal damage by NSAIDs need to be considered in addition to their effects on PGs.

While PGs are known for their favourable properties in relation to the gastrointestinal mucosa, Helicobacter pylori (H. pylori) has been linked to the development of gastritis and peptic ulceration. It is not clear whether an interaction exists between PGs and H. pylori. This subject is considered by a study in this Thesis in which the presence of H. pylori is determined by both histology and bacteriology in patients with antral gastric ulcers (GU) and non-ulcer dyspepsia (NUD). PGs are measured in biopsies taken from the antrum, and at least 2 cm away from the ulcer edge in GU patients. In line with previous findings, this study shows that H. pylori is associated with a significant number of cases with GU or active chronic gastritis. Also PG values are higher in patients with severe gastritis or GU than those with mild gastritis or NUD, when the presence of H. pylori is disregarded. Surprisingly, there is no significant difference between PG values in patients infected with H. pylori or those free of the organism, despite the presence of a greater number of cases with gastritis in the infected group. These findings might encourage

speculation about the presence of at least a partial defect in PG production at the level of the mucosal cells or the neutrophils, which can also synthesise PGs. However, the presence of such defect is not confirmed by this study.

The understanding of the activity of H. pylori was enhanced by the findings of Leunk et al. in 1988, which suggested that H. pylori culture filtrates contained a factor cytotoxic against Vero (mammalian) cell lines. The effect of this factor on the human gastric mucosa or its possible interaction with another mucosal damaging agent, indomethacin, are not clear. These points are addressed by another study in this Thesis. The combination of H. pylori culture filtrate and indomethacin reduces mucosal PGE_2 production and the histological scores to a greater extent than indomethacin alone, in vitro. This might indicate the presence of an interaction between indomethacin and H. pylori culture filtrate. Such interaction appears to be damaging to the human gastric mucosa.

Many studies have attempted to establish a link between H. pylori and the development of structural damage to the gastro-duodenal mucosa. The possible effect of the organisms on the function of the gastric mucosa is not fully understood. A protein isolated from H. pylori culture filtrates inhibited acid secretion by rabbits parietal cells, as shown by Cave and Vargas in 1989. Cyclic AMP acts as a second messenger for several

physiological activities in the gastric mucosa, including acid secretion. In this Thesis, H. pylori protein is isolated from the culture filtrates, and its effect on c.AMP production investigated. H. pylori protein is shown to suppress the generation of c.AMP in the fundic mucosa *in vitro*, intensify its inhibition by indomethacin and prevent its stimulation by histamine. This might explain the acid inhibitory effect of H. pylori protein. It also suggests that this protein has the potential to interfere with other physiological activities mediated by c.AMP.

SECTION 1

BACKGROUND OF THESIS.

INTRODUCTION.

The understanding of the pathogenesis of peptic ulcer disease has been aided by the recent identification of three major factors: prostaglandins, non-steroidal anti-inflammatory drugs, and Helicobacter pylori. This Thesis contains a group of studies covering certain aspects related to these topics. Prostaglandin measurements are common to all sections of this Thesis.

Section 1 reviews past and recent findings in the areas under discussion. It also describes methods and techniques common to various studies in this Thesis. These include prostaglandin radioimmunoassay, identification of H. pylori, preparation of H. pylori culture filtrate, and isolation of H. pylori protein. This section ends with a summary of the aims of this Thesis.

CHAPTER 1.

1. CHEMISTRY AND PHARMACOLOGY OF PROSTAGLANDINS.

1.1. INTRODUCTION AND HISTORICAL BACKGROUND.

Prostaglandins (PGs) constitute a family of chemically related fatty acids that are among the most prevalent naturally occurring compounds; they seem to modulate many biological functions in the body. They are formed by a complex of microsomal enzymes, acting on certain 20-carbon unsaturated fatty acids, particularly eicosatetraenoic or arachidonic acid (AA). Additional products of the cellular metabolism of AA, differing in structure from PGs, include the thromboxanes (TX) and leukotrienes (LT), whose physiological and pathological functions are closely interrelated with those of the PGs.

The existence of the PGs has been known for over half a century. Kurzok and Lieb (1) were the first to observe, in 1930, that strips of human uterus relax or contract when exposed to human semen. In the mid 1930s, von Euler (2) in Sweden and Goldblatt (3) in England independently extracted crude PGs from the seminal fluid of man and vesicular glands of sheep, and reported their smooth-muscle-contracting and vasodepressor activities. In the late 1950s, Bergstrom and Sjovall (4) isolated PGs in a pure form and, in the early 1960s, determined their chemical structure. With the identification of two

unstable cyclic endoperoxides, PGG_2 (5) and PGH_2 (6), the emphasis shifted from the primary PGs (E and F series) to other biologically active products of AA metabolism, resulting in the discovery of TXA_2 (7) and prostacyclin, PGI_2 (8). In addition to the cyclo-oxygenase mechanism, another metabolic pathway, converting AA to hydroperoxides (HPETE and HRTE) (9,10) and then to LTs (11) was identified. The impact of PGs and other products of AA metabolism could be compared only to that of corticosteroids in the late 1940s and 1950s.

1.2. CHEMICAL STRUCTURE AND BIOSYNTHESIS.

The natural PGs are considered analogues of prostanoic acid, a structure with a 20-carbon and 5-membered ring. They fall into several classes designated by letters, indicating different substituents on the ring, alphabetically, in order of their discovery. The main classes are subdivided according to the number of double bonds on the side chain indicated by the subscript 1,2 or 3.

The common precursors of PGs, TXs, and LTs are three naturally occurring eicosapolyenoic acids: trienoic (dihomo-γ-linolenic) acid, tetraenoic (arachidonic) acid (AA) and pentaenoic acid (12). In man, tetraenoic acid (AA) is by far the most common, giving rise to the PGs of the subscript-2 series. It is either derived from dietary linoleic acid or ingested as a constituent of food. After absorption from the gut, it is esterified

and presents ubiquitously in the body as a component of phospholipids of cell membranes and other complex lipids.

The hydrolysis of esterified AA provides the first rate-controlling step in PG formation. Dihomo-γ-linolenic acid, which has one less double bond than AA, gives rise to PGs of the subscript-1 series. Pentaenoic acid is very rare, derives from fatty acids with three double-bonds, and is converted to PGs of the subscript-3 series.

In mammalian tissues, PGs and their precursor acids occur in appreciable quantities only in seminal, menstrual and amniotic fluids. The precursor acids are present as phospholipids in cell membranes. A wide variety of divergent physical, chemical, and neurohormonal factors may activate the enzyme phospholipase A_2, setting free the precursor acids to gain access to enzyme complexes present in most tissues. Once released, fatty acids can follow two pathways: the cyclo-oxygenase pathway gives rise to the PGs and TXs, while the lipoxygenase pathway gives rise to the LTs and other unsaturated hydroxy acids. The metabolic pathway for AA and the structures of selected metabolites are shown in Figure 1.

The chain of events leading to the release and metabolism of AA from the membrane phospholipids can be initiated by a number of factors: nerve stimulation, neurotransmitters (e.g. norepinephrine), neuropeptides (e.g. somatostatin), various humoral agents (e.g.

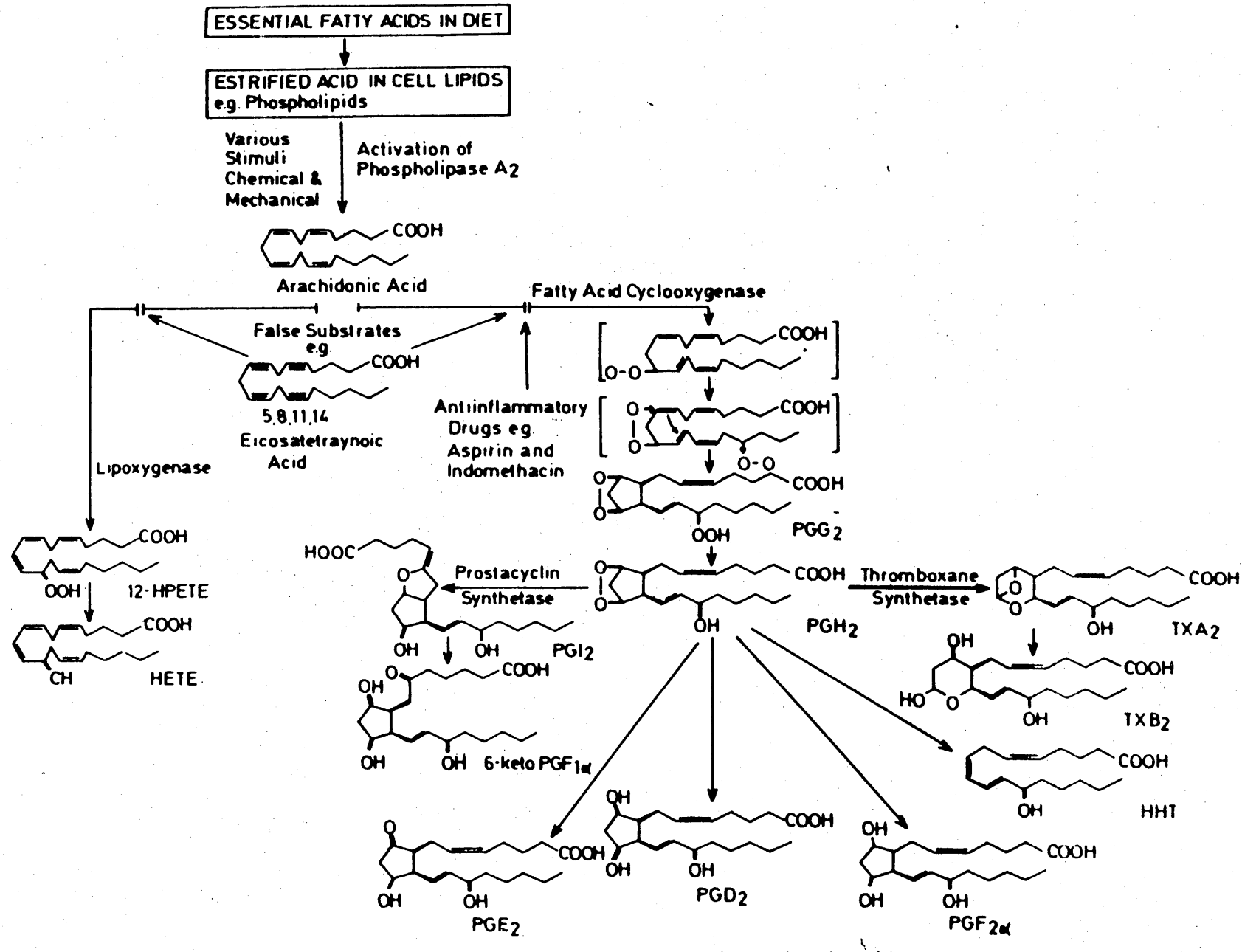


Fig 1. Biosynthesis of the products of arachidonic acid.

bradykinin), hyperosmolar solutions and even mechanical strain. Indeed, almost any deformation of cell membrane,such as stretching a blood vessel, inflation of the lungs or contraction of the intestine, may lead to increased PG formation under physiological conditions (13-15). Numerous pathological conditions may increase PG and TX synthesis. Any damage of tissue, apart from frank trauma, increases the generation of cyclo-oxygenase products (16). Acute cardiac ischaemia favours the synthesis of vasoconstrictor products over normal vasodilator metabolites of AA. Tissue injury from an anaphylactic reaction or oedema may augment PG formation (e.g. in the lungs) and may contribute to vascular permeability. Acute hypoxia or exposure to tobacco smoke in the lungs also increases PG synthesis.

Thus, many kinds of tissue injury may lead to increased generation of PGs and LT products (17). Breakdown of lysosomes will release, among other enzymes, phospholipases, which may hydrolyse AA from the membrane phospholipids.

Not all metabolites are formed in every tissue when AA is released; it depends on the most active enzymes in the tissue involved. All cells contain phospholipids and at least some cyclo-oxygenase and lipoxygenase. Most tissues seem able to synthesise PG endoperoxides from free AA, but the factors controlling their further steps have not been defined. Certain tissues (such as lung, spleen, gastrointestinal tract, thyroid and adrenals) are able to

synthesise the whole range of products, whereas other tissues predominantly produce PGD_2 (mast cells), PGE_2 (seminal vesicles), PGI_2 (vessel wall) or TXA_2 (platelets).

Products of lipoxygenase are direct inhibitors of the cyclo-oxygenase pathway, suggesting the existence of a negative feedback relationship between these two pathways. Similarly the inhibition of TX biosynthesis (e.g. by imidazole derivatives) leads to increased formation of primary PGs (18).

The PG intermediates, PGG_2 and PGH_2, are highly unstable, apparently existing only momentarily in vivo. PGs are rapidly inactivated, the first step being the oxidation of the C-15 hydroxyl group by 15-hydroxy-PG-dehydrogenase (PGDH). This enzyme is widely distributed in many tissues, especially the lungs; consequently, about 95% of PGs of the E and F series, but not D or I, are metabolised during a single passage through the lungs. The 15-keto compound is then reduced by a reductase enzyme (PGR) to the 13,14-dihydro derivatives. Subsequent beta-oxidation and omega-oxidation of the side-chains causes further degradation, giving rise to dicarboxylic acids in the urine. Both PGDH and PGR are intracellular soluble enzymes, so that the substrate must pass through the cell membrane before degradation can proceed. Natural PGE_2 and PGF_2 pass through the membrane and are quickly inactivated. In contrast, methylated PGs (e.g. 16, 16-dimethyl PGE_2) also cross the

membrane but are not the substrate for PGDH; therefore, they are not taken up by the lung tissue and then slowly released unchanged into the circulation. This results in prolonged biological activity. PGI_2, which is the substrate for PGDH in vitro but not for transfer into lung cells, can pass unchanged from the venous to the arterial blood. Such selectivity in PG inactivation is not seen in the gastrointestinal mucosa or in the liver, where all natural PGs are inactivated on passage through the portal circulation. However, methylated PGs can escape inactivation, being resistant to the mucosal or hepatic PGDH; they are active orally and , in part, act from the gastric lumen directly on the gastric glands. PGD_2 and TXA_2 are not degraded by PGDH, although they possess a 15-hydroxyl group. TXA_2 is very unstable and spontaneously converts to TXB_2, which follows the catabolic route of other PGs. PGI_2 is also hydrolysed spontaneously to inactive 6-keto-PGF_1 .

1.3. PHYSIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES.

PG-mediated control of cellular, tissue or organ functions reflects the interactions between different PGs, TXs and LTs; this might explain why some PGs have directly opposing actions in many systems.

1.3.1. The Gastrointestinal Tract - Introduction.

PGs of the E,F and I series are synthesised in substantial amounts throughout the gastrointestinal tract (19,20). There are marked species differences in the

generation of various types of PGs. Quantitatively, the predominant form of PGs in human, feline and canine gastric mucosa seems to be PGE_2 (21), whereas it is PGI_2 in the rat's mucosa (20).

PGs are released under various conditions, such as neurohormonal stimulation of digestive gland secretion, mechanical strain on the gut wall, action of various humorals (acetylcholine, norepinephrine, angiotensin, bradykinin, histamine, etc.), and the presence of hyperosmolar solutions or other irritants in the gut lumen (22).

1.3.2. Effects on Gastric Secretions.

PGs of the E and I series inhibit basal and stimulated gastric acid secretions both in vitro and in vivo. In the dog, either PGE_2 or PGI_2 inhibits gastric acid secretion stimulated by food, histamine, pentagastrin, 2-deoxyglucose, carbachol or reserpine (23,24). PGI_2 was found to be several times more potent than PGE_2 in inhibiting acid secretion when given intravenously (25). With direct intra-arterial administration, PGE_2 is a more potent inhibitor than PGI_2, and, in contrast to PGI_2, causes a reduction in mucosal blood flow (26). Methyl analogues of PGE_2 were found to be many times more potent than native PGE_2 in inhibiting gastric acid secretion in animals and in humans. The gastric inhibitory effect of natural PGs and their methylated analogues has been confirmed in humans. PGE_1

and PGE_2 are reportedly inactive when given orally and are capable of inhibiting histamine- and pentagastrin-induced acid secretion only when infused intravenously (27,28). PGE_2 was later reported to cause a moderate inhibition of gastric secretion when given orally in larger doses (29). In contrast, methyl PGE_2 analogues were found to be active gastric inhibitors after either intravenous or oral administration (30). Their prolonged inhibitory effects on basal and pentagastrin- or meal-stimulated gastric secretion were observed in healthy subjects, in peptic ulcer patients, and in patients with Zollinger-Ellison syndrome.

The mechanisms by which natural PGs and their analogues inhibit gastric acid secretion is still unclear. The possibility of direct inhibition of parietal cells by methyl PGE_2 analogues acting from the gastric lumen is supported by the finding that these agents are capable of inhibiting acid formation in isolated parietal cells, in gastric glands and in isolated gastric mucosa (31). PGE_2 analogue was more effective after topical application to the human gastric mucosa than intraduodenal or jejunal administration (32).

The antisecretory PGs probably operate via special membrane receptors to reduce the formation of cyclic AMP in the parietal cells. They decrease histamine-stimulated accumulation of cyclic AMP in the parietal cells as well as the secretory activity of these cells. They appear to block the stimulatory action of histamine

on cyclic AMP synthesis, thus removing the major pathway for the excitation of parietal cells by histamine and other interacting secretagogues such as acetyl choline or gastrin (33,34).

Another mechanism through which PGs might affect gastric secretion is suppression of gastrin release. Methyl PGE_2 analogues given orally caused a marked suppression of gastrin response to a meal (30).

PGs, and particularly their methyl analogues, when applied topically to the gastric or duodenal mucosa, stimulate mucus-alkaline secretion; this may account, at least in part, for the reduction in luminal acidity observed with administration of these PGs. It may also contribute to the enhancement of mucosal protection (35-37). In contrast to the mucosal alkaline secretion, PGs reduce bicarbonate secretion by the pancreas. PGI_2 in particular was shown to reduce the stimulated pancreatic secretion of bicarbonate and protein enzymes (31).

1.3.3. Mucosal Protection (Cytoprotection).

PG analogues, when given topically or parenterally in non-anti-secretory doses, are capable of preventing the gross mucosal lesions of the stomach and intestine resulting from exposure to various ulcerogens and irritants (38). This protection can be reproduced by intragastric administration of lower concentrations of damaging agents (e.g. 20% ethanol or 5 mM taurocholate). Since these "mild irritants" enhance mucosal PG

biosynthesis, it is likely that their protective action is mediated by endogenous PGs (38-40).

The term cytoprotection is now quite popular in medical literature; however, PGs and mild irritants do not completely protect the mucosa from damage by necrotizing agents. It appears that the mucosa exposed to a necrotizing agent (whether or not pretreated with PG) undergoes extensive damage in the surface epithelium, which is exfoliated and replaced by epithelial cells migrating from the gastric pits. PGs do, however, protect the deeper mucosal layers, thus facilitating prompt recovery of the mucosa. It is likely that the protection by PGs against gastric mucosal bleeding from NSAIDs or ethanol, and speeding of the healing rate of gastroduodenal ulceration in humans are due, at least in part, to the protective properties of PGs.

Cytoprotection is probably not limited to the gastrointestinal mucosa; damage to other digestive organs, such as the liver and the pancreas, can also be reduced by certain PGs (31).

The nature of cytoprotection is unknown but several mechanisms have been postulated, including an increase in mucus-alkaline secretion (35,36), enhancement of mucosal blood flow, and stabilization of lysosomal membranes coupled with a reduction in the release of lysosomal enzymes (41). It has also been suggested that PGs maintain the hydrophobicity of mucosal surface and reverse

the reduction of this hydrophobicity by damaging agents such as aspirin (42). Similar effects have been obtained using phospholipids with surfactant properties (43).

1.3.4. Effects on Intestinal Contractility, Secretions and Blood Supply.

Exogenous PGs exhibit a considerable variability in their action on intestinal smooth muscle, depending on the type of PG, the dose, and the muscle layer studied. In general, PGs of the E and I series inhibit, whereas PGs of the F series stimulate intestinal contractility (44).

PGE and its methylated analogues strongly stimulate intestinal fluid and electrolyte accumulation in the gut lumen (enteropooling), which may result in diarrhoea (45). PGs of the I series exert the opposite effect, reducing the enteropooling action of the E series (46). Diarrhoea is one of the major side effects occurring in patients treated with PGE and its stable analogues (47).

PGs appear to play an important role in the regulation of the gastric circulation and can alter gastric blood flow either by a direct effect on the vascular smooth muscle or, indirectly, by action on the other gastric functions. Systemic or local infusion of PGs of the E and I series or their stable analogues results in an increase in basal gastric blood flow; in contrast, when given during stimulated gastric secretion, they may decrease blood flow as a consequence of the inhibition of gastric acid secretion (22,23). Since

aspirin and indomethacin decrease total gastric blood flow, which is mainly confined to the mucosal-submucosal layer, it has been suggested that local PGs in the gastric wall are important in the control of gastric blood flow (48,49).

Arachidonic acid and PGs of the E and I series infused into the superior mesenteric artery produced a marked intestinal vasodilation, whereas the inhibitors of cyclo-oxygenase show opposite effects (25,50). The same series also increases oxygen consumption in the gut, probably due to stimulation of intestinal transport processes (22). F series PGs constrict the intestinal vascular bed and reduce oxygen consumption. Results of studies using pharmacological doses of PGs indicate that they play an important role in the regulation of resting blood flow and the vascular responses to food (48-50).

Because of their gastric antisecretory and mucosal protective properties, PGE_2 and its methylated analogues have been tried in peptic ulcer therapy (51,52) and in the prevention of gastric toxicity of NSAIDs (47). The results of these clinical trials indicate that PGs are superior to placebo in accelerating ulcer healing or in ulcer prevention; however, side effects, particularly diarrhoea limit their wider clinical use.

1.4. THE ROLE OF DIETARY FATTY ACIDS IN PG PRODUCTION.

In addition to their effect on PG production, polyunsaturated fatty acids in general and omega-3 fatty

acids in particular are thought to affect a wide range of biological processes. They are largely derived from marine animals, and differ from the 18-carbon unsaturated fatty acids common in Western diets (linoleic and oleic acids) in that their first double bond is located between the third and fourth carbon atoms from the methyl end (hence the designation N-3 or omega-3). The two major omega-3 fatty acids are eicosapentaenoic acid (C20:5, omega-3) with five double bonds and docosahexaenoic acid (C22:6, omega-3) with six double bonds. Major effects of these fatty acids include reduction of plasma triglyceride and lipoprotein levels (53,54), reduction of thrombogenecity of platelets in the microcirculation (55) due to effects on the mediators derived from arachidonic acid (56), altered inflammatory and immune cell function (57), and retarded development of atherosclerosis (58).

As for their effect on the gastric mucosa, polyunsaturated fatty acids (linoleic and arachidonic acids), when given to experimental animals, result in increases in PG concentrations in gastric juice (59,60) and these prevent experimental mucosal injury (60). It is unclear, however, whether gastric PG production can still be increased by linoleic or arachidonic acids when given as part of naturally occurring oils that also contain other fatty acids like eicosapentaenoic acid and docosahexaenoic acid which competitively inhibit the utilization of arachidonic acid and its precursors by cyclo-oxygenase (12,61,62).

One of the studies included in this Thesis (Chapter 7) shows that gastric tissue PGE_2 is higher in rats fed on diets containing fish oil, evening primrose oil and olive oil, compared with controls. Also animals fed on fish oil tend to have lower PGE_2 values than those fed on olive oil or evening primrose oil. Gas-liquid chromatography conducted on these three oils demonstrates that fish oil contains large amounts of eicosapentaenoic and docosahexaenoic acids. The inhibitory effects of these acids on the cylclo-oxygenase system (12,61,62) might help explain the tendency of gastric PGE_2 to be low in animals fed on fish oil. Hollander and Tarnawski (63) have recently suggested that the marked increase in the dietary availability of essential fatty acids could be responsible at least in part for the decrease in the incidence of peptic ulcers in the Western world. This could be due to the potential of these diets to stimulate the synthesis of mucosal PGs, especially of the E_2 series (63).

CHAPTER 2

2. PROSTAGLANDIN PRODUCTION IN PEPTIC DISORDERS. MEASUREMENTS AND POSSIBLE SOURCES OF CONFLICT.

2.1. INTRODUCTION.

The finding by some early workers (64,65) of reduced PG synthesis in patients with gastric ulcers had encouraged hopes of devising a therapeutic approach based on treating a deficiency state. The attractions of such an approach stem from the possibility that it would be physiological, and as a result, less likely to have serious side effects. Numerous other publications have documented that NSAIDs caused both PG inhibition and gastric damage. This might have contributed to the early impression that peptic disease, in general, was associated with PG deficiency. However, no conclusive evidence has been shown to prove that a correlation existed between PG inhibition by NSAIDs and the degree of mucosal damage. Opinions have, therefore, varied as to whether PG synthesis was inhibited, un-altered, or increased in various peptic disorders. In addition to the influence of NSAIDs, several other factors need to be considered when PG production is assessed. Such factors, discussed below, can probably account for many sources of conflict in the reported PG measurements.

2.2. PROSTAGLANDIN MEASUREMENTS IN PLASMA AND GASTRIC JUICE.

In 1974, Hindsale et al. (66) measured PGE in plasma and gastric juice, and found that PGE was significantly lower in duodenal ulcer patients, in the basal state, than in normal volunteers. Plasma PGE was also higher during stimulation with betazole. Such findings were disputed by Tonnesen et al. (67) and Baker et al (68) who found that PGE and acid output were higher in patients with duodenal ulcers. In a more comprehensive study, Baker and colleagues (69) measured plasma concentrations of PGE and PGF in patients with gastric ulcers, duodenal ulcers and in subjects without ulcers. No deficiency in plasma PG levels was found. Also, plasma PG concentrations failed to correlate with the parameters of gastric secretion studied, and were unaffected by histamine H_2-receptor blockade or the activity of duodenal ulceration. During combined pentagastrin and insulin secretory studies, the same authors (69) found a significant correlation between the outputs of PGE and acid into gastric juice.

The rapid clearance of PGs from the circulation, up to 90% in a single passage through the lungs and liver (70,71) make it unlikely that changes in gastric PG metabolism will be reflected in the plasma or that plasma PG levels will influence gastric secretory status, with the exception of relatively large PG infusions.

As PGs are synthesised and can be inactivated within the same tissues (72,73), and are rapidly cleared from the circulation, it is likely that their action is a local one. Variations in the gastric output of PGs occur under different conditions (74) and it seems reasonable to speculate that changes in gastric mucosal PG metabolism will be more likely to be paralleled by changes in the gastric juice than in plasma PG levels.

In conclusion, PG concentrations in gastric juice or plasma may be misleading, as mucosal events may not be reflected temporally and PGs themselves are unstable. Moreover, the anatomic origin of PGs, especially those in plasma, is uncertain.

2.3. MEASUREMENT OF PROSTAGLANDINS IN GASTRIC AND DUODENAL TISSUE.

2.3.1 Prostaglandin Synthesis in the Presence of Ulceration.

Several studies have measured PG synthesis in gastric ulcer mucosa. Konturek et al. (64) found decreased PG values and this was in agreement with previous findings by Wright et al. (65). Other workers (75), on the other hand, could not detect any significant differences in PG synthetase activity in gastric mucosal biopsies from patients with inactive gastric ulcer and those from either healthy controls or patients with inactive duodenal ulcer. Work presented in this Thesis (Chapter 11) shows that PGE_2 synthesised by gastric mucosal specimens is higher in

patients with gastric ulcers than those with non-ulcer dyspepsia. This is probably due to the effects of gastritis, discussed below.

Some of the studies employing the technique of cultured mucosal biopsies have shown that PG production in duodenal mucosa from patients with duodenal ulcers is not impaired (76). Biopsies from gastric mucosa obtained from the same patients showed reduced PG production (76). This finding of an abnormality in gastric but not in duodenal mucosa in duodenal ulcer patients, is difficult to interpret. Another study (77) suggested that duodenal PGI_2 synthesis was defective in duodenal ulcer patients. The presence of such a defect could not be confirmed by the findings of a study presented in this Thesis (Chapter 8). Other workers (64) found no difference in gastric mucosal PG content in patients with duodenal ulcer or healthy volunteers. However, when the relationship between post-cibal duodenal PG synthesis and duodenal acid load was examined (78), patients with duodenal ulcers had lower PGI_2 values than healthy controls.

2.3.2. Influence of Local Inflammation.

One possible explanation for the obvious discrepancy in the results of studies described above is the fact that most previous workers have failed to take into account the interaction between local inflammation and PGs. The role played by PGs in mediating the inflammatory responses was described well before the discovery of their activities in

maintaining the mucosal defences. Innumerable publications have indicated that NSAIDs reduce the vasodilatation, oedema and pain of inflammation by inhibiting PG synthesis. It was also shown that, although they did not act directly, PGs enhanced enormously the oedema and pain-producing effects of other mediators, such as histamine, bradykinin and complement component C5a (79-81). In addition, PGs were found to play a role in influencing local c.AMP production (82). PGE_2 and prostacyclin are among the most potent stimulators of c.AMP generation. A rise in c.AMP was associated with a reduction of lymphocyte stimulation, cell mediated cytotoxicity, antibody and lymphokine production, release of lysosomal enzymes and allergic release of histamine (82). Although all the major immuno-inflammatory cell types except lymphocytes are capable of generating PGs after an appropriate stimulus, in general the macrophages seem to be the major source (83,84). Human neutrophils and macrophages produce mainly PGE_2 and TXA_2 as a result of stimulation by various materials, such as phagocytic stimuli (zymosan, antigen-antibody complexes), lymphokines and bacterial endotoxin lipopoly-saccharide. The production of PGs by neutrophils is relatively short lasting, which might indicate a somewhat different function, e.g. vasodilatation, to that of the PGE release from macrophages which occurs over a much longer period. The latter would be consistent with the PGE activity in an

immunoregulatory function. Cultures of macrophages produce both PGE_2 and PGI_2 when exposed to an inflammatory agent such as zymosan (85).

Schlegel et al. (86) were the first to demonstrate that patients with gastritis and gastric ulceration had a higher rate of PG synthesis than subjects with normal mucosa. This was confirmed by subsequent studies (87) including one presented in this Thesis (Chapter 11).

It is also interesting to find that, in the presence of H. pylori, gastritis does not seem to be associated with increased PG production, as shown in Chapter 11.

It has to be emphasized that the above studies (86,87,Chapter 11) simply confirm the involvement of PGs in the inflammatory response. They do not necessarily contradict the hypothesis that PG deficiency may be a factor in the genesis of peptic ulcer. This is because PGs could not be measured in the phase that preceded ulceration.

2.3.3. Tissue Sampling and the Types of Prostaglandin Assays.

The fact that tissue trauma stimulates PG synthesis has been widely recognized (88). Several workers have attempted to minimise the effect of this phenomenon by washing the biopsy specimens before incubation and/or the use of fresh un-homogenized tissue. Among those who washed their biopsies are Wright et al. (65), Konturek et al. (64,89), Hillier et al. (77) and the studies included

in this Thesis. The duration of the washing period is not very critical but it should be standardised to all the specimens examined in a particular study. Generally speaking, the longer the washing period the more likely it is to remove greater amounts of PGs induced by the initial trauma of obtaining the biopsy. In these circumstances it is preferable to consider measures of helping the cells to stay alive, especially if the washing and incubation periods are to continue for many hours. In two of the studies included in this Thesis (Chapters 12 and 13) a standard culture medium, RPMI 1640 (Gibco) is used. Among the workers who used culture media, RPMI 1640 in particular, are Sharon et al. (76) and Rachmilewicz et al. (90). Two sources of trauma can however be identified in their experiments (76,90); the first relates to obtaining the biopsies which were not initially washed, and the second is due to homogenization which involves the breaking up of cells to stimulate further PG synthesis. Homogenization was also used by several other authors (19,20,65,78,91).

Another basic principle that needs to be considered is whether to freeze the biopsies or to keep them fresh till the time of PG measurement. Frozen tissue was used by some workers (77,92,93), but Bennet et al. (19) used both fresh and frozen tissue in the same study. Many other authors used fresh biopsies only (65,76,78,87,90). Freezing specimens helps to store them till the time recruitment is completed, when biopsies from all study

subjects could be assayed together. Fresh tissue, on the other hand, is relatively closer to physiological circumstances within the gastric or duodenal mucosa. The effect of freezing is clarified in Chapter 6 of this Thesis. Frozen specimens produce more PGE_2 and PGI_2 than fresh ones. This could be related to the destruction of cell membranes within frozen tissue. The issue could become complicated by the inclusion of other procedures such as homogenization (76,90) or the addition of extrinsic precursors (20,78,87,91) to the PG assay. To homogenize a fresh specimen probably removes its relative physiological advantage over a frozen one because of the trauma involved. Also, the addition of extrinsic precursors only allows for measurement of the capacity of the cyclo-oxygenase enzyme to utilize them; it does not necessarily measure PG synthesis from intrinsic precursors stored within the cell membranes.

In this Thesis, extrinsic precursors are not added when frozen biopsies are studied. Neither is homogenization used in studies involving fresh tissue.

Previous studies have also differed in the type of PG assay used. Bioassays and column chromatography were used by some early workers (19,86) while the majority of others, including this Thesis, relied on radioimmunoassays. Assay insensitivity was previously recognized as a potential source of problems in tissue PG measurements (94).

Another area that needs clarification is whether an assay is measuring PG concentration or synthesis. Some authors (65) spoke of mucosal levels and this is difficult to interpret since PGs are not stored. To prove that PG synthesis has taken place, it was suggested (95) that evidence should be shown that the assay could detect PG inhibition by cyclo-oxygenase inhibitors. In this Thesis, and in addition to unpublished preliminary data, evidence is presented showing that PG inhibition takes place in response to naproxen (Chapter 10) and indomethacin (Chapters 12, 13). Also, the assay used can detect stimulation of PG synthesis by histamine (Chapter 13).

In conclusion, there are many problems still surrounding PG measurements. Satisfactory interpretation of the findings of a given measurement could be facilitated by adequate description of the procedures used, standardizing these procedures to all the study groups, and by describing the tendency of PGs measured to rise, fall, or stay unchanged, rather than comparing individual tissue concentrations. Physiological values of tissue PGs have not been measured yet.

2.4. Prostaglandin Measurement in this Thesis.

The overall process of measuring tissue PGs can be divided into the following stages: the washing period, the incubation period, and the radioimmunoassay. Measures to stabilize PGs are observed during all stages.

The following is a general discussion of these stages, and individual variations will be mentioned in the appropriate Chapters.

Some studies in this Thesis use frozen specimens while others use fresh ones. The implications of this issue have been discussed in the previous section, in the context of other principles of tissue sampling.

Immediately before biopsies are incubated, they are washed in phosphate buffered saline, or in RPMI 1640 if tissue culture techniques are to be used subsequently. The purpose of the washing period was to minimise measurement of PGs induced by trauma. Its duration varies between studies but it is standardized to all specimens within the individual studies.

The washing supernatant is removed and the specimens are then incubated in either fresh buffer, or culture medium with or without test materials such as H. pylori culture filtrate. The supernatants yielded from this process are added to an equal volume of methyloximating (MOX) agent to stabilize their content of PGs (96). As mentioned earlier, a cause of many problems in PG measurement has been the low concentrations of PGs in many body fluids, together with the instability of PGs with a -ketol ring structure (PGE series). Using the oximating solution, PGs are reliably and conveniently converted to their methyloximes in high yield (greater than 95% for room temperature oximation) even in complex biological

matrices such as plasma (96). The exact degree of conversion is difficult to assess since no radio label is 100% pure, but T.L.C. analysis of the product showed less than 2% of the radioactivity corresponding to the unchanged PG. Data from the examination of the two isomers of PGE_2 suggest that in this case it is only the major methyloxime isomer which is recognized. There are many advantages of having stable derivatives of PGs for a radioimmunoassay: the samples are particularly stable during extended storage and during the assay procedure itself. The presence of oximating solution (50%) also inhibits bacterial growth, and increases both the sensitivity and the specificity of the assay (96,97). The reagents such as label and standards are also stable, and the stability of the substrate is important during iodination procedures to prepare ^{125}I labelled compounds.

During the radioimmunoassay, alliquots are assayed in duplicate. They all carry code numbers to facilitate randomization. Intra-assay variations are 14.8 for PGE_2, 11.0 for 6-oxo-PGF_1 (the stable metabolite of PGI_2), and 5.0 for TXB_2 (the stable metabolite of TXA_2). Inter-assay variations are 13.5 - 26% for PGE_2, 13.0% for 6-oxo-PGF_1, and 5.6% for TXB_2. Cross-reactions of the anti-sera are as follows: PGE_2 (MOX) anti-serum with E_1, 53%; PGE_3, 31%; PGB_2, 0.2%; 15-oxo-PGE_2, 0.25%. 6-oxo-F_1 (MOX) anti-serum with TXB_2, 0.02%; PGE_2, 0.01%; PGE_1, 0.01%. TXB_2 anti-serum with PGD_2, 0.02%; 6-oxo E_1, 0.02%; PGE_2, 0.02%. The sensitivity of the PG assays

(as defined by the amount distinguishable from zero with a 95% confidence limit) is 2 pg in all assays. Other details are as previously described (96,97). Results are expressed as ng or pg per mg protein or tissue weight. Tissue protein is measured by the Lowry method (98). It is worth noting that there is a correlation between the weight of mucosal biopsies and their protein content (65). To express PG values per either parameter will therefore make no significant difference to the overall interpretation of results.

CHAPTER 3

3. GASTRIC AND DUODENAL COMPLICATIONS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS.

3.1. INTRODUCTION.

Numerous studies, including some reported in this Thesis, have dealt with the issue of gastric and duodenal side effects related to the use of non-steroidal anti-inflammatory drugs. Opinions have, however, varied as to whether these agents do increase the frequency of complications like perforation, bleeding or death. The purpose of this chapter is to assess the evidence for and against the involvement of these drugs in causing serious side effects and to discuss the possible mechanisms by which they affect the gastric and duodenal mucosa.

3.2. DO NON-STEROIDAL ANTI-INFLAMMATORY DRUGS INCREASE THE RISK OF SERIOUS GASTRIC AND DUODENAL COMPLICATIONS? EVIDENCE FOR AND AGAINST.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed group of drugs. In 1983, it was estimated that sufficient prescriptions were written to provide 1.29% of the adult population of the United States with NSAIDs, and usage patterns projected the continuation of this trend (99). In the United Kingdom, the total number of prescriptions for NSAIDs rose from 7.6 in 1967 to 22 million in 1985 (100). This rise covered individuals of all ages, but the rates of

prescription were consistently greater in elderly people; it was estimated that every individual over the age of 65 could on average receive at least one prescription for an NSAID in any 1 year (100). It is interesting to notice that the Food and Drug Administration (FDA) in the United States and the Committee on Safety of Medicines (CSM) in Britain receive more reports of adverse effects ascribed to the use of NSAIDs than of those thought to have been caused by any other form of treatment (101-103). It was also suggested that gastrointestinal events were the most common side effects of NSAIDs (104). In a study by Caruso et al., the overall incidence of endoscopically confirmed gastric lesions during treatment with anti-inflammatory drugs was 31% (105). Despite the importance of these observations, a lot of controversy has surrounded the evidence for an increased risk of serious complications in association with the intake of NSAIDs. Several factors have to be considered in this context, but the main source of controversy probably relates to the methodology involved in the measurement of the above risk.

In the early stages of evaluating the possible toxicity of a new NSAID, it is almost always the case that an animal model is used. Experiments involving such models usually measure the number and size of ulcers or erosions induced by the agent in question, often under a stressful environment. The dose of the drug tested, per animal weight, is very large in most cases. These experiments might not necessarily reflect the natural

history of peptic ulcer disease in man, and species specific responses can be of crucial importance. Therefore, the capacity of an individual NSAID to cause ulceration under these circumstances might be of limited relevance to man.

An important source of data on the toxicity of NSAIDs, which is relied on by the pharmaceutical companies, the FDA, and the CSM, comes from the so called "Phase Three Trials". These are double-blind, controlled trials, usually of relatively brief (three months or less) duration. The test drug is compared to a standard drug or, less commonly to a placebo. These studies produce highly reproducible findings; for example, the proportion of subjects with gastric or duodenal side effects after taking an individual NSAID does not vary greatly from one study to another. The limitations of these studies is that they involve relatively small numbers of patients, so that rare but serious side effects would be missed. Another major problem is that the subjects studied are select populations, thus excluding many individuals with a history of gastro-intestinal problems. More importantly, those older than age 65 or 70 years are almost never included. This can be considered a serious omission for reasons described below.

Another means of assessing drug toxicity involves voluntary reporting by clinicians to a regulatory agency, such as the CSM, of a reaction thought to have been caused by a drug. The voluntary nature of this system may

result in an under-estimation of the common or expected toxicity with a relative over-estimation of the bizarre effects. Also, the CSM regularly informs clinicians of the introduction of new agents, and requests them to watch for, and report any side effects they may cause. For one reason or another, there is a tendency to report more on the newly introduced agents than on the established ones.

The 1980s saw the publication of several studies that attempted to estimate the risk of upper gastrointestinal bleeding, perforation, or death from aspirin or non-aspirin NSAIDs. These studies have often come up with conflicting conclusions.

An important study was reported by Coggon and colleagues in 1982, in which they retrospectively assessed aspirin intake in patients with haematemesis and melaena and in controls (106). In order to validate their data further, they chose age-and sex-matched community controls, and used paracetamol as a positive control. By this, Coggon et al. felt that they could then obtain an unbiased estimate of ordinary community drug use, and that the paracetamol data enabled them to estimate the extent to which patients who developed haematemesis and melaena might ordinarily be taking analgesics. They assumed that paracetamol intake was not associated with upper gastrointestinal complications. This study (106) found that, compared to community controls, more patients with haematemesis and melaena took aspirin, paracetamol and other NSAIDs. Coggon et al. estimated that about one-

third of aspirin intake in patients with bleeding was equivalent to that in controls and was by deduction non-causal; another one-third by reference to parallel increases in paracetamol intake was non-causal but consequential upon the presence of the bleeding lesion. The remaining one-third was unexplained and likely to be causal. Among chronic users of aspirin in the Nottingham community, they also estimated that 1 in every 1000 would be likely to be admitted with haematemesis and melaena each year. This figure is in agreement with that suggested by data reported in the Boston Collaborative Study (107).

In another study, Armstrong and Blower (108) aimed at relating NSAID ingestion to all cases of peptic ulceration with perforation or bleeding, who either died or underwent emergency surgery in the South Cheshire Health District. During a three year period, they found that 80% of those dying from ulcer complications had received NSAIDs. Another investigation aiming at identifying all cases of death from peptic ulcer and relating these to use of NSAIDs was conducted by Catford and Simpson (109). They reported the findings of an enquiry into deaths from peptic ulcer in Hampshire between 1981-3. Information was sought by questionnaire about all those whose death certificates indicated peptic ulceration. During the data collection phase, industrial action was undertaken by the Registrars of Births and Deaths. This resulted in many death notifications being forwarded too late to

gather complete information. Nevertheless, where information was available about those dying in hospital, approximately 50% had been on NSAIDs. Series of cases, suggesting high exposure to NSAIDs amongst patients with peptic ulcer disease or its complications, have also been reported by Collier and Pain (110), and O'Brien and Burnham (111). Caution is advised in interpreting such findings because case series might emphasize the unusual, patients with one disease could be more susceptible to having another, and because patients with an indication for NSAID intake might be more prone to gastro-duodenal damage due to any cause. More importantly, peptic ulcer disease is common in the general population and one should be aware of the possibility that a simple coincidence might take place between two common events: NSAID intake (99,100) and peptic ulcer disease.

While the studies described above have strongly suggested the presence of a high risk of peptic ulcer complications in patients on NSAIDs, there are studies that have concluded that the risk is either low (112) or negligible (113).

Carson et al. (112) studied upper gastro-intestinal bleeding in approximately 92,000 subjects. Using data from almost 50,000 subjects prescribed NSAIDs, they found a rate of bleeding of 0.33% in the first 30 days compared with 0.22% for controls, for a relative risk of 1.5%. Thus, the risk of upper gastro-intestinal bleeding in the first month of NSAID treatment, according to Carson et al.

is 50% over the expected rate. In other words, in two-thirds of patients with upper G.I. bleeding within a month of starting NSAID therapy, the cause is unrelated to NSAID intake. It is important to note that Carson et al. excluded from their analysis patients with a history of upper G.I. bleeding. It would be crucial to look at this group of patients to determine the risk of NSAID-induced bleeding in them. Moreover, the analysis only included bleeding within the first month. Later bleeding and "silent" ulcers would have been missed.

In another study, Jick et al. (113) examined the frequency of hospital admission for perforated peptic ulcer in relation to the use of NSAID at the Group Health Cooperative of Puget Sound, Seattle, U.S.A. Their study found that 54 people were admitted to hospital with well-documented perforated peptic ulcer. Among these cases, 6 had presented a prescription for an NSAID within 90 days before hospital admission, resulting in a crude rate of 6 hospital admissions per 23.4 million person-days at risk (0.26 per million person-days at risk) among NSAID users. The crude hospital admission rate among non-users was 48 per 559 million person-days at risk (0.09 per million person-days at risk). Jick et al. concluded that the frequency of hospital admission for perforated ulcer was not measurably affected by concurrent use of NSAIDs at the Group Health Cooperative of Puget Sound. However, as pointed out by Jick et al., their crude estimates were confounded by age which is strongly positively associated

with both rate of hospital admission and NSAID use (100). Adjusted for age and sex, the rate ratio for NSAID users compared with non-users was 1.6 (95% confidence interval 0.68-37).

The other study that disputed the suggestion of an increased risk of ulcer complications in NSAID users was reported by Inman and Rawson (114,115) in 1985. They assessed the results of more than 50,000 patients treated with benoxaprofen, fenbufen, zomepirac, indomethacin or piroxicam. It covered a period equivalent to 90,000 patient years of observation, about half during and half after stopping or changing treatment. They estimated that the frequency of upper gastro-intestinal bleeding or perforation ranged from three to six cases per 1,000 patient years, only a minority being attributed to NSAIDs by the reporting doctor. According to their data, these complications occurred with equal frequency after the patients stopped or switched NSAIDs and were very rarely caused by these drugs. The methodology involved in collecting and analysing the data of Inman's study might explain why NSAID use appeared free from ulcer complications. Although a large number of subjects were included in the study as a whole, the numbers of those taking the individual NSAID might not have been big enough to detect an increased risk. Also, the study included subjects of all ages, and this overlooks the possibility that ulcer complications could have been predominant in older patients. In addition, only 50% of questionnaires

sent to general practitioners were returned, and these were mailed up to a year after the prescriptions were issued initially.

Many of the limitations of the above studies (108-115) were addressed by a study reported by Somerville et al. (116) in 1986. Four hundred and six of 903 patients admitted to two Nottingham hospitals with suspected upper gastro-intestinal bleeding were diagnosed as having a bleeding gastric or duodenal ulcer. Of these, 327 patients were questioned and the 230 cases aged 60 or over were matched with both community and hospital inpatient controls. This study found that the use of non-aspirin NSAIDs amongst the bleeders increased with age, particularly over the age of 60, when at least one-third of patients were takers. Bleeders were found to be at least twice as likely to be NSAID takers as controls, the relative risk being 3.8 and 2.7, compared respectively with hospital and community controls. The differences between cases and either type of control were highly significant.

It is not clear why the elderly should be so seriously affected by the adverse effects of NSAIDs (108-116). Large numbers of prescriptions are written for this age group, probably because of the increasing incidence of osteoarthritis with age, while those with rheumatoid arthritis are on the whole younger. About half of the NSAIDs prescribed are for the over 60s (100). Not only is the incidence of gastro-intestinal

complications high in the elderly, but the outcome in terms of mortality is much worse, the case fatality rate being three times greater in the over 70s in Armstrong and Blower's series (108). In addition to the high rate of NSAID prescription (100), there are other factors that might help explain the susceptibility of the elderly to NSAID related peptic complications. Sonnenberg (117) found a significant correlation between geographical variations in salt consumption and mortality from gastric ulcers. He suggested that dietary intake of salt was a risk factor in mortality from gastric ulcer. Sonnenberg has also argued for a cohort effect (118), starting at an age below 5 years for both gastric and duodenal ulcers. This effect implies that important determinants for the development of peptic ulcer disease occur very early in the life of a cohort and that it is these early determinants that are changing with time. It could be speculated that subjects born in the early years of the 20th century were exposed to one or more factors that predisposed them to ulcer formation later in their lives. This interesting proposition is probably too simple to explain all the variations of peptic ulcer disease or its complications.

In conclusion, opinions have differed as to whether NSAIDs are causally related to serious complications of peptic ulcers. Such difference could be due to the methodology involved in various studies. On balance, the evidence for a causal relationship has been more

convincing. The importance of even a small rise in the relative risk is emphasized by the high rate of prescribing NSAIDs, particularly in elderly subjects.

3.3. INTERFERENCE WITH PROSTAGLANDIN SYNTHESIS BY NSAIDs.

It is generally agreed that NSAIDs such as indomethacin inhibit PG biosynthesis by inhibiting cyclo-oxygenase activity, the enzyme which is responsible for converting arachidonic acid substrate to PG intermediate PGG_2. This is subsequently converted to the endoperoxide intermediate PGH_2 which by the action of specific PG synthetase enzyme is converted to a large number of possible PGs or their derivatives (Figure 1). NSAIDs are considered not to have any major effects on the enzymes involved in the lipoxygenase system, and therefore the production of the leukotrienes would only be affected by these drugs to the extent that further arachidonic acid substrate would be available for leukotriene biosynthesis on account of the decreased PG production.

The potencies of various NSAIDs in inhibiting PG synthesis have been indicated in previous reports (119). Indomethacin, naproxen and meclofenamic acid are powerful inhibitors of PG synthesis, phenylbutazone is a moderate inhibitor, while salicylic acid, aspirin and benoxaprofen are only weak inhibitors. Evidence is presented in this Thesis indicating that etodolac does not seem to suppress PG synthesis in the gastric or duodenal mucosa of patients

with rheumatoid arthritis who took the drug for 4 weeks. The mechanism by which this agent causes gastric or duodenal side effects probably involves one or more of the non-PG dependent processes discussed below. Other drugs act as prodrugs that show activity through their metabolites: one of these agents is suldinac whose sulphide metabolite is able to exert a potent inhibiting effect on PG synthesis (120).

In addition to their direct effect on the cyclo-oxygenase enzyme, NSAIDs can stabilize lysosomes and inhibit the release of lysosomal enzymes including lipases (121). This will interfere with the release of arachidonic acid from cell membranes (mediated by phospholipases) and in turn can inhibit the production of PGs, especially when high concentrations of NSAIDs are present.

A further feature regarding the effect of NSAIDs on PG production which should be noted is that certain drugs may show selectivity in terms of which PGs are mainly affected. Previous observations with sheep vesicular tissue incubated in Cu^{+2}-containing medium indicated that salicylic acid caused a preferential reduction in PGE_2 synthesis relative to PGF_2 synthesis (122); the latter in fact was stimulated in the presence of Cu^{+2}. In contrast, indomethacin suppressed the production of PGE_2, PGI_2 and PGF_2 to the same extent. This phenomenon of selective inhibition might be relevant to the action of etodolac. By decreasing rheumatoid disease activity one

speculates that it suppresses synovial PG production, thus displaying a form of tissue selectivity, bearing in mind that it does not affect PGs of the stomach or duodenum. Alternatively, one could also speculate that etodolac selectively suppresses other PG compounds not measured in the study described in this Thesis. Assuming this is true, it is unlikely to be of great significance because PGE_2, PGI_2, and TXA_2 are the major PG groups in the human gastric and duodenal mucosa.

3.4. OTHER MECHANISMS OF MUCOSAL TOXICITY.

In addition to their capacity to interfere with PG synthesis, NSAIDs have several other activities which can lead to mucosal damage. These actions should not be considered as totally separate from each other. For example, the capacity of indomethacin to decrease mucosal blood flow (44,123,124) is not necessarily unrelated to its inhibitory effect on PG synthesis, which in turn can influence blood flow (125). Like indomethacin, aspirin was found to decrease mucosal blood flow (124), although an earlier report suggested the reverse (126).

Aspirin (127), indomethacin (125) and fenoprofen (128) were also found to cause disruption of the gastric mucosal barrier in a manner that allows back diffusion of hydrogen ion with its damaging consequences. Aspirin and indomethacin also increase basal (129) and maximally stimulated gastric acid secretion (124,130), which may contribute to their unwanted effects on the gastric and

duodenal mucosa.

Several studies have suggested that NSAIDs can affect the rate of secretion and/or the characteristics of the mucus layer in the stomach. Aspirin (131) and indomethacin (132) were found to inhibit mucus secretion. It was also suggested that aspirin could increase pepsin-mediated proteolysis of mucus, decrease mucus viscosity and increase the permeability of mucus to hydrogen ion (133). Indomethacin was also found to inhibit active bicarbonate secretion by the gastric mucosa (134).

Another mechanism, that received little attention in the past, relates to the possibility that bacteria may mediate NSAID toxicity. In the intestine, it was observed that the ulcerogenic effect of NSAIDs could be reduced by antibiotic treatment (135). Germ-free animals were also found to be resistant to indomethacin-induced intestinal lesions (136). It was postulated that enteric bacterial β-glucuronidase hydrolyses the acylglucuronides of NSAIDs released into the intestinal tract from the bile, and the free acids then irritate the mucosal surface (135). Interest in these important observations has been revived by the recent entry into the argument of another organism: Helicobacter pylori. It is not clear whether NSAIDs interact with H. pylori in the gastric mucosa. Evidence is presented in this Thesis showing that human gastric antral specimens exposed to a mixture of indomethacin plus H. pylori culture filtrate have lower PGE_2 values and histological grades than biopsies

incubated with indomethacin or the culture filtrate alone. The results of this *in vitro* study suggest that an interaction may exist between indomethacin and H. pylori culture filtrate. Such interaction appears to be damaging to the gastric mucosa as judged by the histological findings and PGE_2 measurements.

CHAPTER 4

4. HELICOBACTER PYLORI AND PEPTIC ULCER DISEASE.

4.1. INTRODUCTION AND HISTORICAL BACKGROUND.

In 1983, an organism was "rediscovered" by Warren and Marshall (137). It was initially called Campylobacter pyloridis but the name was later changed to Campylobacter pylori and more recently to Helicobacter pylori (H. pylori). This organism appears to be ubiquitous and the population seems to be continuously exposed to it. The possibility that H. pylori might be the cause of peptic ulcer disease (138) has generated a lot of interest.

The bacterium has been isolated worldwide, usually from the stomach, especially from the antrum and less often from the corpus fundus area (139).

H. pylori is a microaerophilic organism which grows best in blood agar in a humid atmosphere. It appears as a curved, spiral or S-shaped Gram-negative rod, 2.2 - 3 um long and 0.5 um in diameter in fresh specimens of gastric mucus. The organism has a smooth coating and four to five flagella projecting from one end. These flagella are sheathed and have terminal bulbs (140).

4.2. CLINICAL EVIDENCE OF PATHOGENICITY OF H. PYLORI.

The evidence that H. pylori is a true pathogen has been increasing; several observations indicate that the

organism is not simply a commensal or an opportunist which colonizes inflamed gastric mucosa.

First, many centres all over the world have produced data confirming the close association between H. pylori and the presence of active chronic gastritis and peptic ulcer disease (141-143).

Second, some investigators attempted to fulfil Koch's postulates by self-inoculation with the organism. Postulate three (the organism in pure culture must produce the same disease in a susceptible animal) and postulate four (the organism should be found in the diseased areas produced in the susceptible animal) were fulfilled by Marshall. Seven days after ingesting 10^9 organisms, he developed a mild gastrointestinal illness and, on antral biopsy, was found to have acute gastritis. Cultures of antral mucosa grew H. pylori (144).

Third, additional evidence may be derived from observations of sudden hypochlorhydria. An observation of hypochlorhydriac gastritis in 17 of 37 volunteers who underwent acid secretion studies with a pH electrode that had not been sterilized between experiments appeared, in retrospect, to have been caused by H. pylori contamination (145).

Fourth, it was reported that a patient with Zollinger-Ellison syndrome suddenly became achlorhydric after an episode of general malaise and mild fever (146). Gastric histology revealed marked active inflammation and,

in retrospect, evidence of H. pylori infection. This patient was re-examined some 10 years later. He still had evidence of massive H. pylori infection, together with active chronic gastritis (147). The course followed by the above patient is consistent with Marshall's Hypothesis (144) which suggests, among other things, that if H. pylori is not cleared by natural immune processes, patients with persistent gastrointestinal disturbance will also develop achlorhydria.

4.3. THE ASSOCIATION BETWEEN H. PYLORI, GASTRITIS AND, GASTRIC ULCERS.

After H. pylori was described in chronic gastritis by Warren and Marshall (137), data produced by subsequent investigators have pointed to a very close, if not causal, link between H. pylori and what is now known as Type B gastritis (see below). In a recent review of world literature (148) of H. pylori - associated gastritis, in over 1,000 patients, those with gastritis had a 75% carriage rate of H. pylori compared with under 10% in those without gastritis. Of 54 asymptomatic volunteers studied, 11 had both gastritis and H. pylori compared with none of the 43 patients without gastritis. H. pylori appears to be associated with a specific gastritis that predominantly affects the antrum and involves infiltration of the epithelium and interstitial tissue with neutrophilic polymorphs, lymphocytes, and plasma cells (137). A similar histological picture was originally called active chronic gastritis by Whitehead in 1972

(149), or Type B gastritis by Strickland and Mackay in 1973 (150). This is to be distinguished from autoimmune gastritis (Type A), the combined form (Type AB), and chemical gastritis (Type C). Autoimmune gastritis is known to affect mainly gastric body mucosa; it is associated with hypochlorhydria, antiparietal cell antibodies in the serum, and pernicious anaemia. Type AB gastritis was suggested by Glass and Pitchumoni (151) to indicate the presence of patchy involvement, of both antrum and body, with multifocal atrophy and intestinal metaplasia; it is thought to carry an increased risk of gastric ulceration and the development of carcinoma. Chemical gastritis (Type C) has been recently suggested to describe the histological picture induced by enterogastric reflux (152) or other chemicals like NSAIDs (153). It results from persistent injury to the surface epithelium and is characterized by compensatory fovealar hyperplasia and a vascular and exudative response evidenced by capillary dilatation, congestion and oedema of the lamina propria. There is no notable increase in inflammatory cells.

There are two further characteristics of Type B gastritis that are worth mentioning. The first relates to the finding of lymphoid follicles, frequently seen when the inflammatory cell infiltrate extends through the full thickness of the mucosa, and by separating the glandular elements, may give a false impression of atrophy (154).

These reactive lymphoid follicles are virtually confined to H. pylori positive gastritis (155).

The second characteristic relates to the development of intestinal metaplasia. This might take the form of small intestinal or colonic type mucosa, and probably results from persistent chronic inflammation. Its importance stems from the suggestion that it might represent a premalignant condition (156). It has also been observed that H. pylori organisms do not adhere to intestinal epithelium and this could explain the low prevalence of H. pylori in Type A gastritis (157).

As mentioned above, there are numerous studies suggesting a strong link between active chronic gastritis and H. pylori. Estimates of positivity for the organisms in this condition have ranged from 70% (158) to more than 90% (159,160). Similarly, previous studies have shown that the prevalence of H. pylori in gastric ulceration has generally varied between 63% (142) and 77% (141). In some studies, the prevalence was as high as 86% (161) and 98% (162). Many gastroenterologists and histopathologists believe that there is a strong link between gastritis and gastric ulceration (150,163-165). It is also believed that gastritis is the fundamental change upon which other damaging factors act to induce ulceration. Gastritis in this context does not need to be associated with H. pylori in all cases. This has been illustrated by the works of O'Connor et al. (166), who studied biopsies from 54 patients with chronic benign gastric

ulcer. They found 72% of the patients to be positive for H. pylori. Eight of the 15 patients who were negative, had histological evidence of chemical gastritis. In other words, the gastritis found in association with benign gastric ulcers may arise from different aetiologies, unlike the antral gastritis associated with duodenal ulcers; the latter tends to be the specific type associated with H. pylori. This can probably explain the apparent difference in the prevalence of H. pylori in gastric as opposed to duodenal ulcers, being commoner in the latter entity (see below).

4.4. H. PYLORI AND DUODENAL ULCERATION.

Several studies have suggested the presence of a very close association between H. pylori and duodenal ulceration (139,140,162,166). The prevalence ranged between 80-100%. H. pylori was identified largely by studying biopsy specimens taken from the gastric antrum and/or body. An explanation is therefore needed as to how duodenal ulceration could be related to an organism that colonizes the gastric mucosa.

Gastric metaplasia, the presence of gastric epithelium in the duodenum, is found in up to 64% of normal individuals (167) and in up to 100% of cases with duodenal ulceration or inflammation (168,169). H. pylori was also found in the duodenum (170) only in patients with both active duodenitis and gastric metaplasia. Wyatt et al. (170) found that only patients with a low fasting

gastric juice pH had gastric metaplasia. It was suggested that such metaplastic change could be protective against acid (169). The ability of the gastric epithelium within the duodenum to protect against acid might become undermined when it is infected with H. pylori (170). Acid with or without other damaging or predisposing factors might therefore lead to duodenal inflammation and ulceration (170). It should be emphasized that this sequence of events remains purely speculative. A definite aetiological link between H. pylori and duodenal ulceration has yet to be established.

4.5. H. PYLORI AND NON-ULCER DYSPEPSIA.

The possible association between non-ulcer dyspepsia (N.U.D.) and H. pylori was first suggested by Marshall et al. in 1985 (139). Such association does not seem to be as strong as that between H. pylori and gastric or duodenal ulcers. In this Thesis, H. pylori is found in 10 out of 26 (38%) of subjects identified as having N.U.D. This is slightly lower than that found in a previous study (171), which identified the organisms in 45% of subjects. N.U.D. is defined in this Thesis as having upper abdominal complaints such as pain, nausea, vomiting and heartburn for a minimum of a month, normal endoscopic findings or minor mucosal erythema, normal upper abdominal ultrasound scan and without the intake of antibiotics within a month, or ulcer healing drugs within a week of endoscopy. The identification of this type of dyspepsia might have some therapeutic significance. A recent study (172) has found

that short term De-Nol therapy improved N.U.D.-associated gastritis and symptoms, in addition to eradicating H. pylori in 83% of patients infected with the organisms.

4.6. MECHANISMS OF INTERACTION BETWEEN H. PYLORI AND THE GASTRIC MUCOSA.

The strong association between H. pylori and peptic disease has led many workers to explore the possibility that these organisms have the potential to cause direct damage, or at least to predispose to it. The complexity of this issue has also led some investigators to put forward their own hypotheses as to how H. pylori interacts with the gastric mucosa.

It was postulated (144) that H. pylori causes an acute antral gastritis that may resolve or persist as chronic gastritis with hypochlorhydria lasting for up to 12 months. As immunity develops, the inflammation regresses and acid secretion returns to normal. During this, an imbalance may occur between mucosal resistance and acid secretion which theoretically may lead to the development of a peptic ulcer.

Although H. pylori seems able to establish itself in subjects with normal acid secretion in certain situations (173,174), its acquisition by ingestion in the experimental state may require transient acid suppression (144,175,176). It was not possible to identify any consistent relationship between H. pylori and acid secretion or serum gastrin (177). Recently, Levi and

colleagues (178) claimed that subjects infected with H. pylori had higher serum gastrin levels than non-infected individuals. They postulated that hypergastrinaemia in these circumstances could lead to hyperacidity and, possibly, ulceration.

Since H. pylori possesses high levels of urease activity it was thought that such property caused hydrogen ion back-diffusion in the gastric epithelium and that this was a predisposing factor in gastric ulcer formation (179). It was also proposed that a function of the urease may be to create an insulating neutral-to-alkaline blanket around the organism to protect it from the lethal effects of gastric acid (159). Subsequent studies have also suggested that the very high urease activity is likely to be important in colonization of the host, and that protection from acid is unlikely to be a primary role, as the neutral habitat of H. pylori-related organism St1 is the non-acid-secreting tissue of the small intestine (180). In addition, the fact that H. pylori resides deep in the gastric mucus layer does not necessarily imply that the organisms are trying to avoid the effects of acid. Sarosiek et al. (181) have recently shown that incubation of H. pylori filtrate with gastric mucus led to a gradual loss in mucus viscosity. This had a detrimental effect on the ability of mucus to retard the diffusion of hydrogen ions. They suggested that the degenerative changes produced in gastric mucus gel by H. pylori may be

a contributing factor in the pathogenesis of gastritis and peptic ulcer.

When opsonic activity of human serum against H. pylori was assessed, it was found that opsonisation depended on the alternative pathway of complement activation and not on antibody (182). These findings suggested that antibody played no role in protection against H. pylori, and that the presence of antibody in patients sera was mainly of diagnostic value.

The nature and significance of the interaction that may exist between mucosal prostaglandins and H. pylori have not been fully clear. The importance of this area of research stems from the possibility that PGs and H. pylori might exert contrasting effects on mucosal defences. This issue was first addressed by work included in this Thesis (183-185, Chapter 11). Mucosal synthesis of PGE_2, PGI_2, and TXA_2 was measured in subjects with gastric ulcers and non-ulcer dyspepsia. PGE_2 and PGI_2 were higher in patients with severe gastritis regardless of the presence of H. pylori. PG values in patients infected with the organisms were comparable to those in subjects without H. pylori despite the presence of a higher proportion of cases of severe gastritis in the infected group. This encouraged speculations about the possibility of at least a partial defect in PG synthesis in the presence of H. pylori (183). Goren et al. (186) later suggested that patients with H. pylori had low values of 6-keto-PGF_1 . The significance of such finding

is not clear, given the fact that PGE_2, the major mucosal PG compound, was not suppressed to a significant degree (187).

Another important aspect of H. pylori interaction with the gastric mucosa is the possibility that the organisms might have cytotoxic activity. Leunk et al. (188) suggested that H. pylori culture filtrate contained a factor that caused cytopathic (cytotoxic) changes in Vero (mammalian) cell lines. Cave and Vargas (189) have recently shown that a protein isolated from H. pylori culture filtrate could inhibit acid secretion by rabbit parietal cells. Studies included in this Thesis (Chapter 12) show that H. pylori culture filtrate does not affect the viability of the human gastric mucosa in vitro or its PGE_2 production after 48 hours of incubation. When combined with indomethacin, it reduces both PGE_2 and mucosal histological scores to a greater extent than indomethacin or the filtrate applied separately. This implies that an interaction may exist between H. pylori culture filtrate and indomethacin capable of causing damaging effects to the gastric mucosa in vitro. A similar trend is noted when H. pylori protein was applied in combination with indomethacin (Chapter 13). This Thesis also shows that H. pylori protein is capable of suppressing cyclic AMP production by the human fundic mucosa. It also intensifies the inhibitory effect of indomethacin and prevents the stimulatory effect of histamine on both PGE_2 and c.AMP production. Given the

central role played by c.AMP in mediating various physiological activities, these findings suggest that H. pylori protein has the potential to influence at least some functional aspects of the human gastric fundic mucosa.

4.7. SOME METHODOLOGICAL CONSIDERATIONS IN H. PYLORI RESEARCH.

4.7.1. Identification of H. pylori.

The methods of identifying H. pylori can be divided into those requiring gastric biopsies and those that do not. Several approaches have been proposed to identify the organisms in gastric biopsy specimens. H. pylori can be cultured in fresh specimens (159) or identified by histological examination after being stained with a modified Giemsa (190). The observation that H. pylori produces high urease activity was utilized in devising new tests for its diagnosis. When biopsy specimens are placed in Christensen's medium colour changes are observed indicating the presence of H. pylori (191). A result can be obtained within an hour, but recent modification of the technique makes it possible to get a result after one minute (192). Another immediate technique involves the use of monoclonal antibodies and immunofluorescence, giving results within an hour (193).

Tests that do not require gastric biopsies include breath tests and serum antibodies. Two breath tests were described: the ^{13}carbon (194), and the cheaper ^{14}carbon

breath test (195). In these tests, carbon-labelled urea is given orally. The urea is metabolised and an isotope hydrogen will be detected in the breath if H. pylori is present. Antibodies to H. pylori can also be detected in the blood of infected subjects by either enzyme-linked immunosorbent assay (ELISA)(196), or by complement fixation (182). It has to be emphasised, however, that the titre of these antibodies does not correlate with the severity of the gastritis and that their presence is valuable only as a diagnostic tool (182).

4.7.2. Methods of Identifying H. pylori in this Thesis.

Two tests are used to detect the presence of H. pylori in this Thesis, both requiring gastric biopsies. Specimens are assessed by both histology and bacteriology. Such an approach takes advantage of the fact that study subjects had to be endoscoped in order to define the precise nature of their peptic disease. In addition, the histological assessment provides the opportunity to define the type and the severity of gastritis, and to exclude malignancy in patients with dyspepsia in general and in those with gastric ulcers in particular. The presence of malignancy would exclude patients from entering into any of the studies described in this Thesis, for understandable reasons. The numbers and the sites of gastric biopsies assessed for H. pylori will be indicated in the appropriate chapters.

4.7.3. Culture of Gastric Biopsies:

Culture techniques are modified from those described by Marshall et al.(139). Tissue is processed within three hours of endoscopy. Specimens are macerated in a 5 ml glass Potter tube with a PTFE tipped piston (McQuilken, Glasgow, UK), with a small amount of 0.9% saline. The resulting suspension is then plated onto Columbia agar base with 7% horse blood (Oxoid Ltd, Basingstoke, UK), heated blood agar (Oxoid Ltd), and Skirrow's Campylobacter selective medium containing vancomycin 10 mg/l, trimethoprim 5 mg/l and polymyxin B 2500 iu/l (Oxoid Ltd). All strains isolated grow on all three media. Incubation is performed in sealed culture jars (Don Whitley Scientific Ltd, Shipley, UK), with catalysts and 'Campypak' gas generators (BBL, Cockeysville, USA) to provide humid microaerophilic conditions. At 37°C, H. pylori appears as small translucent colonies approximately one millimetre in diameter at between 48 and 72 hours. Identification of H. pylori is confirmed by its characteristic appearance on Gram's stain, and positivity for oxidase and urease production (A S Rosco, Taastrup, Denmark).

4.7.4. Histopathology:

Specimens are fixed in formalin buffered saline, embedded in paraffin wax and 5 um sections prepared for light microscopy in the usual fashion. Sections are stained with Haematoxylin and Eosin, or Cresyl Violet

(197) The latter makes H. pylori more obvious on gastric sections.

Sections are assessed by light microscopy in the usual way for the features of chronic gastritis as graded by Whitehead et al.(149). Particular attention is paid to the presence or absence of Helicobacter-like organisms and the activity of the gastritis as indicated by the intensity of the polymorphonuclear leucocyte infiltrate in the epithelium and in the lamina propria (198).

4.7.5. Collection and Concentration of H. pylori Culture Filtrate:

Columbia blood agar containing a heavy growth of H. pylori is inoculated into 20 ml Brain Heart Infusion Broth (BHI); 0.5 - 1 ml of this is inoculated onto sloppy agar plates, and spread by gentle rolling. The plates are incubated at 37°C in a glass jar along with platinum catalyst and a BBL Campypak to provide a micro-aerophilic environment for growth. The jar is left for 4 days, as this was found from preliminary experiments to be the optimum time for active filtrate production. The plates are later frozen at -20°C for 3 days to kill the bacteria. After this they are thawed at room temperature (20°C) for 6 hours. Fluid is then pipetted off and the remaining agar spun at 2000 rpm/10 minutes to remove any further fluid. This fluid is driven through 0.4 um and then 0.2 um filters to remove all dead bacteria and agar. The resultant liquid is concentrated by transferring it to

dialysis tubing (Sigma) capable of retaining molecules of high molecular weight (above 12,000 kd). The tubing is sealed, covered entirely by laying in a bed of polyethylene glycol and left for 5½ hours. The concentrated culture filtrate was found to have cytopathic activity against Vero cells, in line with previous findings (188). For determination of the titre of cytopathic activity, doubling dilutions of the test samples are made in tissue culture medium and added to wells with cultured Vero cells. The titre is 1:32, i.e. 32 units of H. pylori filtrate/ml; this is estimated to be derived from 5×10^9 organisms.

4.7.6. Isolation of H. pylori Protein:

The culture filtrate described above is purified using G200 Sephadex Column (Pharmacia). Protein fractions are separated by their size as they flow through the column. They are collected overnight at an optical density of 280 nm. Two clear peaks of absorbance occur (Figure 2); the small peak which appears first, and the second much larger peak, which tends to be brownish in colour, probably because of serum proteins coming off the column at the same time. The fractions corresponding to the peaks are taken and concentrated again in polyethylene glycol. The small peak protein (H. pylori protein) is collected and divided into 100 ul alliquots. These are kept frozen at -20°C. To determine the titre of cytopathic activity of this protein, doubling dilutions of the test

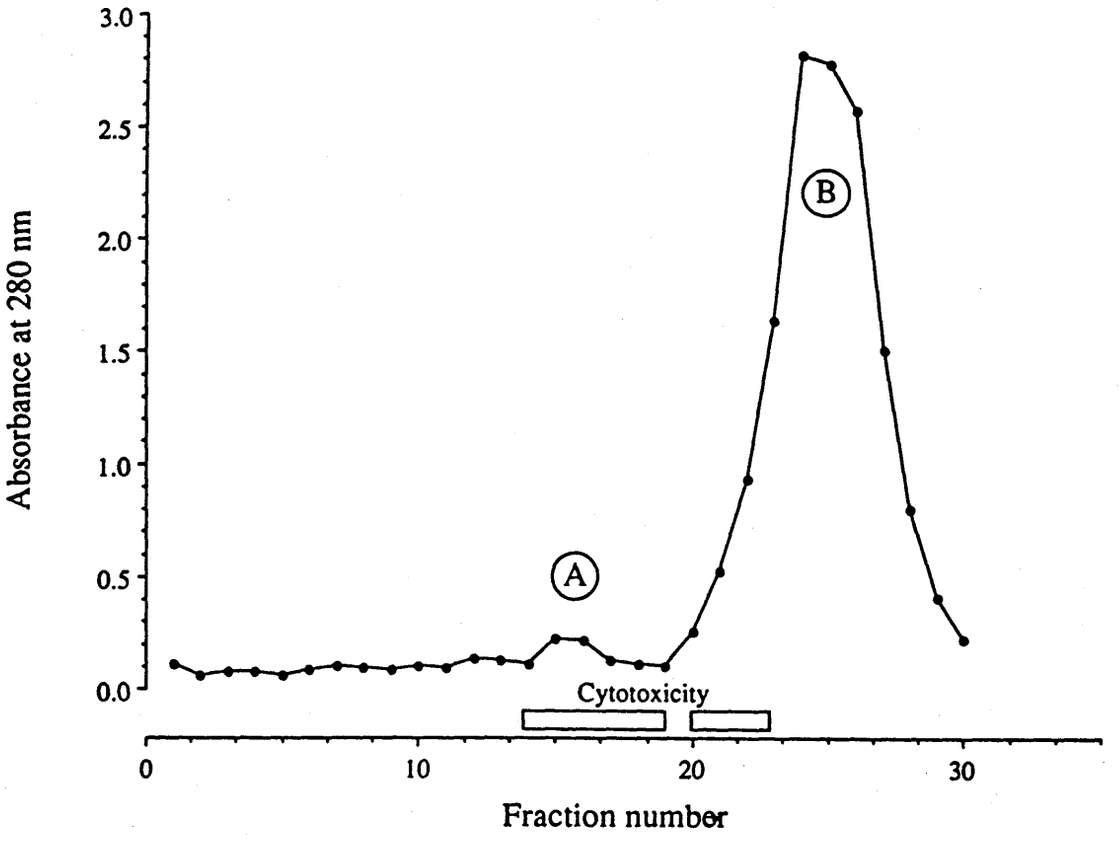


Fig. 2. Isolation of H. pylori protein (A) at an optical density of 280 mm, with its range of cytotoxicity. (B) represents serum proteins which are also present in the control fluid.

samples are made in tissue culture medium and added to wells with cultured Vero cells. The titre is 1:16, i.e., 16 units of H. pylori protein/1 ml; this is estimated to be derived from 5 x 10^9 H. pylori organisms

4.7.7. H. pylori Culture Control Fluid:

The purpose of using this fluid is to correct for the possibility that the effect of H. pylori culture filtrate or protein might be due to one of the materials used during its preparation. The control fluid is prepared in the same way as the culture filtrate or protein, but without the addition of bacteria. Un-inoculated sloppy agar plates are frozen, later thawed, and the resulting fluid collected. This is concentrated as described above. When the control fluid goes through the G200 column, only one large peak is obtained. This was found to have no cytopathic effect against Vero cells.

4.7.8. Gastric Tissue Culture Medium:

The following medium is used in this Thesis when gastric biopsies are incubated with H. pylori culture filtrate or protein.

RPMI 1640 (Gibco) is strengthened with 50 m molar HEPES/1 to act as an extra buffer, 292 mg/1 glutamine and 10 ml of 100 m molar/1 pyruvate as precursors for anaerobic metabolism. Also, penicillin 50 u/ml, streptomycin 50 ug/ml, gentamicin 50 ug/ml and amphotericin B 1 ug/ml are added in order to inhibit bacterial and fungal growth. Leupeptin (Sigma) is used to

prevent tissue digestion by pepsin or trypsin. In addition, isobutyl methylxanthine 1 mmol/l is added in order to stabilise c.AMP generated during incubation.

CHAPTER 5

5. AIMS OF THESIS.

This Thesis aims at investigating the changes in gastric and/or duodenal mucosal PG synthesis in a variety of conditions related to the pathogenesis of peptic ulcer disease.

Some methodological aspects of PG assays are examined in Chapter 6. A basic question in PG measurement is whether to process tissues fresh or frozen. Chapter 6 aims at clarifying the effects of freezing gastric and duodenal biopsies on their ultrastructure and capacity to synthesis PGs.

Gastric prostaglandin synthesis was previously found to increase in response to the intake of individual essential fatty acids such as arachidonic and linoleic acids. Chapter 7 aims at studying the effects of these fatty acids on PG production when given as parts of naturally occurring oils such as fish oil, evening primrose oil and olive oil. The basic fatty acid constituents of these oils are also analysed, using gas-liquid-chromatography. This is because the oils studied might contain other fatty acids that could inhibit the cyclo-oxygenase pathway.

Previous workers studying PG synthesis in the presence of duodenal ulceration have come up with different conclusions. The aim of Chapter 8 is to measure duodenal mucosal PG production in patients with duodenal ulcers. This might help verify the presence or absence of deficient PG production in these cases, which in turn might have therapeutic implications.

Established NSAIDs, such as indomethacin, were previously found to inhibit PG synthesis in the gastric mucosa. When these agents were studied, they were often given to laboratory animals or to healthy humans, in short courses or as one large single dose. There have been indications that NSAIDs with potent anti-arthritic activity resulted in maximal peptic damage. Section 3 aims at investigating the gastric and duodenal mucosal side effects of two NSAIDs: naproxen, with established anti-arthritic activity, and etodolac, the first representative of a new class of NSAIDs, the pyrano-carboxylic acids. Mucosal PG synthesis is measured at base-line, and four weeks after naproxen and etodolac are taken in therapeutic doses by patients with active rheumatoid arthritis. Such design is thought to overcome many of the problems encountered by previous studies.

While PGs are recognized for their favourable effects on the gastric mucosa, H. pylori has been linked to the development of peptic damage. It is not known whether an interaction exists between H. pylori and mucosal PGs. The aim of Chapter 11 is to measure PG synthesis in the

presence of H. pylori in patients with gastric ulcers and non-ulcer dyspepsia. While acknowledging the importance of including patients with duodenal ulcers in a similar study, it is worth noting that PG measurements in these cases is likely to be complicated by two factors: the rarity of duodenal ulcer patients who do not harbour H. pylori, and the presence of gastric epithelium in the duodenal mucosa, known as gastric metaplasia.

The possibility that H. pylori produces a cytotoxic factor is investigated in this Thesis. Chapter 12 aims at studying the effect of H. pylori culture filtrate on the human gastric mucosa. It also investigates the possible interaction between H. pylori culture filtrate and another mucosal damaging agent, indomethacin, in vitro. Mucosal histological scores and PGE_2 production are used to quantify the response of gastric tissue to these agents.

Although H. pylori has been linked to the development of peptic ulcer disease, its effect on the function of the gastric mucosa is not fully understood. A protein isolated from H. pylori culture filtrate has been shown to inhibit gastric acid secretion by rabbit parietal cells. Chapter 13 aims at investigating the effect of H. pylori protein on the synthesis of human gastric mucosal cyclic AMP, which acts as a second messenger for a variety of gastric functions including acid secretion. PGE_2 synthesis is also measured in Chapter 13 because PGs are known to interact with c.AMP.

SECTION 2

MUCOSAL PROSTAGLANDIN SYNTHESIS IN RESPONSE TO TISSUE SAMPLING, DIET, AND DUODENAL ULCERATION.

SECTION 2. MUCOSAL PROSTAGLANDIN SYNTHESIS IN RESPONSE TO TISSUE SAMPLING, DIET, AND DUODENAL ULCERATION.

INTRODUCTION.

This section deals with three basic issues related to PG measurement and metabolism. Chapter 6 investigates the consequences of freezing mucosal specimens, as far as their ultrastructure and capacity to synthesize PGs are concerned. Chapter 7 measures the effect on gastric tissue PGE_2 and gastric histology of diets containing 3 naturally occurring oils: fish oil, evening primrose oil and olive oil. The composition of these oils is also analysed, looking for fatty acids that might inhibit PG synthesis. Chapter 8 measures duodenal mucosal PGs in patients with duodenal ulceration, in order to verify the presence or absence of deficient PG production in these patients.

CHAPTER 6.

6. EFFECTS OF FREEZING GASTRO-DUODENAL BIOPSIES ON THEIR ULTRASTRUCTURE AND CAPACITY TO SYNTHESIS PROSTAGLANDINS.

6.1. SUMMARY.

The effect of freezing endoscopic gastric and duodenal biopsies prior to prostaglandin (PG) assays on their ultrastructure and PG synthesis was studied in 6 subjects. Frozen samples produced more PGI_2 and PGE_2 than fresh ones in gastric and duodenal tissue. Freezing also resulted in disintegration of the cell membranes, considered as the probable sources of PG precursors, in all cells within the individual biopsies. These findings indicate that freezing gastric and duodenal biopsies before PG measurements results in extensive tissue homogenization, which in turn increases PG generation.

6.2. INTRODUCTION.

Gastric and duodenal PG measurements have been beset with technical pitfalls and further work in this area has been recommended (199). The differences in the quantity and type of PGs measured may be partly explained by the differences in the types of assays used (19,65,86). The effect of various ways of dealing with tissue samples prior to carrying out the PG assay is likely to be of importance though this is still not clear. For instance, duodenal mucosal PGI_2 in duodenal ulcer patients was found

to be normal in a study using fresh specimens (76) and deficient by another using frozen biopsies (77). The aim of this double-blind study is to investigate the effect of freezing gastric and duodenal biopsies on their capacity to synthesise PGE_2 and PGI_2. Ultrastructural assessments are also performed to look for changes that might explain the potential of frozen tissue to synthesise PGs.

6.3. SUBJECTS AND METHODS.

Six subjects (5 females and 1 male) aged 32-45 years, were included after ensuring that they took no medications, had normal upper gastrointestinal endoscopy, and no histological evidence of gastritis or duodenitis. They were all non-smokers. Their blood tests showed normal hepatic and renal function, normal haemoglobin and normal platelet and blood cell counts. There was no suggestion of bleeding tendencies. Informed consents were obtained and the study was approved by the local Ethical Committee.

7.5 - 15 mg Diazemuls (diazepam) was given intravenously prior to endoscopy. Two pairs of biopsies were taken from the gastric antrum and two pairs from the first part of the duodenum of each subject. One pair was sent for electronmicroscopy and the other for PG synthesis. Each pair consisted of one fresh biopsy and one frozen biopsy taken within 1 cm of each other. The biopsies were assigned to either fresh or frozen groups at random. They were kept for 30 minutes in liquid nitrogen (-190^{o}C,

frozen biopsies) or ice (0 - 4°C, fresh biopsies) after which they were processed for PG measurement.

PG synthesis was checked after washing the individual biopsies in 0.5 ml of phosphate buffered saline for 30 minutes at 20°C to minimize measurements of compounds induced by trauma. The supernatant was discarded and 0.5 ml of fresh phosphate buffer was added at each of the following stages of incubation: at 20°C for 30 minutes, and at 37°C for 5, 15 and 30 minutes respectively. After each stage the supernatant was removed and added to an equal volume of methyloximation agent (96), prior to radioimmunoassay. The same radioimmunoassay was carried out on the methyloximated supernatants of both fresh and frozen samples. Protein estimation was made using the Lowry method (98). The results are expressed in ng/mg protein. PGI_2 was measured as its stable metabolite (6-oxo-PGF_1). All specimens were assayed in duplicate. Details of the radioimmuno-assay are as described in Chapter 2.

Preliminary studies showed that the mean (SD) initial wet weights of unwashed pairs of fresh and frozen biopsies (5 $\pm$ 1.6 mg) dropped only in insignificant amounts over 5 half hourly consecutive periods of incubation, the first of which being equivalent to a washing period. There were no significant differences in the changes in weight between fresh and frozen specimens.

On arrival at the electronmicroscopy laboratory, the

biopsies were diced into blocks approximately 1 mm^3. Five blocks per biopsy were processed for electron-microscopy. Light microscopy on 2u thick sections was then used to identify areas for ultrastructural examination. 50 nm thick sections were then cut to cover a 3 mm diameter grid. All biopsies were presented at random with no differentiation between fresh and frozen specimens.

Statistical analysis included the Wilcoxon's signed ranks test.

6.4. RESULTS.

As seen in Figures 3-6, greater amounts of PG were produced by biopsies processed after having been frozen prior to incubation.

The electronmicroscopic features of two pairs of gastric and duodenal biopsies are shown in Figures 7-10.

The damage caused by freezing involved all the cells inspected; it was superadded to the effect of other sources of trauma such as that of obtaining the biopsies. Since the procedures employed in taking the biopsies, fixing and processing the tissues were standard throughout, it was not considered necessary to conduct any statistical analysis on cells; damage by freezing was obviously confined to frozen biopsies only (changes seen in 100% frozen cells against 0% in fresh cells).

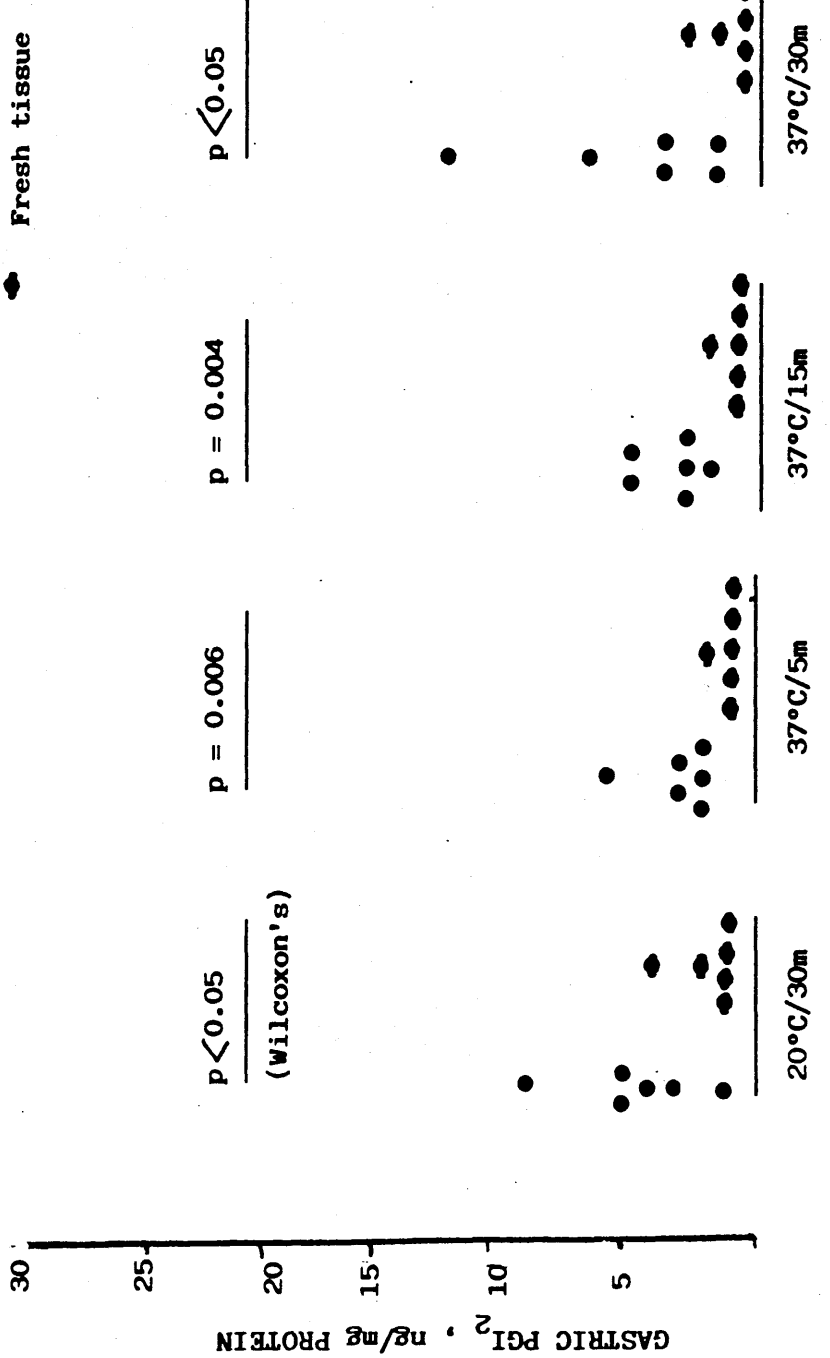


Fig. 3. Gastric PGI_2 produced by fresh and frozen tissue at progressive stages of incubation.

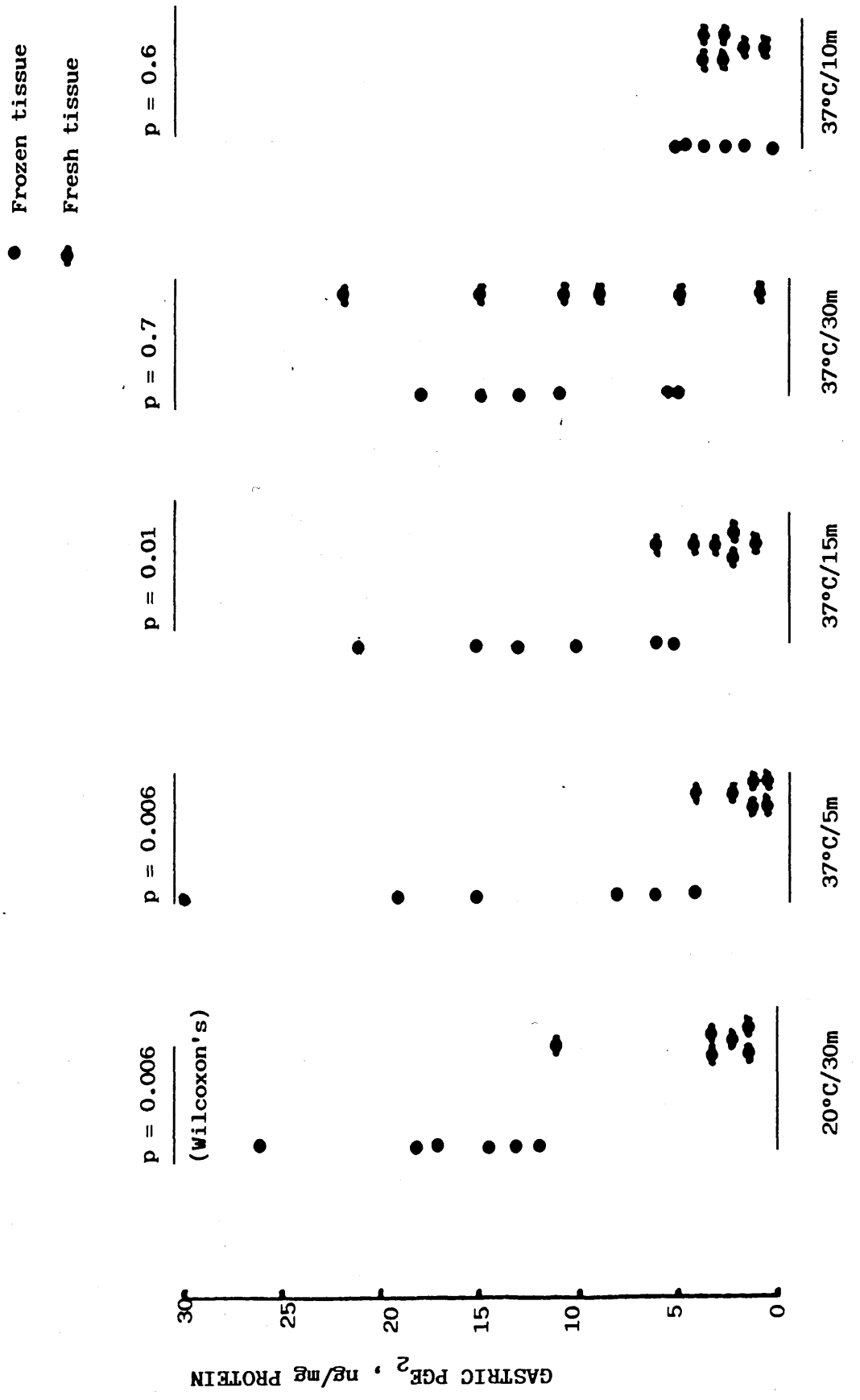


Fig. 4 Gastric PGE_2 produced by fresh and frozen tissue at progressive stages of incubation.

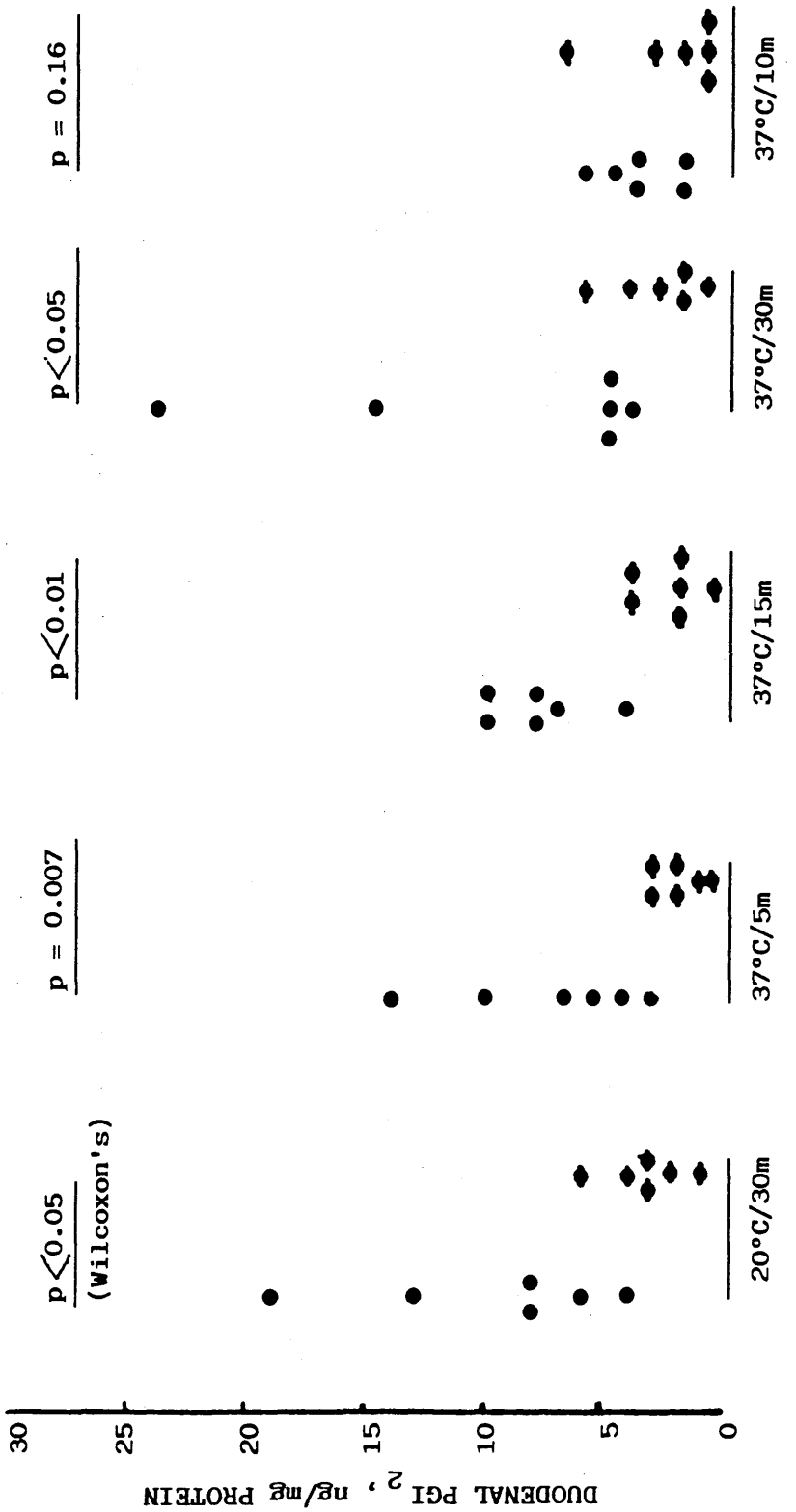


Fig. 5 Duodenal PGI_2 produced by fresh and frozen tissue at progressive stages of incubation.

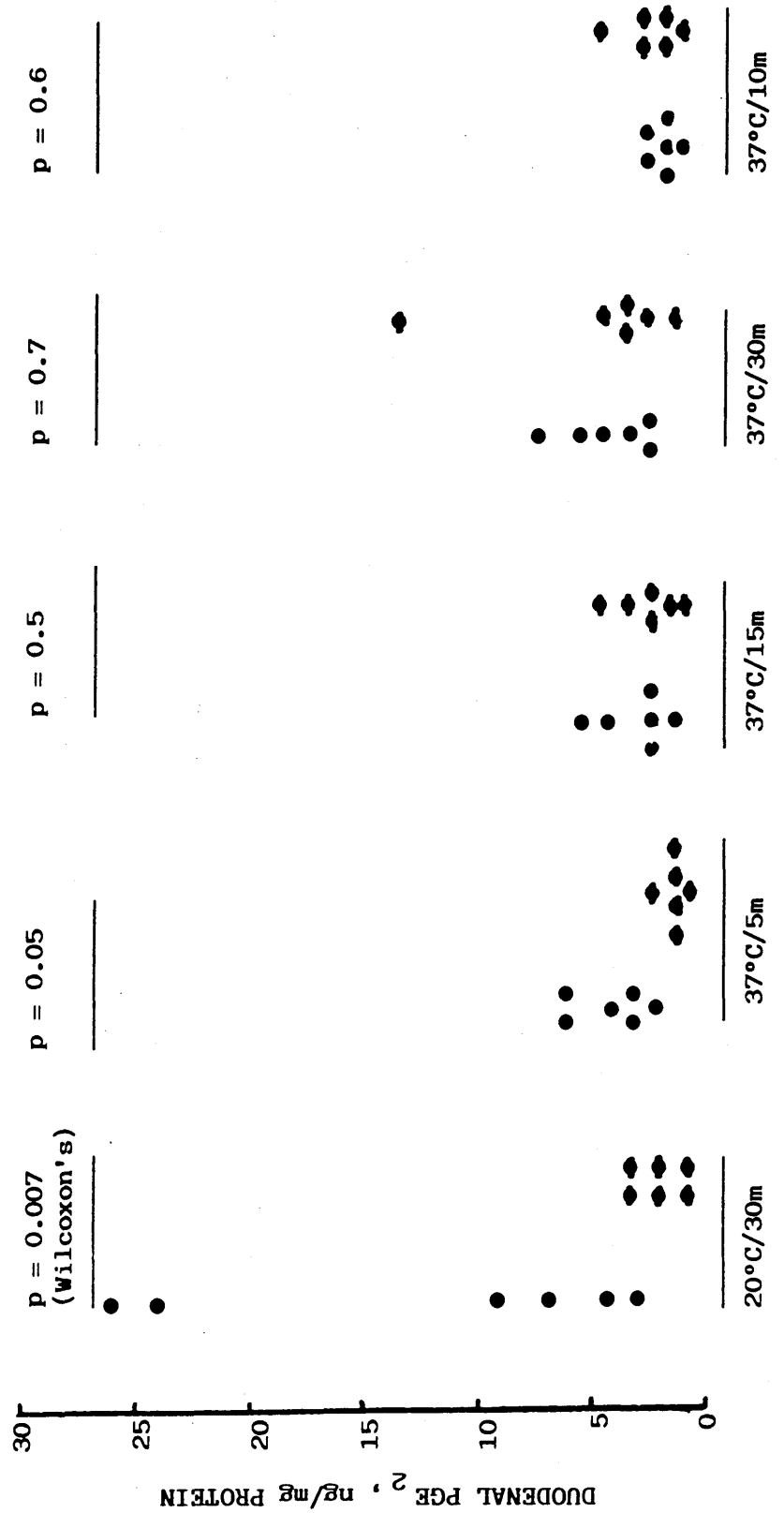


Fig. 6 Duodenal PGE_2 produced by fresh and frozen tissue at progressive stages of incubation.

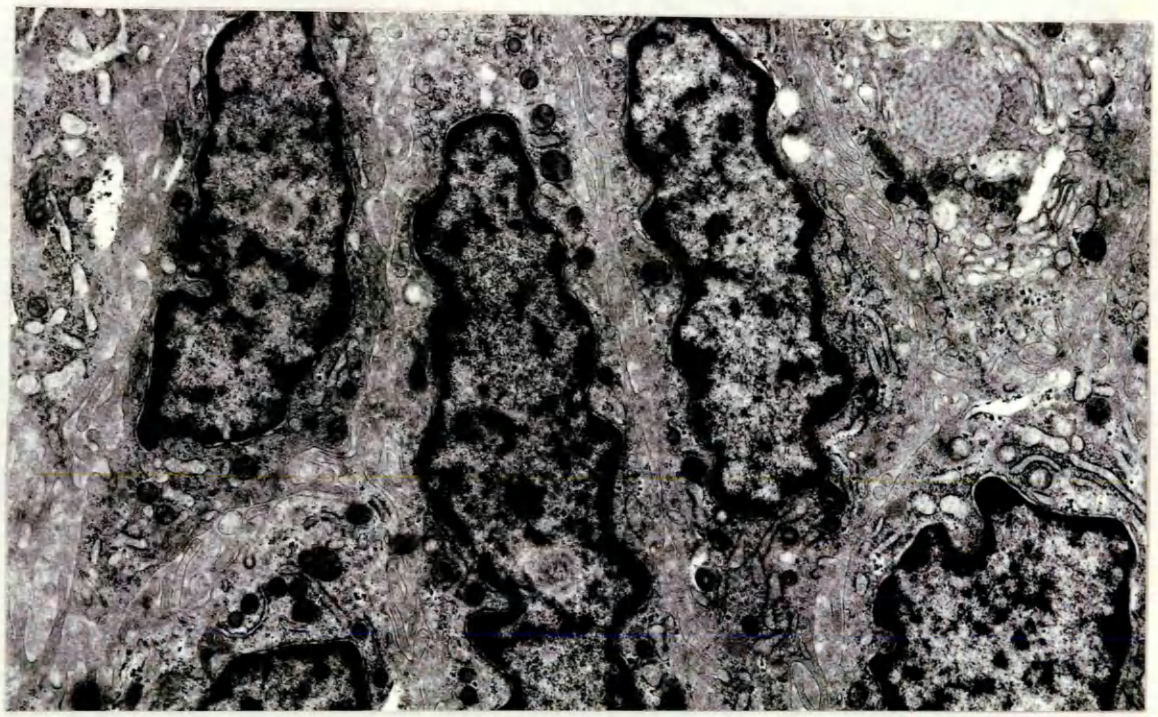


Figure 7. Fresh gastric biopsy (x14160); Healthy looking cells with well defined nucleus, cytoplasmic organelles and cell membranes

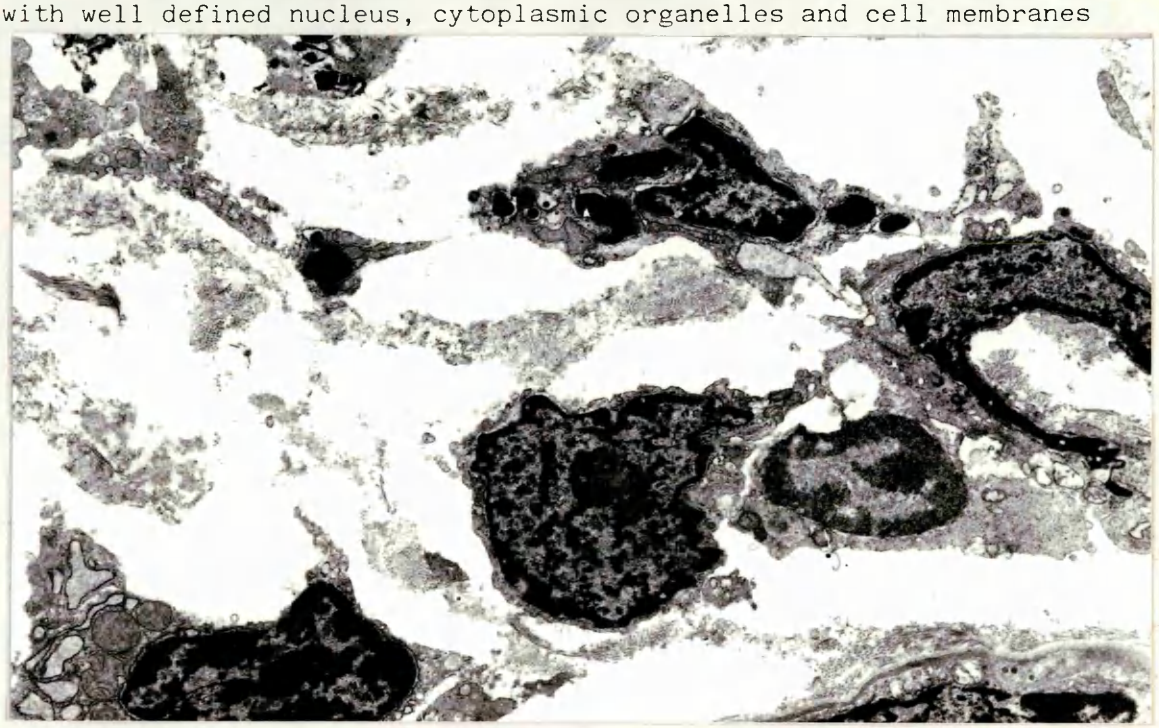


Figure 8. Frozen gastric biopsy (x8640); Shrunken cytoplasm and nuclei, and poorly defined cell membranes

Figure 9. Fresh duodenal biopsy (x18,000); Good cellular preservation with junctional complexes and microvilli surrounding the lumen

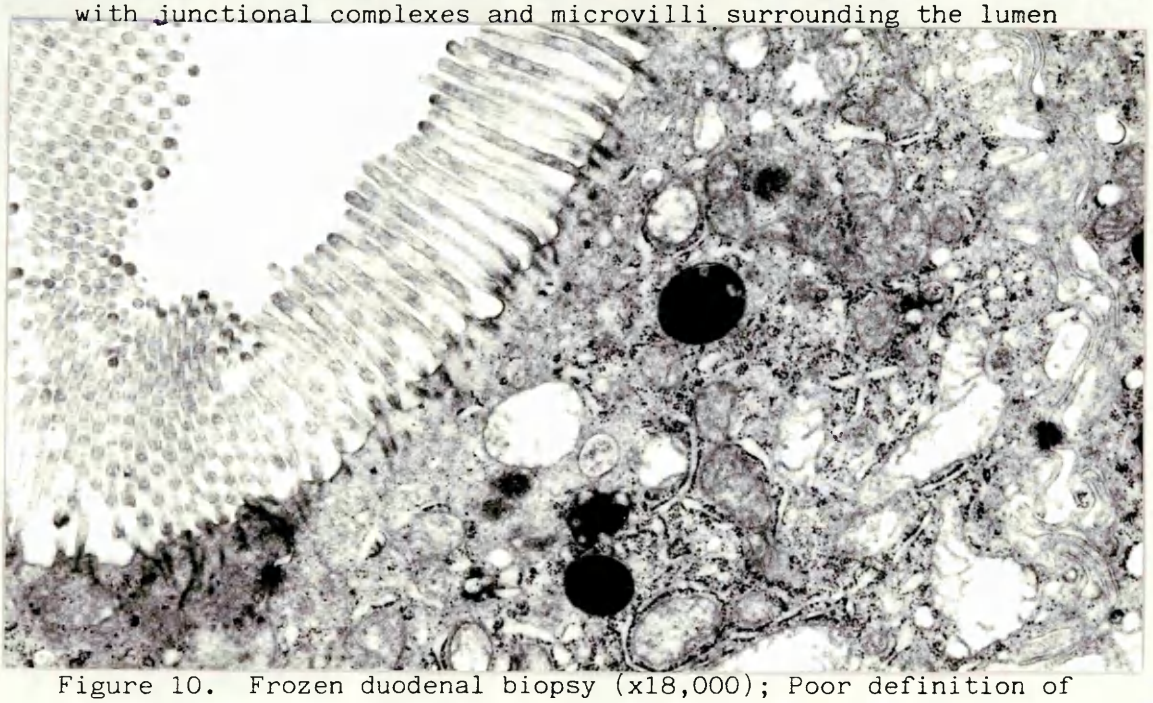


Figure 10. Frozen duodenal biopsy (x18,000); Poor definition of junctional membranes with ice crystal formation within the cytoplasm

6.5. DISCUSSION.

The ultrastructural changes seen in the frozen specimens are consistent with those described elsewhere (200). The effect of freezing on cell functions is not new, and cryopreservatives were suggested about 40 years ago when Polge, Smith and Parkes (201) showed that glycerol afforded considerable protection to spermatozoa during freezing and thawing. It was later shown that freezing can result in the loosening of acrosomal membranes of the spermatozoa because of "thermal shock" and ice crystal formation within the cytoplasm (202). These changes are thought to take place within minutes of freezing, hence the term "thermal shock" (202). The results of this study are in agreement with these findings despite the fact that gastro-duodenal cell membranes are thinner and, theoretically, more flexible. However, while other cellular functions might be adversely affected by freezing PG synthesis appears to be enhanced; this is probably due to accumulation of larger amounts of arachidonic acid precursors derived from the phospholipids of the damaged cell membranes, though this study did not measure such precursors.

Where homogenization of tissue prior to testing for PG synthesis is to be considered, freezing seems to offer a more effective and convenient option, as it can result in rupture of 100% cells. This is more extensive than using a Dounce (manual homogenizer, 67% cytolysis) and as effective as Potter's motorized homogenizer which produces

95% cytolysis (91,203).

Age, sex, smoking and medications can probably influence PG synthesis. Correction for these factors was made by studying paired specimens. Other sources of variation in reported PG measurements also include the type of assay used (19,65,86), and, as shown by the results, whether the tissues are frozen or not prior to incubation.

In this study the tissues were washed for 30 minutes prior to incubation in order to remove PGs synthesised in response to the initial trauma of obtaining the biopsy, or at least to minimize their measurements; I agree with other workers (77) that a shorter period, e.g. 5-15 minutes, could still be helpful as long as the timing is standardized for all specimens studied within the same experiment. Also, the incubation period was divided into the above stages simply to assess the rate of PG synthesis; such division is not essential in other circumstances, and one or two stages can be adequate. There are no general guide-lines as to whether specimens should be processed fresh or after freezing them in the context of measurement of endogenous PG synthesis. As mentioned in Chapter 2, freezing can be used to facilitate storage of specimens till all study subjects have been recruited and the study completed. Specimens can then be processed together and this helps eliminate the influence of many environmental and operator-dependent factors. Freezing specimens and the subsequent rise in their PG

synthesis should not influence the overall interpretation of results provided that all specimens assessed in a particular study are kept frozen prior to PG measurement. Fresh tissue can be used when it is of crucial importance to keep as many cells as possible alive during incubation, as in Chapters 12 and 13. To homogenize fresh tissue (19,20,65,78,91) probably removes its relative advantage over frozen tissue because of the damage caused by homogenization. If fresh unhomogenized tissue is to be used, two issues have to be considered: keeping the specimens viable during incubation, and stabilizing PG compounds till all specimens have been obtained and the study completed.

In conclusion, freezing gastric and duodenal specimens increases their capacity to produce PGs. This is probably due to the breakdown of cell membranes. These results emphasize the importance of standardizing tissue sampling to all specimens used in a particular study. In other words, biopsies can be used fresh or after freezing, provided that either procedure is standardized to all specimens or study groups.

CHAPTER 7.

7. THE EFFECT OF DIETS CONTAINING FISH OIL, EVENING PRIMROSE OIL AND OLIVE OIL ON PROSTAGLANDIN E_2 SYNTHESIS AND HISTOLOGY OF RAT STOMACH.

7.1. SUMMARY.

Arachidonic and linoleic acids were previously shown to have protective effects on the gastric mucosa by inducing prostaglandin (PG) synthesis. The natural sources of these acids like fish, evening primrose and olive oils might however contain components capable of blocking PG synthesis.

To investigate the effects of these oils on gastric PGE_2 and the mucosal thickness, 4 groups of rats were fed on fat-free diet, 7% fish oil, 7% evening primrose oil (E.P.O.) and 7% olive oil diets. Compared to a median PGE_2 of 2 ng/mg protein in the control group, the median PGE_2 in rats fed on fish oil was 9 ($p<0.01$), E.P.O. 50 ($p<0.01$) and olive oil 31 ng/mg protein ($p<0.001$). No significant differences were noted in the mucosal heights of the 4 groups.

These results suggest that diets containing any of these oils can increase gastric PGE_2 synthesis. Such diets may be potentially useful in the prevention of peptic ulcers, given the known protective effects of PGE_2.

7.2. INTRODUCTION.

Polyunsaturated fatty acids like arachidonic and linoleic acids, when given to experimental animals, result in increases in PG concentrations in gastric juice (59,60) which in turn have trophic effects on the gastric mucosa (38) and can prevent experimental mucosal injury (60).

It is not clear whether gastric PG production can still be increased by these acids when taken as part of naturally occurring oils that also contain other fatty acids like eicosapentaenoic acid and docosahexaenoic acid; these competitively inhibit the utilization of arachidonic acid and its precursors by cyclo-oxygenase (12,61,62). The aim of this study was to assess the effect on gastric tissue PGE_2 and the mucosal thickness of diets containing fish oil, EPO, and olive oil, as this might have some clinical and nutritional implications. The basic fatty acid composition of each oil was also determined. The choice of these oils was largely influenced by the recent interest in the apparently useful roles of fish oil and EPO in various systemic diseases (204-210). Little is known about the value of these oils and the more widely used olive oil in peptic ulcer disease. They are, however, known to contain one or more of the fatty acids mentioned above.

7.3. METHODS.

Four-week old male Lewis rats were used. They weighed 100-120 g, and were kept in cages with 12 hour

light and 12 hour dark cycles. They were allowed free access to water and only one kind of diet to which they were randomly allocated.

Diets were prepared by Special Diets Services Limited, Witham, Essex, CM8 3AD, England. Five hundred grams of fat free diet were mixed with 40 ml of each of the following oils separately: cod liver oil (density of 0.93 g/ml), EPO (0.926 g/ml) and Spanish olive oil (0.916 g/ml). The oil component of each diet was therefore equivalent to 7%. Animals were randomly divided into four groups according to the type of diet they would receive: fat-free diet (n=7, control group), 7% fish oil diet (n=7), 7% EPO (n=10) and 7% olive oil diet (n=13). Diets were renewed and consumed within 2-5 days of preparation.

After 6 weeks of taking these diets the animals were sacrificed in ether. Each stomach was removed, opened along the greater curve and its contents washed off under a stream of cold water. Full thickness biopsies (5-10 mg) were taken from the antral region, immediately frozen in dry ice (-96^{o}C) and later stored at -70^{oC} till the time of PG measurements. No attempt was made to dissect the gastric mucosa to avoid traumatizing the tissue, which could subsequently affect PG synthesis. The rest of the stomach was fixed in formalin/saline solution prior to histological assessment. All specimens, including the biopsies, were given code numbers to facilitate

randomization.

Before testing for PG synthesis, the biopsies were thawed, weighed, and washed in 1 ml of phosphate buffered saline per biopsy for 5 minutes at 20°C to minimize measurement of PGs induced by trauma. The supernatant was removed and 1 ml of fresh phosphate buffer was added. Tissues were then incubated at 37°C for 60 minutes. The supernatant was removed and mixed with an equal volume of methyloximating agent (MOX) (96), left at 20°C overnight and kept at 4°C till the time of the radio-immunoassay. The biopsies were taken for protein measurement (98) and the results were expressed in nanograms of PGE_2/mg protein. Details of the radioimmunoassay are as described in Chapter 2. For histological assessment, 2 longitudinal sections were made along the greater curve. They were processed routinely and stained with haematoxylin and eosin. The antral mucosal thickness was determined by taking the mean of 5-10 (average 7) measurements from the luminal surface of the antrum to the upper border of the muscularis mucosae using an eye piece graticule on a calibrated microscope. The antrum was chosen for histology because PGs were measured in antral tissue. The three oils used were analysed for their basic fatty acid ingredients by gas-liquid-chromatography (G.L.C.). Statistical analyses involved the Mann-Whitney and Kruskal-Wallis tests where appropriate.

The study was carried out according to local ethical and Home Office regulations governing experiments on

animals.

7.4. RESULTS.

After 6 weeks of taking different diets, the amount of weight gained by the four groups of rats were comparable (final weights of 200-250 g/rat) without any significant differences between the groups.

The basic fatty acid constituents of the oils studied are shown in Table 1. As expected, fish oil is rich in eicosapentaenoic and docosahexaenoic acids, while EPO and olive oil contain large amounts of linoleic and oleic acids, respectively. In addition, each of these oils contains, in smaller degrees, some other fatty acids which can be important in deciding the amount of PGs produced by the gastric tissue.

The amounts of PGE_2 produced by various groups of rats and the mucosal thickness of these groups are shown in Figures 11 and 12. The diets used did not seem to have any significant effect on the mucosal thickness after 6 weeks, but all oil diets resulted in higher PGE_2 values than the control group. It is also worth mentioning that the histological features were normal in all groups.

Since the mucosa was not dissected in the antral biopsies, the PGE_2 measured reflects the activity of other tissues as well as the mucosa. This should not influence the interpretation of the results because the procedures were standardized in all groups.

TABLE 1

BASIC FATTY ACID CONSTITUENTS OF FISH, EVENING PRIMROSE AND OLIVE OILS AS DETERMINED BY GAS-LIQUID-CHROMATOGRAPHY.
(% of total weight)

Fatty Acids	Fish Oil	Evening Primrose Oil	Olive Oil
Myristic Acid [$C_{14:0}$]	13.3	-	0.2
Palmic Acid [$C_{16:0}$]	0.3	5	10
Palmit-oleic Acid [$C_{16:1}$]	12.2	-	0.2
Hexadecdienoic Acid [$C_{16:2}$]	13.3	-	-
Stearic Acid [$C_{18:0}$]	3.8	1.7	2.6
Oleic Acid [$C_{18:1}$]	16	9.5	80
Linoleic Acid [$C_{18:2}$]	1.2	77	6.4
Linolenic Acid [$C_{18:3}$]	1	4	0.5
Arachidonic Acid[$C_{20:4}$]	5.6	-	-
Eicosapentaenoic Acid [$C_{20:5}$]	27	-	-
Docosapentaenoic Acid [$C_{22:5}$]	2.2	-	-
Docosahexaenoic Acid [$C_{22:6}$]	3.7	-	-

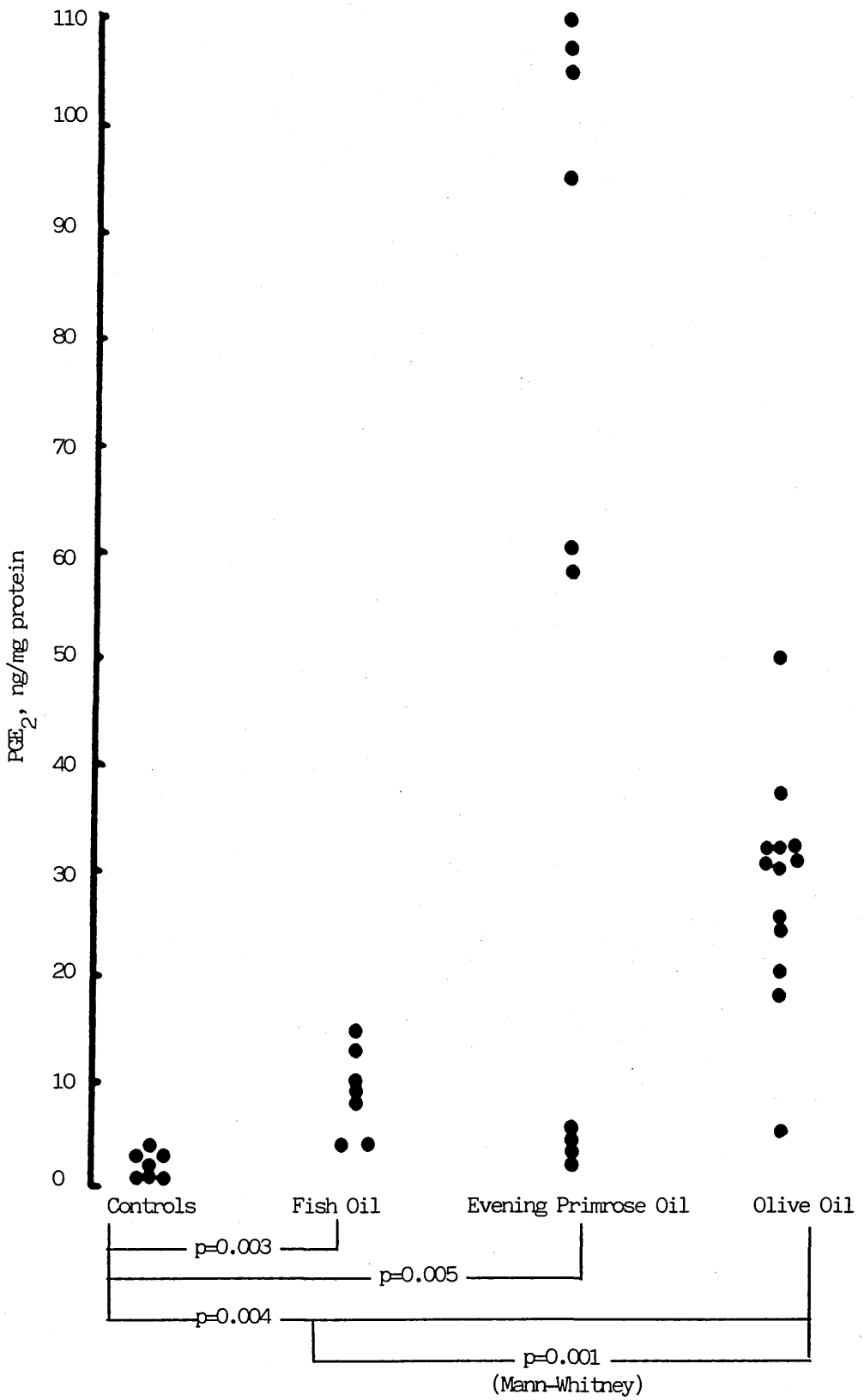


Fig. 11. Gastric tissue PGE_2 produced by study groups fed on fish oil, evening primrose oil and olive oil, as compared with controls.

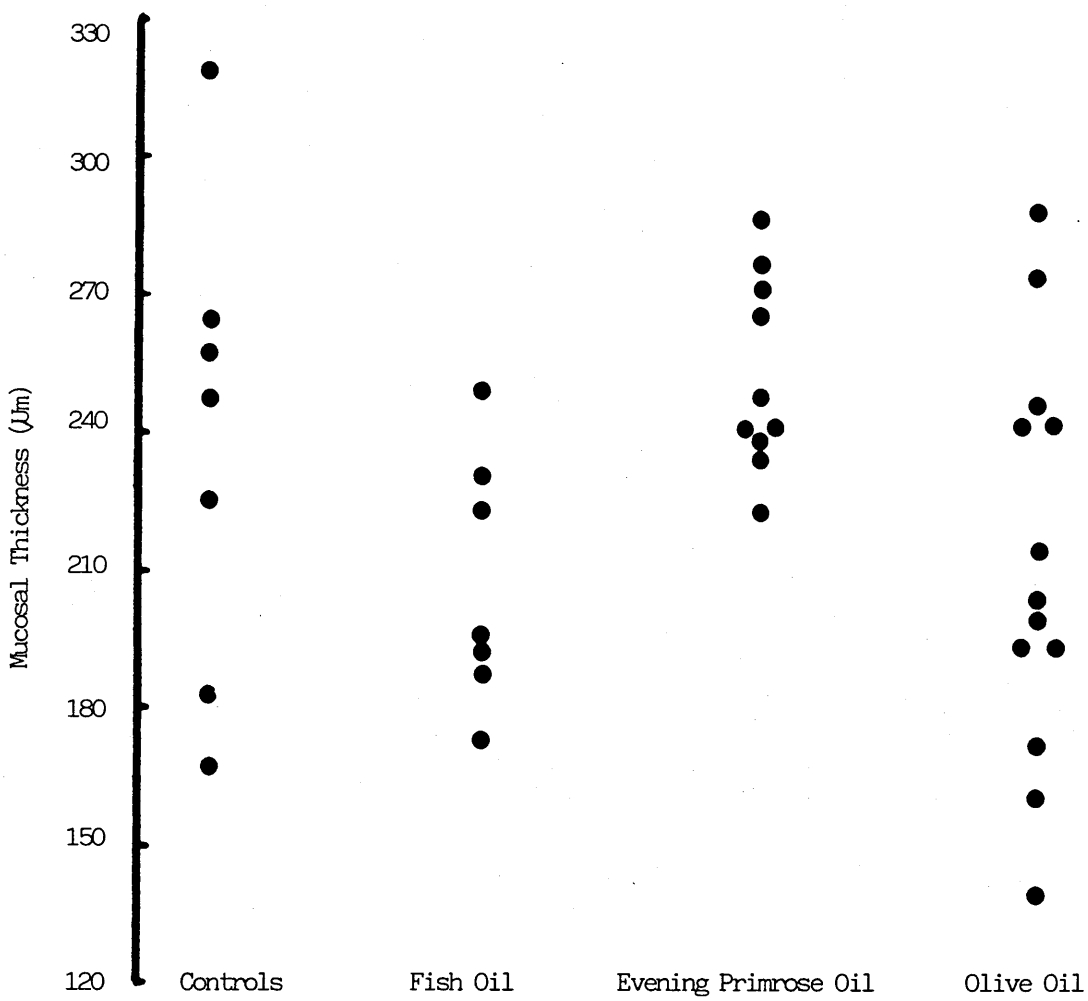


Fig. 12. Gastric mucosal thickness in study groups fed on fish oil, evening primrose oil and olive oil, as compared with controls.

7.5. DISCUSSION.

These results show that all the oils studied, including olive oil, are capable of increasing gastric PGE_2 synthesis. The relatively low PGE_2 production by the fish oil group could be as a result of the synthesis of PGE series with one or two extra bonds induced by highly unsaturated precursor acids (eicosapentaenoic and docosahexaenoic acids). Also, products from these precursors would not be detected as PGE_2. In this assay, there was 31% cross-reaction between PGE_2 antiserum and PGE_3, the more likely product of fish oil. Since it is important to measure prostanoids induced by fish oil ingestion, a procedure like gas chromatography-mass spectroscopy would stand a better chance in distinguishing between PGE_2 and PGE_3 than radioimmunoassay because of its high specificity.

The interaction between components of fish oil and PG synthesis might have more desirable effects in systems other than the gastric mucosa. Eicosapentaenoic acid is both a substrate (61) and an inhibitor of the cyclo-oxygenase pathway (62), whereas docosahexaenoic acid is simply an inhibitor (62). Thromboxane A_3 derived from eicosapentaenoic acid is less active in aggregating platelets than conventional TXA_2, an effect which helps prevent coronary heart diseases (12,61,62). It was recently suggested that eicosapentaenoic acid can compete with arachidonic acid not only for metabolism by the cyclo-oxygenase pathway but also for metabolism by the

lipoxygenase pathway to leukotrienes, hence its beneficial effect in modifying immune and inflammatory responses (205). The animals were not subjected to any gastric injurious agents in this study. This is because many studies have already established the mucosal protective effect of PGs in general and PGE_2 in particular against experimental injuries (211). Ethical issues were also considered in this context.

The study groups varied in numbers of animals as a result of randomization, but I accept that it is better to use equal numbers where possible.

In addition to their organ protective properties, PGs are said to exert a trophic effect on the gastric mucosa (38). An attempt was made to demonstrate this feature by measuring the mucosal thickness, but there was no significant differences between various diet groups. One reason for this might be because of the possibility that the 6- week study was too short for gross structural changes to evolve.

It has been proposed that the marked increase in the dietary availability of essential fatty acids could be responsible at least in part for the marked decrease in the incidence and virulence of peptic ulcer disease in the Western World, probably because such diets are capable of inducing PG synthesis (63). Corn oil and safflower oil are the main sources of essential fatty acids in Western societies; they contain about 70% by weight linoleic acid

which is comparable to that contained in EPO. It could therefore be speculated that corn and safflower oils might have an effect on gastric PGE_2 production similar to that of EPO.

In view of the above hypothesis (63), these results may have some clinical and nutritional implications. This is particularly true in the case of olive oil, provided that the results can be confirmed in humans. The incidence of peptic ulcer disease is also low in Italy (Bianchi Porro G, personal communication), where the consumption of olive oil is high. The rarity of peptic ulcer disease in Italy could be related to the ability of olive oil diets to stimulate gastric PG production, probably because of the presence of linoleic and linolenic acid in olive oil.

Moreover, olive oil is easier to produce, and is probably more palatable than fish oil or EPO. For these reasons, compliance with its intake might be easier to achieve. However, further studies are needed to see if these findings apply to humans.

CHAPTER 8.

8. DUODENAL MUCOSAL PROSTAGLANDIN SYNTHESIS IN PATIENTS WITH DUODENAL ULCERS.

8.1. SUMMARY.

The capacity of the duodenal mucosa to synthesise PGE_2, PGI_2 and thromboxane A_2 was tested in short term incubates of biopsies taken from duodenal ulcer patients and a group of controls without ulcers. The values of all prostaglandins tested were similar in both groups without any statistically significant differences. It was not possible to confirm findings of previous reports suggestive of a defect in the potential of human duodenal mucosa to synthesise prostaglandins in cases of duodenal ulceration. These findings might help explain, at least in part, the failure of the so-called "cytoprotective" doses of prostaglandin analogues in the treatment of duodenal ulcers.

8.2. INTRODUCTION.

A balance, probably dynamic in nature, normally exists between the duodenal mucosal resistance and potentially injurious luminal milieu, with mucosal resistance adapting to luminal conditions. Mucosal resistance is not exclusively a function of individual surface cells, but rather a multifactorial property of the various mucosal layers, enabling the tissue to contain and quickly repair injury. The chief factors determining

mucosal resistance are believed to be: the state of the adherent mucus layer, mucosal bicarbonate secretion, blood flow and regenerative capacity. Local prostaglandin (PG) synthesis modulates some of these factors, but other mechanisms are also involved.

Ulcers occur when an imbalance allows luminal injurious factors to predominate over mucosal resistance. The chief injurious factors are acid and peptic activity. Bile reflux, drugs and stasis may alter the luminal milieu and act together with acid-peptic activity to induce injury, weaken mucosal resistance, or both.

Disturbances of protective mechanisms or enhanced injurious potential - some possibly genetically determined, others acquired - have been identified in ulcer patients. Since PGs modulate several key mucosal protective mechanisms, and some exogenous PGs or their derivatives accelerate healing of peptic ulcers, it follows that PG deficiency may contribute to the development of peptic ulcers. The evidence that such deficiency exists in duodenal ulcers has been indirect and has given rise to some disagreement amongst previous workers (64,76-78). Moreover, recent studies (51,212) have shown that PG analogues healed duodenal ulcers only when given in acid-inhibitory doses which are higher than those needed for cytoprotection, despite the multitude of favourable properties of such analogues (38).

The aim of this study is to see if a deficiency

exists in the capacity of the duodenal mucosa of patients with duodenal ulcers to synthesis PGs, as compared with a group of control subjects without ulcers.

8.3. PATIENTS AND METHODS:

Patients with duodenal ulcers were entered into the study if they were 18-70 years old at the time of the diagnosis of their ulcers by endoscopy. They were excluded if they took ulcer healing agents or NSAIDs within a week of the endoscopy or if they had any other systemic illness or infection. Patients with a history of previous gastric surgery or heavy alcohol intake (more than 10 units/week) were also excluded.

Control subjects were recruited after presenting with an indication for upper gastrointestinal endoscopy. The endoscopy and all other subsequent tests were normal. They were excluded if they had any of the drugs or the exclusion criteria for duodenal ulcer patients mentioned above. Subjects outside the age range of 18-70 were also excluded.

The endoscopy was performed after a 12-hour fast, and the intravenous administration of 5-15 mg diazepam.

Single biopsies weighing 5-10 mg were taken from the first part of the duodenum of the control subjects, and 2 cm away from the ulcer edge in duodenal ulcer patients. They were immediately frozen in dry ice ($-96^{o}C$) and later kept at $-70^{o}C$ till the time of PG assay.

Before testing for PG synthesis, the specimens were

thawed, weighed, and washed in 0.5 ml per biopsy of phosphate buffered saline for 5 minutes. The supernatant was discarded as it is considered to contain PGs induced by trauma. The specimens were then incubated at 20°C for 30 minutes and at 37°C for another 30 minutes. 0.5 ml of fresh buffer was added at the start of each stage of incubation, and the supernatant was removed at the end for methyloximation (details of this process, the subsequent radioimmunoassay and protein estimation are as described in Chapter 2). All specimens were assayed in duplicate under double-blind conditions.

Three classes of PGs were measured: PGE_2, PGI_2 (as 6-oxo-PGF_1), and thromboxane A_2 (as TXB_2). The results are expressed in ng/mg protein.

The Mann-Whitney test was used for statistical analysis.

Informed consents were obtained and the study was approved by the local Ethical Committee.

8.4. RESULTS.

A total of 27 duodenal ulcer patients and 21 control subjects were included; their demographic data are shown in Table 2. Both groups seem to be comparable as far as their ages and smoking habits are concerned. As expected, there are more males with duodenal ulcers, but the control group contains more females, possibly because the basic diagnosis in this group is that of probable irritable bowel syndrome.

TABLE 2

DEMOGRAPHIC DATA OF DUODENAL ULCER PATIENTS
AND THE CONTROL SUBJECTS.

	DUODENAL ULCERS	CONTROLS
Males	18	7
Females	9	14
Age (years) Median (range)	31 (29-42)	33 (23-42)
Smoking (cigarettes/day		
Nil	6	9
less than 10	4	3
10-20	9	5
more than 20	8	4
Total Number	27	21

The total amounts of PGs synthesised by both groups after 60 minutes of incubation are shown in Figure 13. There are no significant differences between duodenal PG synthesis in ulcer patients, as compared to the control group.

8.5. DISCUSSION.

The results show no evidence of any deficiency in the synthesis of PGE_2, PGI_2 or TXB_2 in the presence of duodenal ulceration; on the contrary PG values in ulcer patients tended to be higher than in the control group, possibly due to the presence of an element of duodenitis in the non-ulcerated or "healthy" looking mucosa from which the biopsies were taken. As in previous studies, however, no histological assessment was performed on representative duodenal tissue.

These findings are in agreement with those of some (76,78) but not all previous studies (77). In addition to their finding of unsuppressed PG values in duodenal ulcer patients, Ahlquist et al. (78) also showed that duodenal mucosal PGs in such patients failed to rise in response to the challenge with an acid load which might indicate a defect in the mechanism of mucosal adaptive cytoprotection.

Other workers showed low values of gastric antral PG synthesis in patients with duodenal ulcers (76), but the relevance of such finding is unclear.

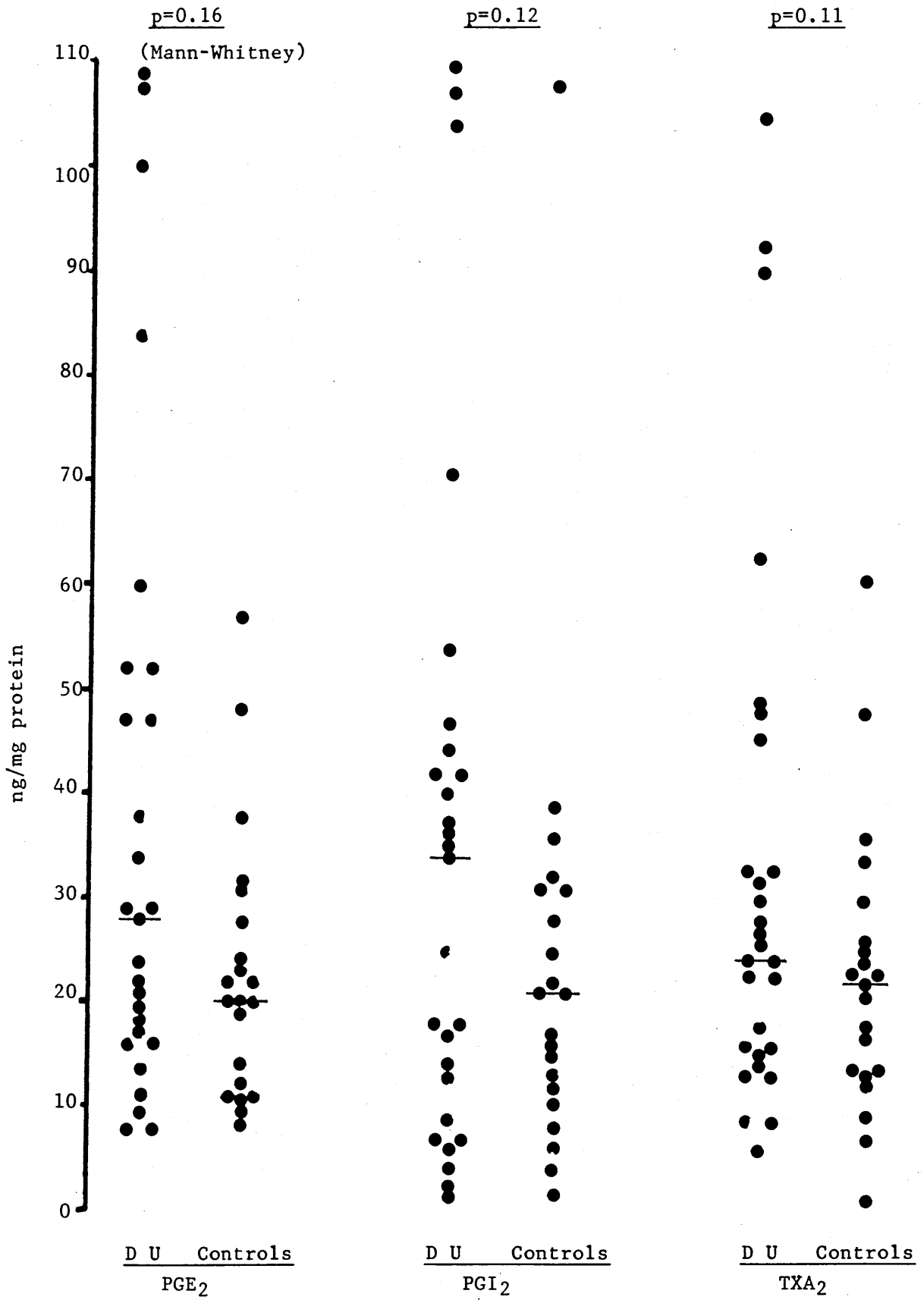


Fig. 13. Duodenal mucosal prostaglandins in patients with duodenal ulcer and controls.

While agreeing with another previous report (77) that PGE_2 and TXB_2 synthesis remain intact in duodenal ulcers, its finding of a low PGI_2 value could not be confirmed; the method used in that report to assess PG synthesis was in many respects similar to the one used by this study, at least as far as handling of the tissues is concerned. It is believed, however, that the assay used in this study has the added advantage of a greater degree of PG compounds stabilization by methyl-oximation (96).

The subjects investigated in the study were not on any therapy for at least one week before the biopsies were taken. The concurrent intake of some NSAIDs could have resulted in suppression of PG values, as in the case of findings obtained from experimental models using NSAIDs. This might have been the origin of the idea that PG synthesis is low in duodenal ulcers, which could not be confirmed by many subsequent studies including this one. This also highlights the importance of taking into consideration the drug history of patients included in PG assessments.

In conclusion, no evidence was found of PG deficiency in the duodenal mucosa of patients with duodenal ulcers. Such finding might be relevant to the interpretation of the results of other studies investigating the response of duodenal ulcers to the intake of PG analogues. As mentioned earlier, these agents were effective only in acid-inhibitory doses (51,212), which are higher than the amounts needed for cytoprotection, possibly because PG

synthesis is maintained in duodenal ulcer patients, as shown by this study.

SECTION 3.

ANTI-ARTHRITIC AND GASTRO-DUODENAL RESPONSES TO NAPROXEN AND ETODOLAC THERAPY. EFFECTS ON ENDOSCOPIC APPEARANCE, HISTOLOGY, AND PROSTAGLANDIN SYNTHESIS.

SECTION 3. ANTI-ARTHRITIC AND GASTRO-DUODENAL RESPONSES TO NAPROXEN AND ETODOLAC THERAPY. EFFECTS ON ENDOSCOPIC APPEARANCE, HISTOLOGY, AND PROSTAGLANDIN SYNTHESIS.

INTRODUCTION.

This section investigates a range of activities of two NSAIDs: a new preparation, etodolac, is compared to an established agent, naproxen. From the point of view of patients with an indication for their use, such as active rheumatoid arthritis, the anti-arthritic activity of these agents probably comes first in the order of priorities. Gastro-duodenal side effects, especially dyspeptic symptoms, might come second. These parameters are measured in Chapter 9. Mucosal PGs are measured in Chapter 10, to see if this could explain the differences between naproxen and etodolac regarding their effects on the gastric and duodenal mucosa. To put PG results in context, and to facilitate their interpretation, other responses are also included such as the anti-arthritic activities, the endoscopic scores and histology.

CHAPTER 9

9. EVALUATION OF THE EFFICACY AND COMPARATIVE EFFECTS ON GASTRIC AND DUODENAL MUCOSA OF ETODOLAC AND NAPROXEN IN PATIENTS WITH RHEUMATOID ARTHRITIS USING ENDOSCOPY.

9.1. SUMMARY.

The therapeutic efficacy and upper gastrointestinal side effects of a twice daily dose of 300 mg etodolac and 500 mg naproxen were compared in 30 hospital out-patients with active rheumatoid arthritis. Drugs were taken for 4 weeks in a double-blind, randomised, parallel group, single centre study. Rheumatological, endoscopic and laboratory assessments were performed at the start and on completion of the study.

Mucosal lesions developed in only 3 patients (20%) of the etodolac group and they all had low endoscopy scores, compared to 8 patients (53%) of the naproxen group with significantly worse endoscopy scores ($p<0.05$). Lesions were asymptomatic in all but one patient (on naproxen), and the gastric antrum was the commonest region involved. Of the 11 patients who developed endoscopic abnormalities in both groups, 7 (64%) were moderate to heavy smokers. Etodolac had a similar anti-arthritic effect to naproxen, but it appears to be better tolerated by the upper gastrointestinal mucosa as shown by endoscopy.

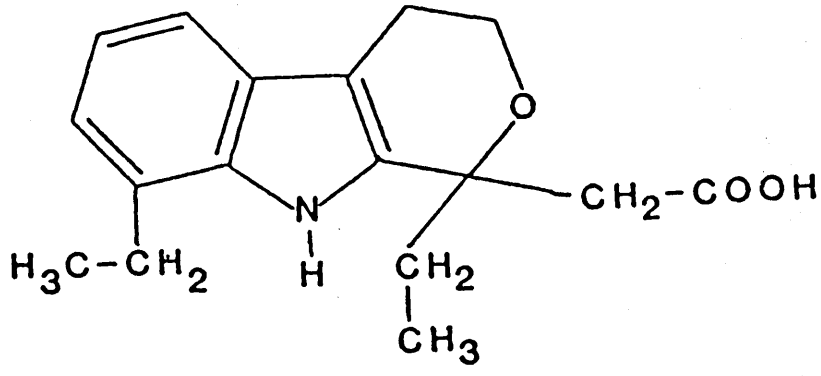
9.2. INTRODUCTION.

There is a continuous demand for an alternative anti-inflammatory therapy with a lesser damaging effect on the gastro-duodenal mucosa in patients with chronic arthritic diseases like rheumatoid arthritis. The unreliability of upper abdominal symptoms as indicators of NSAID-induced mucosal damage makes it important to use endoscopy for assessment of the possible side effects of NSAIDs especially the newer preparations. Etodolac (Lodine$^{(R)}$, Ultradol$^{(R)}$) is the first of a new class of non-steroidal anti-inflammatory drugs, the pyranocarboxylic acids, which has been shown to be effective in relieving symptoms of rheumatoid arthritis (213,214) and osteoarthritis. The chemistry of etodolac is shown in Figure 14. Comparing it to naproxen, a propionic acid derivative with established efficacy in inflammatory arthritic disorders (215), previous studies found no clinically significant differences in efficacy or in the recorded number of complaints in rheumatoid arthritis (216), although normal volunteers taking etodolac showed better endoscopic scores than those taking naproxen (217). This study aims at comparing the two drugs in patients with active rheumatoid arthritis in respect of their anti-arthritic activity and their effect on the gastro-duodenal mucosa as judged by endoscopy over a 4 week period.

9.3. PATIENTS AND METHODS.

Informed and written consents were obtained, and

CHEMISTRY OF ETODOLAC



Etodolac = Ultradol© = Lodine = AY-24,236

Chemical Name: (±)1,8-Diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid

Molecular Formula: $C_{17}H_{21}NO_3$

Molecular Weight: 287.35

Fig. 14. Chemical properties of etodolac.

patients were included only after ensuring that they fulfilled the criteria set by the American Rheumatism Association for the diagnosis of rheumatoid arthritis, if they were in Functional Class I, II, or III, and if they showed evidence of disease activity as suggested by all of the following: (i) number of tender or painful joints on motion - 5 or more, (ii) number of swollen joints - 3 or more, and (iii) duration of morning stiffness - 30 minutes or more.

Patients were excluded if their arthritis started before the age of 16, if they had any other systemic disease that involved the kidneys, liver or cardio-vascular systems, if they were known to have allergic reactions or disease, or if they had a definite or presumptive diagnosis of peptic ulceration. Drugs like sulphasalazine, systemic or intra-articular steroids, cytotoxics, and peptic ulcer healing agents were not allowed and patients taking them prior to the study were excluded. Patients receiving gold, salts, penicillamine or antimalarials were admitted provided that: (a) these agents were started at least 6 months prior to entry into the study, (b) dosage regime had remained fixed for the 2 months prior to entry into the study and would not be changed for the duration of the study and, (c) dosage of the antimalarial did not exceed 400 mg of hydroxychloroquine per day.

Suitable patients were asked to discontinue their current non-steroidal anti-inflammatory drugs and were

provided with paracetamol to take during a wash out period of about 4-7 days, after which they reported for their first visit and endoscopy. Both naproxen and etodolac were presented as grey capsules that looked identical to one another. They carried code numbers to facilitate randomization. Study medications were delivered in a double-blind fashion at doses of 300 mg twice daily of etodolac and 500 mg twice daily of naproxen together with a bottle of paracetamol, as an 'escape' analgesic, for a total of 4 weeks. The number of unused medications including paracetamol was noted by tablet count on the second visit on completing the study. Patients were dropped from the study if their initial endoscopy showed any abnormality, if they demonstrated non-compliance, developed intercurrent illness, did not get adequate relief from the study medication, or if they experienced serious adverse reactions.

9.3.1. Clinical Assessments:

These were performed on day one, immediately before issuing the study medications, and four weeks later. They included: (i) general medical history and physical examination, (ii) duration of morning stiffness (in minutes), (iii) grip strength (in mmHg), (iv) investigator and (v) patients evaluation of global condition (very good, good, fair, poor, very poor), (vi) articular index (218), (vii) laboratory tests including full blood count, biochemical screen, urinalysis, ESR, and (viii) endoscopy. Endoscopy was performed by a single operator (myself) on

both visits, and findings recorded on a 0 - 5 scale, modified from Lanza (217), for the oesophagus, duodenum and each region in the stomach; 0 score: normal, 1: any erythematous change, 2: submucosal haemorrhage, 3: single erosion, 4: multiple erosions, and 5: frank ulceration.

9.3.2. Statistical Analyses:

The significance of the degree of change in results of the clinical assessments between the two visits within each group, was tested using the Wilcoxon ranked and sign tests. Endoscopy scores were compared using the Mann-Whitney test between the two groups.

9.4. RESULTS.

Thirty-two patients entered the study, but two were excluded from the analysis; one patient who failed to attend the final visit and one who took medications prohibited by the protocol. It is also worth mentioning that 3 patients were not entered because of initial endoscopic abnormalities. Minor endoscopic lesions could have also existed in some of the study patients, but must have spontaneously disappeared during the washout period. The demographic data for the remaining 30 patients is presented in Table 3; the two groups were not significantly different in their baseline characteristics.

9.4.1. Compliance:

Patients in the naproxen group took a median of 90% of the number of naproxen tablets supplied (82-94%, interquartile ranges) and 57% (15-74%) of the paracetamol

TABLE 3

DEMOGRAPHIC DATA FOR 30 PATIENTS ANALYSED:
MEDIAN (INTERQUARTILE RANGES) FOR AGE AND
DURATION OF ARTHRITIS.

PATIENTS	NAPROXEN	ETODOLAC
Number studied	15	15
Sex: Male	5	4
Female	10	
Age (years)	57 (45-65)	50 (41-63)
Duration of Rheumatoid Arthritis (years)	6 (4-8)	11 (5-17)
On 2nd line drugs	10	8
Previous NSAID intake	12	12
Smokers: Light (1)	1	2
Moderate (2)	4	5
Heavy (3)	2	1
Alcohol	8	7

(1): less than 5 cigarettes/day
(2): 5-20 cigarettes/day
(3): more than 20 cigarettes/day.

provided; this is compared to 87% (86-90%) of etodolac and30% (3-49%) of paracetamol, respectively in the etodolac group. This suggests that compliance was fairly good in both groups but patients on etodolac needed less paracetamol.

9.4.2. Efficacy Assessment (Table 4).

Baseline values were comparable in both groups without any statistically significant differences. Significant improvements were observed in the duration of morning stiffness and articular index in both treatment groups. Patients treated with naproxen also showed a significant improvement in right-hand grip strength, which was not found in the etodolac group. When the results for this parameter were compared between the two groups, there was a significant advantage for naproxen ($p=0.03$, Mann-Whitney).

9.4.3. Endoscopy Results (Table 5).

None of the patients receiving etodolac had an endoscopic score greater than 3 (single erosion in one area) at the 4 week assessment. Indeed, twelve out of the fifteen patients in the group had a score of zero (normal mucosa in all regions) at that time. This is in contrast to the naproxen group where one patient developed frank ulceration, and a further six had multiple erosions. These differences in the scores between the two groups were statistically significant ($p<0.05$).

It is of interest to note that only two patients

TABLE 4

DURATION OF MORNING STIFFNESS, GRIP STRENGTH, ARTICULAR INDEX: MEDIAN AND INTERQUARTILE RANGES.

	NAPROXEN		ETODOLAC	
	WEEK 0	WEEK 4	WEEK 0	WEEK 4
Duration of morning stiffness (minutes)	90(60-180)	50(5-120)**	60(50-120)	30(5-60)**
Grip Strength(mmHg)				
Right hand	81(59-104)	103(63-163)*	73(61-111)	78(70-112)
Left hand	77(69-129)	101(72-150)	87(66-114)	94(68-133)
Articular index	13(10-21)	10(7-11)*	13(9-17)	7(4-12)*
ESR (mm/hr)	18.5(7-34)	17(9-27)	22(7-39)	18(6-34)

* = $0.01 < p < 0.05$

** = $0.001 < p < 0.01$

TABLE 5

DISTRIBUTION OF PATIENTS ACCORDING TO FINAL ENDOSCOPY SCORES PER EACH ANATOMICAL REGION.

	NAPROXEN (n=15)						ETODOLAC (n=15)					
SCORE	0	1	2	3	4	5	0	1	2	3	4	5
Oesophagus	15	0	0	0	0	0	15	0	0	0	0	0
Greater Curve	14	0	1	0	0	0	13	1	0	1	0	0
Lesser Curve	14	0	1	0	0	0	15	0	0	0	0	0
Antrum	9	0	0	1	4	1	14	0	0	1	0	0
Pylorus	13	0	0	1	1	0	15	0	0	0	0	0
Duodenum (first part)	14	0	0	0	1	0	15	0	0	0	0	0

complained of GI symptoms, both receiving naproxen, and only one of them showed lesions on endoscopy. Also, the majority of lesions were in the stomach, particularly in the antrum, with only one patient having multiple erosions in the duodenum.

Seven of the eleven patients (64%) who developed abnormal endoscopic findings were moderate to heavy smokers (Table 3). In four of them (57%) the lesions were found in the gastric antrum.

9.4.4. Laboratory Evaluations:

No significant changes or abnormalities were noted in any of the laboratory evaluations. The ESR did not change between the baseline and the 4 week visit.

9.5. DISCUSSION.

The efficacy of etodolac, 300 mg twice daily, seems to be comparable to that of naproxen 500 mg twice daily, but the numbers of patients involved in this study are too small to make a strong statement regarding efficacy. The overall results are in agreement with a similar recent study (216) that included a slightly bigger number of patients.

The results, also, show that etodolac is tolerated better by the stomach than naproxen as revealed by the endoscopic findings. A similar effect on the gastro-duodenal mucosa was also found in normal volunteers who took the drugs for a shorter period (217,219). The reason for such an effect is unclear, but it is

interesting to note that previous workers demonstrated that the amount of gastrointestinal microbleeding produced by etodolac did not differ from that seen with placebo and was significantly less than that seen with some other NSAIDs (220-222). It was also shown that etodolac, at a low dose, reduced gastric PGE_2 levels significantly less than naproxen or piroxicam in rats (223), though at a higher dose all three drugs produced similar reductions in PGE_2 levels. It is not known whether these findings in rats can be extended to humans, and further studies in this field may be worthwhile. Some of these aspects will be considered in Chapter 10.

These results are also in agreement with previous studies suggesting that patients taking non-steroidal anti-inflammatory drugs tend to develop asymptomatic lesions (224,225), and that such lesions affect the stomach in most cases (226), particularly the antrum (227). It also seems that smokers tend to develop their lesions in the antrum more than any other region in the stomach (227). It is clear however that the findings here are based on a short term study, and long term data on the safety of both naproxen and etodolac are required.

In conclusion, etodolac appears to have a less damaging effect on the stomach. These results, together with the findings of previous microbleeding studies, identify etodolac as a potentially useful agent in the treatment of rheumatoid arthritis.

CHAPTER 10.

10. THE EFFECT ON GASTRIC AND DUODENAL MUCOSAL PROSTAGLANDINS OF REPEATED INTAKE OF THERAPEUTIC DOSES OF NAPROXEN AND ETODOLAC IN RHEUMATOID ARTHRITIS.

10.1. SUMMARY.

The synthesis of gastric and duodenal mucosal prostaglandin E_2 (PGE_2), PGI_2, Thromboxane (TXB_2) during a 60 minute incubation of biopsy specimens, the degree of endoscopic and histological damage, and the anti-inflammatory response were all studied after a four-week double-blind study of therapeutic doses of 2 non-steroidal anti-inflammatory drugs, naproxen and etodolac, received by 27 patients with active rheumatoid arthritis (13 receiving naproxen, 14 etodolac). Post-treatment PG values were not different from the baseline levels when all the patients were analysed as one group. Subgroup analysis showed that naproxen suppressed gastric PGE_2 from a median of 29 to 9 ng/mg protein ($p<0.01$), duodenal PGE_2 from 34 to 11 ($p<0.01$) and duodenal PGI_2 from 62 to 15 ng/mg protein ($p<0.05$). No overall suppression occurred with etodolac. Also, on the second assessment patients receiving naproxen had lower gastric and duodenal PGE_2 and PGI_2, but higher values of duodenal TXB_2, than patients receiving etodolac. Both drugs had similar anti-arthritic activity and caused microscopic gastritis in similar proportions of patients. No correlation was

detected between PG values and the mucosal damage which developed in 7 patients on naproxen (54%) and 3 patients on etodolac (21%).

These findings indicate that, unlike naproxen, etodolac does not seem to affect gastric or duodenal PG synthesis; other mechanisms of injury need to be considered.

10.2. INTRODUCTION.

It has been increasingly recognized that NSAIDs and prostaglandins (PGs) have opposite effects on the defensive mechanisms of the gastric mucosa (38,228). One of the theories proposed to explain the mechanism of NSAIDs induced damage is their ability to suppress mucosal PG synthesis (89,229). Most knowledge in this field has come from animal work (223,229-232), or from studies of young, healthy human volunteers given single doses or short term courses of aspirin or indomethacin (91,233). When patients with rheumatic disorders were studied (90), basal values of the ability of the gastro-duodenal mucosa to synthesise PGs were not known, and the precise nature of their disease was not adequately described.

Etodolac is a member of a new class of NSAIDs, the pyranocarboxylic acids; it has been reported to be better tolerated by the stomach than naproxen, a propionic acid derivative with established efficacy in arthritis, but both agents were reported to have a comparable anti-arthritic activity (216,217, Chapter 9 in this Thesis). A

recent animal study has suggested that the different effect of these two agents on the gastric mucosa could be due to the sparing of gastric PG synthesis by etodolac (223).

The purpose of this prospective double-blind single centre study is to assess the effect of four weeks' treatment with therapeutic doses of naproxen and etodolac in patients with active rheumatoid arthritis on gastro-duodenal mucosal PG synthesis, anti-inflammatory activity, and endoscopic and histological changes.

10.3. SUBJECTS, MATERIALS AND METHODS.

10.3.1. Rheumatoid Arthritis Patients:

The patients studied were 18-70 years old, with active rheumatoid arthritis according to the criteria of the American Rheumatism Association. Patients receiving second-line agents, gold, penicillamine or hydroxy-chloroquine but not sulphasalazine, were included if such drugs were previously started 6 months or longer prior to the study, and the doses had been unchanged for 2 months before the study. Sulphasalazine was excluded because 5-10% of its 5-aminosalicylic acid component is absorbed, which might affect PG production, although there is no evidence for this. Subjects on NSAIDs underwent a washout period of at least 5-7 days prior to receiving the study medications, during which time paracetamol was used as an analgesic agent. Preliminary work showed that there was no significant difference in gastric or duodenal

mucosal PG synthesis between arthritic patients who stopped receiving NSAIDs for 4 days and controls who were not receiving NSAIDs. Patients were excluded if they had any abdominal complaints, a history of peptic ulceration or any systemic diseases. Those taking cytotoxic agents, steroids, or ulcer healing drugs were also excluded.

10.3.2. Study Medications:

Naproxen 500 mg bd and etodolac 300 mg bd, prepared as identical capsules, were given in a double-blind randomised design for a period of 4 weeks, with paracetamol used as a baseline analgesic. Compliance was checked using a tablet count.

10.3.3. Assessments:

Assessments were made on 2 visits, just before the start of the study drugs and on completion 4 weeks later. They included a general medical history and examination, assessment of the activity of the rheumatoid arthritis, and endoscopy. Rheumatoid disease activity was assessed by measuring the ESR, grip strength (mmHg), the Ritchie articular index (218), the duration of morning stiffness, and both the patients and investigator's evaluation of the global condition. Endoscopy was performed after the intravenous administration of 5-15 mg of diazepam. Endoscopic abnormalities were graded according to a (0-5) scale modified from Lanza (219); 0 score: normal, 1: any erythematous changes, 2: sub-mucosal

haemorrhage, 3: single erosion, 4: multiple erosions, and 5: frank ulceration.

Patients showing any abnormal endoscopic findings at the initial visit were not admitted to the study. On both visits biopsies weighing 5-10 mg were taken from healthy looking mucosa in the gastric antrum and the first part of the duodenum for prostaglandin assays and for histology. All assessments were done under double-blind conditions. Suitable patients were given the study medications within 12 hours of completing the initial assessment.

10.3.4. Histology:

Specimens were fixed in formalin buffered saline, embedded in paraffin wax and 5 um sections prepared for light microscopy. Sections were stained with Haematoxylin and Eosin. Histological appearances were broadly divided into mild or severe inflammation: mild inflammation referred to the presence of few inflammatory cells in the lamina propria, while severe inflammation meant that there was extensive inflammatory infiltration of the lamina propria, glands and crypts (149).

10.3.5. Prostaglandin Assays:

Biopsy specimens were taken and immediately frozen in liquid nitrogen and stored at -70^{o}C. Each specimen was later thawed, weighed and washed in 0.5 ml of phosphate buffer per biopsy for 5 minutes at room temperature to remove the debris and minimize measurement of PGs induced by trauma. The supernatant was removed

and specimens were incubated at 20°C for 30 minutes and at 37°C for another 30 minutes. Fresh phosphate buffer (0.5 ml) was added at the start of each step of incubation, and the supernatant removed at the end of each stage to be mixed with an equal volume (1:1) of methyloximation agent (96), left overnight at room temperature, and stored after that at 4°C till the time of radioimmunoassay. Three PGs were measured: PGE_2, PGI_2,and Thromboxane A_2. PGI_2 and Thromboxane A_2 were measured as their stable metabolites 6-oxo-PGF_1 and TXB_2 respectively. Full details of the radioimmunoassay are described in Chapter 2. The protein content of each biopsy was measured as described by Lowry (98), and the results of prostaglandin synthesis are expressed in ng PG/mg protein.

10.3.6. Statistical Analyses:

These were performed using the Wilcoxon signed ranks and the Mann-Whitney tests, where appropriate. P values of less than 0.05 were regarded as significant. Correlation between PG values and endoscopic scores was tested using Spearman's rank-correlation coefficient.

Informed consents were obtained and the study was approved by the local Ethical Committee.

10.4. RESULTS.

Twenty-seven patients completed the study; 13 (9 females, 4 males) median age of 60 years, were found to have been on naproxen, and 14 (10 females, 4 males) median age of 50 on etodolac. Three further patients were not

entered into the study because their initial endoscopy was abnormal and 2 other patients dropped out before completing the study owing to protocol violations. In the naproxen group, 6 patients smoked, 9 were on second-line drugs and 10 had previous exposure to NSAIDs, compared to 7, 8, and 11 patients in the etodolac group respectively. Compliance was good and comparable in both groups (median of 89% of naproxen tablets and 87% of etodolac provided were taken).

10.4.1. PG Synthesis:

Baseline values were similar in both groups. Taking all rheumatoid patients as one group, there was no significant change in gastric or duodenal PG values before or after NSAID treatment. Significant differences became noticeable after patients were classified according to whether they received naproxen or etodolac (Figures 15-17); gastric PGE_2, duodenal PGE_2 and PGI_2 were all suppressed by naproxen. Etodolac seemed to have no effect on prostaglandin values. In addition, compared with etodolac patients on the second assessment, naproxen patients had lower gastric and duodenal PGE_2 and PGI_2 but higher values of duodenal TXB_2.

10.4.2. Anti-arthritic Activity.

The extent of improvement in the indices of rheumatoid disease activity is shown in Tables 6 and 7; all variables improved after treatment but not necessarily to a significant degree, apart from the duration of

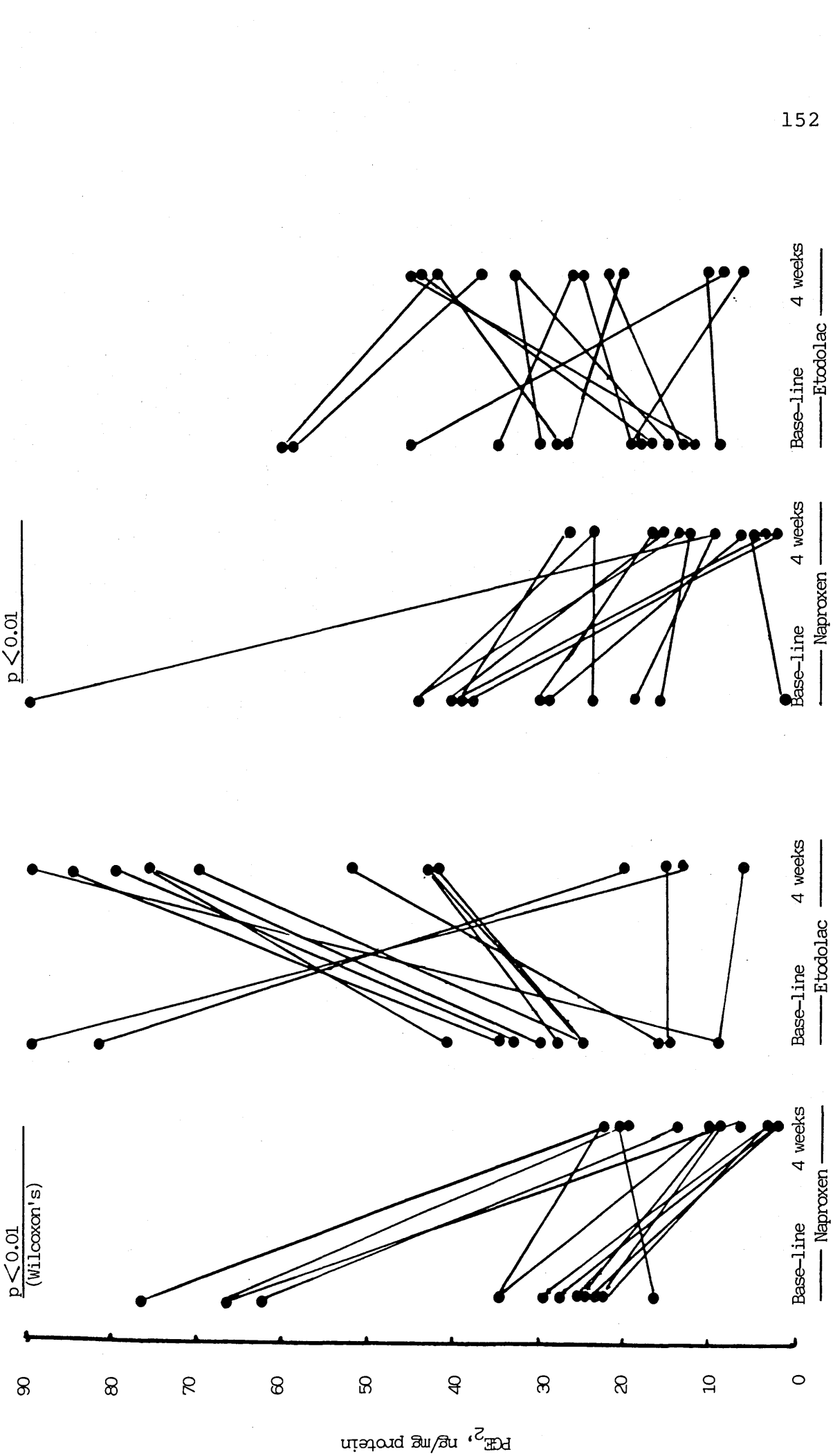


Fig. 15. Gastric and duodenal PGE_2 at base-line and after 4 weeks of treatment with naproxen or etodolac.

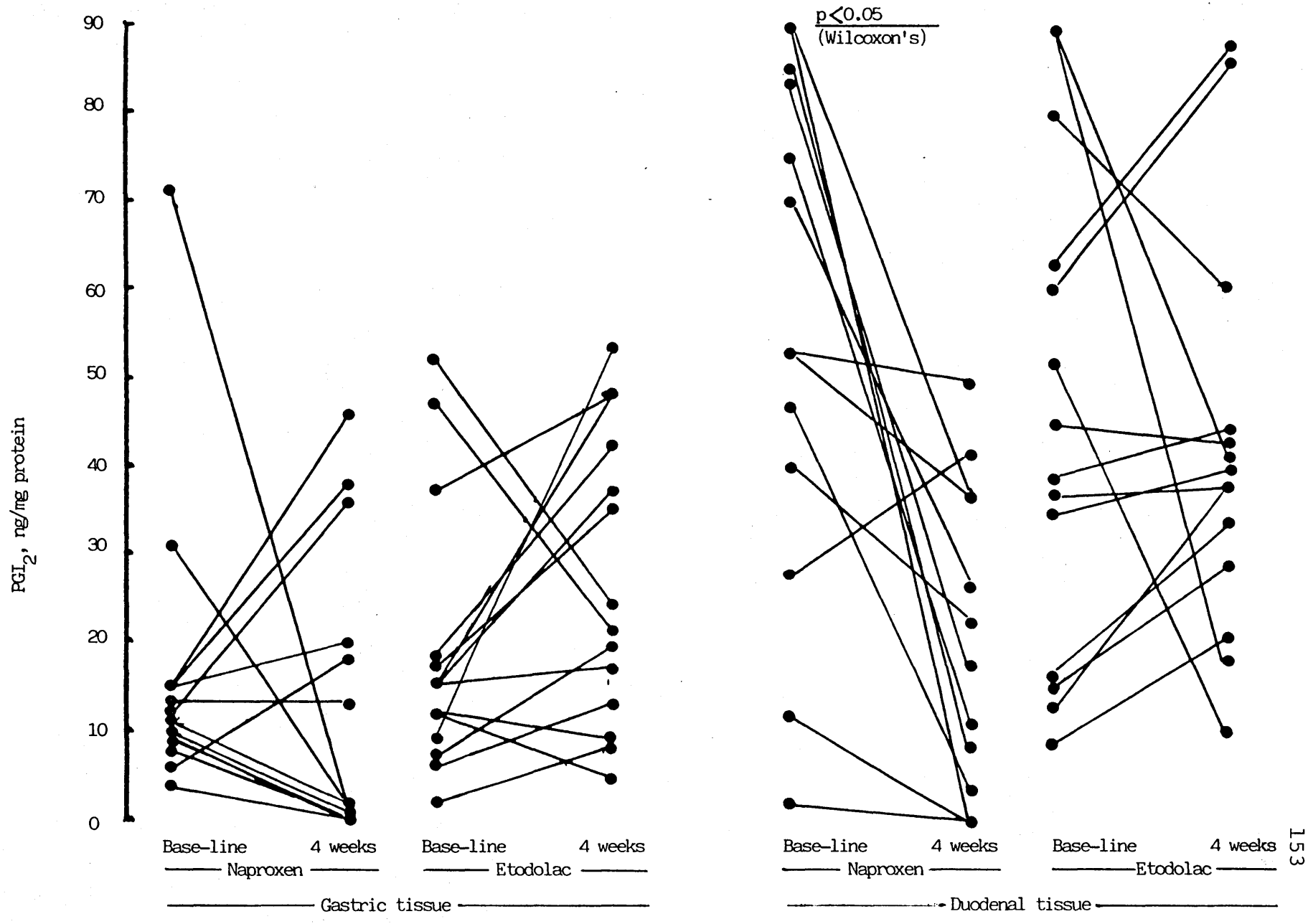


Fig. 16. Gastric and duodenal PGI_2 at base-line and after 4 weeks of treatment with naproxen or etodolac.

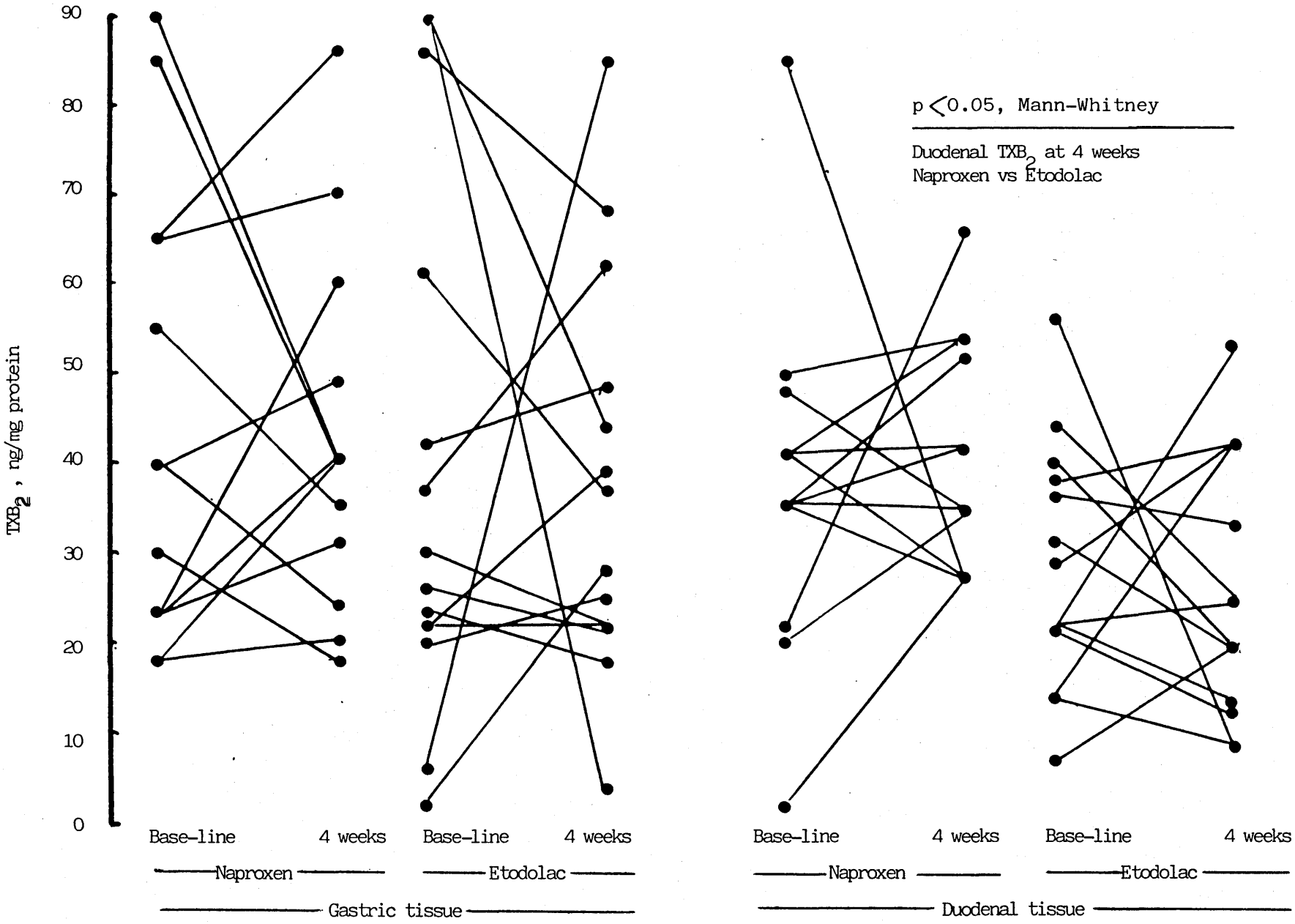


Fig. 17. Gastric and duodenal TXB_2 at base-line and after 4 weeks of treatment with naproxen or etodolac.

TABLE 6.

DURATION OF MORNING STIFFNESS, GRIP STRENGTH, ARTICULAR INDEX AND ESR BEFORE AND AFTER TREATMENT: MEDIAN AND INTERQUARTILE RANGES.

	ALL RHEUMATOID PATIENTS (n=27		NAPROXEN GROUP (n=13)		ETODOLAC GROUP (n=14)	
	Baseline	4 weeks	Baseline	4 weeks	Baseline	4 weeks
Duration of morning stiffness (minutes)	60 (60-120)	30* (5-60)	90 (60-150)	50** (17-90)	60 (48-105)	30** (12-55)
Grip Strength (mmHg)						
Right hand	74 (60-105)	83 (63-135)	74 (60-102)	102 (60-154)	76 (59-126)	81 (71-120)
Left hand	80 (68-116)	94 (72-144)	77 (65-129)	93 (67-174)	88 (67-130)	96 (70-139
Articular Index	13 (10-19)	8*** (5-11)	16 (10-22)	10 (7-12)	13 (9-18)	6 (3-11)
ESR (mm/hr)	22 (7-39)	17 (8-33)	23 (10-36)	17 (9-34)	18 (7-41)	16 (5-35)

Significant improvement: * $p<0.001$, ** $p<0.01$, *** $p<0.05$

TABLE 7

PAIN INTENSITY(*), INVESTIGATOR'S OPINION(**), AND PATIENT'S SELF-ASSESSMENT(**), MEDIAN AND INTERQUARTILE RANGES.

	ALL RHEUMATOID PATIENTS (n=27		PATIENTS ON NAPROXEN (n=13)		PATIENTS ON ETODOLAC (n=14)	
	Baseline	4 weeks	Baseline	4 weeks	Baseline	4 weeks
Pain Intensity	3 (3-4)	3 (2-3)	3 (3-4)	3 (2-4)	3 (3-4)	3 (2-3)
Investigators Opinion	3 (2-3)	2 (2-3)	3 (2-3)	2 (2-3)	3 (2-3)	2 (2-3)
Patients' self Assessment	3 (2-3)	3 (2-3)	3 (2-3)	3 (2-4)	3 (2-3)	3 (2-3)

(*) 1: none, 2: mild, 3: moderate, 4: severe, 5: very severe.

(**) 1: very good, 2: good, 3: fair, 4: poor, 5: very poor.

morning stiffness ($p<0.001$) and the articular index ($p<0.05$). The overall results indicate that, in this small group of patients, naproxen and etodolac have a similar anti-inflammatory activity.

10.4.3. Endoscopic and Histological Changes:

The second endoscopy was abnormal in 7 patients on naproxen (54%) with a median score of 2 (0-4), (interquartile ranges), compared to 3 on etodolac (21%) and a score of 0 (0-1) ($p<0.05$). Lesions developed in the stomach in all 10 cases except 1 (on naproxen) who had them in both the stomach and the duodenum. Only 3 patients (on naproxen) developed upper abdominal complaints, and the rest were all asymptomatic. PGs were not suppressed in the 3 patients with endoscopic abnormalities due to etodolac. The number of patients with severe inflammation in their gastric biopsies rose from 3 (23%) to 10 (76%) after taking naproxen, and from 4 (35%) to 11 (79%) after etodolac therapy. There was no correlation between PG values and the degree of endoscopic damage ($r= -0.3196$ for PGE_2, -0.3793 for PGI_2 and -0.2339 for TXB_2 in the entire population of rheumatoid patients). Also, no significant correlation was found between PGE_2/TXB_2 ratio and the endoscopic scores ($r= -0.2495$). Both gastric and duodenal PGE_2/TXB_2 ratios were significantly higher in patients receiving etodolac than in those who took naproxen ($p<0.005$). From these results it seems that there should be some form of negative correlation between endoscopic scores and PGE_2/TXB_2 ratio,

as patients receiving naproxen appear to have lower ratios and higher scores. This could not be proved, however, as no correlations were significant.

10.5. DISCUSSION.

This study demonstrates that, unlike naproxen, etodolac does not suppress gastric or duodenal PG synthesis. In this respect these results disagree with most of the available data obtained from studies on gastric PGs in patients with rheumatic diseases or those receiving NSAID therapy; such studies (87,90) have shown that the NSAIDs tested do suppress gastric PGs. The effects of individual NSAIDs were not known in those reports, baseline PGs were not measured, and the number of patients taking the same agent was small.

Patients on naproxen had both a greater number of endoscopic abnormalities and lower PG values. Possibly, these two events were inter-related, but this study was unable, like others (91), to demonstrate a correlation between the endoscopic scores and PG values. Gastritis (87) does not adequately explain the sparing of PG by etodolac, as inflammation was present in similar numbers of patients who took either agent. The significance of this gastritis is unclear; it was not evident on endoscopic examination, and was only revealed by histology.

Possibly, agents like etodolac might be selective in their effects on various tissues and different types of

PGs. Such an effect was previously described with salicylic acid which caused preferential reduction in PGE_2 in sheep vesicular tissue, while indomethacin suppressed all classes of PGs (122). As mentioned in Chapter 3, NSAIDs vary in their capacity to inhibit PG synthesis (119,234). Indomethacin, naproxen and meclofenamic acid are powerful inhibitors of PG synthesis, phenylbutazone is a moderate inhibitor, while salicylic acid, aspirin and benoxaprofen are only weak inhibitors. Other drugs act as prodrugs that show activity through their metabolites: one of these agents is suldinac whose sulphide metabolite is a potent PG inhibitor (120). Animal studies (223) have also shown that acute exposure to naproxen or piroxicam reduced gastric PGE_2 to a greater extent than etodolac, although at high doses all three drugs produced similar reductions in PGE_2.

The fact that PGs were not suppressed in patients who developed endoscopic abnormalities due to etodolac might mean that the mucosa recovered its capacity to synthesise PG prior to full healing of erosions, though this study has not shown that such capacity was lost to begin with, or that mechanisms other than PG deficiency were involved in causing gastric damage: in theory, these could include increased mucosal permeability (235), interference with active ion transport (236), redistribution of mucosal blood flow (237), capillary stasis (238), or by interfering with the mucus layer (239).

Little is known about the duodenal mucosal PG

response to the intake of NSAIDs, as most studies concentrated on their effect on the gastric mucosa (91,229-231,233), possibly owing to the fact that the stomach is more commonly affected by NSAIDs than the duodenum as shown by this study and by others (226). Like one of the previous studies on patients with duodenal ulcers (77), these results might suggest that the duodenal mucosal potential to synthesise PGI_2 becomes limited in the presence of ulceration or when subjected to naproxen. Duodenal PGE_2 was also suppressed in the patients taking naproxen, but not in those of Hillier et al. (77), the difference probably being due to the fact that their patients did not take NSAIDs. However, patients with duodenal ulcers studied in Chapter 8 had no PG deficiency in the absence of NSAIDs.

It is also interesting to find that patients who took naproxen had higher values of TXB_2 (the stable metabolite of TXA_2) than those receiving etodolac. Animal studies have suggested that vasoconstriction with TXA_2 induces ulceration of the gastric mucosa (240), and that the selective inhibition of its synthesis results in gastric mucosal protection (241). The effect of TXA_2 on the duodenal mucosa was not clarified by those workers. This study found that both gastric and duodenal PGE_2/TXB_2 ratios were higher in patients taking etodolac than in those receiving naproxen. The significance of this is not fully clear but it may explain, at least in part, the greater damaging effect of naproxen, though this study

could not show a significant correlation between PGE_2/TXB_2 ratio and the endoscopic scores. The above studies (240,241) also demonstrate that effective inhibition of TXA_2 requires the use of specific inhibitors of thromboxane synthetase enzyme. Several studies have shown that TXA_2 synthesis might be maintained even when other cyclo-oxygenase products are suppressed (77,87,90).

In conclusion, after 4 weeks of regular intake in therapeutic doses, naproxen suppressed gastric PGE_2, duodenal PGE_2 and PGI_2, while etodolac did not. At the same time, etodolac caused a lesser degree of endoscopic damage than naproxen; this could be related to their different effects on PGs, though there was no correlation between PG values and endoscopic scores. If it is assumed that more NSAIDs assessed along the same lines of this study would have a similar behaviour to either naproxen or etodolac, these results may indicate that not all NSAIDs suppress gastric or duodenal PGs. Other modes of interaction between NSAIDs and the gastric mucosa need to be considered.

SECTION 4.

HELICOBACTER PYLORI AND THE GASTRIC MUCOSA. INTERACTION WITH INDOMETHACIN, AND EFFECTS ON THE PRODUCTION OF PROSTAGLANDINS AND CYCLIC AMP.

SECTION 4. HELICOBACTER PYLORI AND THE GASTRIC MUCOSA. INTERACTION WITH INDOMETHACIN AND THE EFFECTS ON THE PRODUCTION OF PROSTAGLANDINS AND CYCLIC AMP.

INTRODUCTION.

This section investigates some aspects of the interaction between H. pylori and the gastric mucosa, with special emphasis on the possible role of PGs. Chapter 11 measures gastric PG synthesis in patients with gastric ulcers and non-ulcer dyspepsia, in the presence or absence of H. pylori. It also highlights the influence of gastritis on PG production. Some aspects of the three main topics covered in this Thesis, PGs, NSAIDs, and H. pylori, are assessed together in Chapters 12 and 13. Chapter 12 investigates the possible interaction between H. pylori culture filtrate and indomethacin, using mucosal histology and PGE_2 production as indices of such interaction. In Chapter 13, H. pylori culture filtrate is purified and a protein is obtained. The effect of this protein on mucosal cyclic AMP and PGE_2 production is also investigated.

11. GASTRIC MUCOSAL PROSTAGLANDIN SYNTHESIS IN THE PRESENCE OF HELICOBACTER PYLORI IN PATIENTS WITH GASTRIC ULCERS AND NON-ULCER DYSPEPSIA.

11.1. SUMMARY.

It is not clear whether Helicobater pylori (H. pylori) affects gastric mucosal prostaglandins (PG). In a double-blind study gastric PG synthesis was measured in 22 patients with benign gastric antral ulcers (GU) and 26 with non-ulcer dyspepsia (NUD). The presence of H. pylori was determined by histology and bacteriology: 26 (16 GU + 10 NUD) had H. pylori, and 22 (6 GU + 16 NUD) did not. Severe gastritis was found in 36% of subjects without H. pylori and 77% of subjects infected with H. pylori (X^2 = 8.64, $p<0.01$). Patients with severe gastritis (regardless of the presence of H. pylori) had significantly higher PGE_2 and PGI_2 values than those with mild gastritis. No significant differences in PG values were found between subjects infected with H. pylori and those who did not have the organisms.

11.2. INTRODUCTION.

Both endogenous and exogenous PGs are thought to have a unique combination of favourable effects on the gastrointestinal mucosa (38,211,242-244). They inhibit acid secretion, are trophic to the gastric mucosa and are 'cytoprotective' against various experimental injuries. On the other hand, H. pylori has been associated with gastritis and peptic ulceration (138,159,245,246).

However, the effect on endogenous PG synthesis of the presence of H. pylori remains unclear. The aim of this double-blind study is to assess gastric mucosal PG synthesis in patients with GU and NUD who have H. pylori compared to those who do not have the organisms.

11.3. PATIENTS AND METHODS.

11.3.1. Patients:

They were recruited between 1987 - 1988, having been referred for investigations of upper abdominal complaints. They were included if they were 18-75 years old, without any history of gastric surgery, and in the absence of concurrent infection or systemic illness. They were excluded if their alcohol intake was heavy (more than 10 units/week, one unit equals half pint or 1 measure of spirit) or if they took ulcer healing agents, non-steroidal anti-inflammatory drugs within a week, or antibiotics within a month of endoscopy. NUD was defined as the presence of upper abdominal symptoms for at least one month, normal upper gastrointestinal endoscopy or mucosal erythematous changes, normal upper abdominal ultrasound, and without the intake of the drugs mentioned above. Patients with GU were included only if their ulcers were antral and benign as proved by histology. Being H. pylori positive meant that the organisms had to be seen on histology and grown by bacteriological methods in gastric biopsies taken from the study patients. Informed consents were obtained from all patients, and the

study was approved by the local Ethical Committee. Patients fasted for up to 12 hours prior to endoscopy and received 7.5 - 15 mg diazepam intravenously just before the procedure.

Fifty-two patients were initially entered in the study: 26 had GU, 15 males and 11 females with a median age of 44 years and included 12 smokers. The other 26 patients had NUD, 6 males and 20 females with a median age of 33 years and included 16 smokers.

11.3.2. Gastric biopsies and their disposition.

Seven endoscopic biopsies were obtained from each patient, four from the gastric antrum and three from the body, with 2 mm Olympus biopsy forceps. Antral biopsies were taken from sites approximately 5 cm from the pylorus. Biopsies from the gastric body were taken from the greater curve aspect. The above samples were disposed of as follows:

Single biopsies weighing 5-6 mg were taken at random from healthy looking mucosa in the antrum, at least 2 cm away from the ulcer edge in those with GU. They were immediately frozen in dry ice ($-96^{o}C$) and stored at $-70^{o}C$ till the time of PG assay. Two biopsies from the antrum and two from the body were placed in 10% formalised saline for histopathological assessment. One specimen from each area was placed in 0.9% saline for bacteriological culture. The specimens were assigned for bacteriology or pathology at random.

In addition to the above seven biopsies, an average of five other specimens were taken from the ulcer edge and base in order to exclude malignancy in patients with GU.

11.3.3. Identification of H. pylori.

Bacteriological culture techniques and histopathology were used to identify H. pylori, as described in Chapter 4. In addition, the histological appearances were broadly divided into un-inflamed/mild gastritis (normal or with minimal polymorphonuclear infiltration up to the lamina propria), or inflamed/severe gastritis (moderate to intense infiltration of the lamina propria, glands and crypts with neutrophils and polymorphonuclear cells). Such arbitrary classification helps overcome the difficulty in finding biopsies absolutely devoid of inflammatory cells, that can subsequently affect PG synthesis and measurement (84,87).

11.3.4. Measurement of Prostaglandin Synthesis:

Frozen biopsies were thawed, weighed, washed for 5 minutes in 0.5 ml of phosphate buffered saline and the supernatant was discarded to minimize measurements of PGs induced by trauma. Fresh buffer was added and biopsies were incubated for 30 minutes at 20°C and for another 30 minutes at 37°C. Each time the supernatant was taken and added to an equal volume (1:1) of methyloximation agent (MOX) to stabilize the compounds synthesised (96) till the time of the radioimmunoassay. All samples were assayed in duplicate. Other details of PG assay are as described

in Chapter 2. The results are expressed in ng PG/mg protein contained in each biopsy after incubation. The protein was measured by the Lowry method (98). PGI_2 and Thromboxane A_2 were measured as their stable metabolites, 6-oxo-PGF_1 and Thromboxane B_2 (TXB_2) respectively. All assessments were performed under double-blind conditions, using code numbers.

11.3.5. Statistical Analysis:

The Mann-Whitney and X^2 tests were used where appropriate.

11.4. RESULTS.

Four patients with GU were found to be positive for H. pylori on histology but negative on bacteriological examination; they were dropped from the study as, by definition, subjects had to be positive or negative for H. pylori both by histology and bacteriology. Data on the remaining 48 patients (22 GU and 26 NUD) are shown in Table 8 and Figures 18-20.

Patients positive for H. pylori had more severe gastritis (active chronic gastritis with intense polymorphonuclear infiltration, see methods): 20 patients (77%) compared to 8 (36%) in the H. pylori negative group (X^2=8.64, $p<0.01$). In addition, severe antral gastritis was present in 8 patients (31%) with NUD and 20 GU cases, 91% of the total GU (X^2=16.93,$p<0.001$). The proportion of patients with severe gastritis in the GU subgroup (91%) was higher than that in the group of patients with H.

TABLE 8

CHARACTERISTICS OF PATIENTS POSITIVE OR NEGATIVE FOR HELICOBACTER PYLORI, GASTRIC ULCERS AND NON-ULCER DYSPEPSIA.

	H. pylori NEGATIVE			H. pylori POSITIVE		
	GU	NUD	Total HP Negative	GU	NUD	Total HP Positive
Total Number of Patients	6	16	22	16*	10	26
Sex:						
Males	5	5	10	8	1	9
Females	1	11	12	8	9	17
Age (years)	50	38	46	46	28	37
Number of Smokers	3	10	13	9	6	15
Gastritis:						
Mild	1	13	14	1	5	6
Severe	5	3	8	15	5	20**

Significant rise: * $X^2=5.4$, $p<0.05$ (compared to H. pylori negative GU)

** $X^2=8.64$, $p<0.01$ (compared to H. pylori negative severe gastritis)

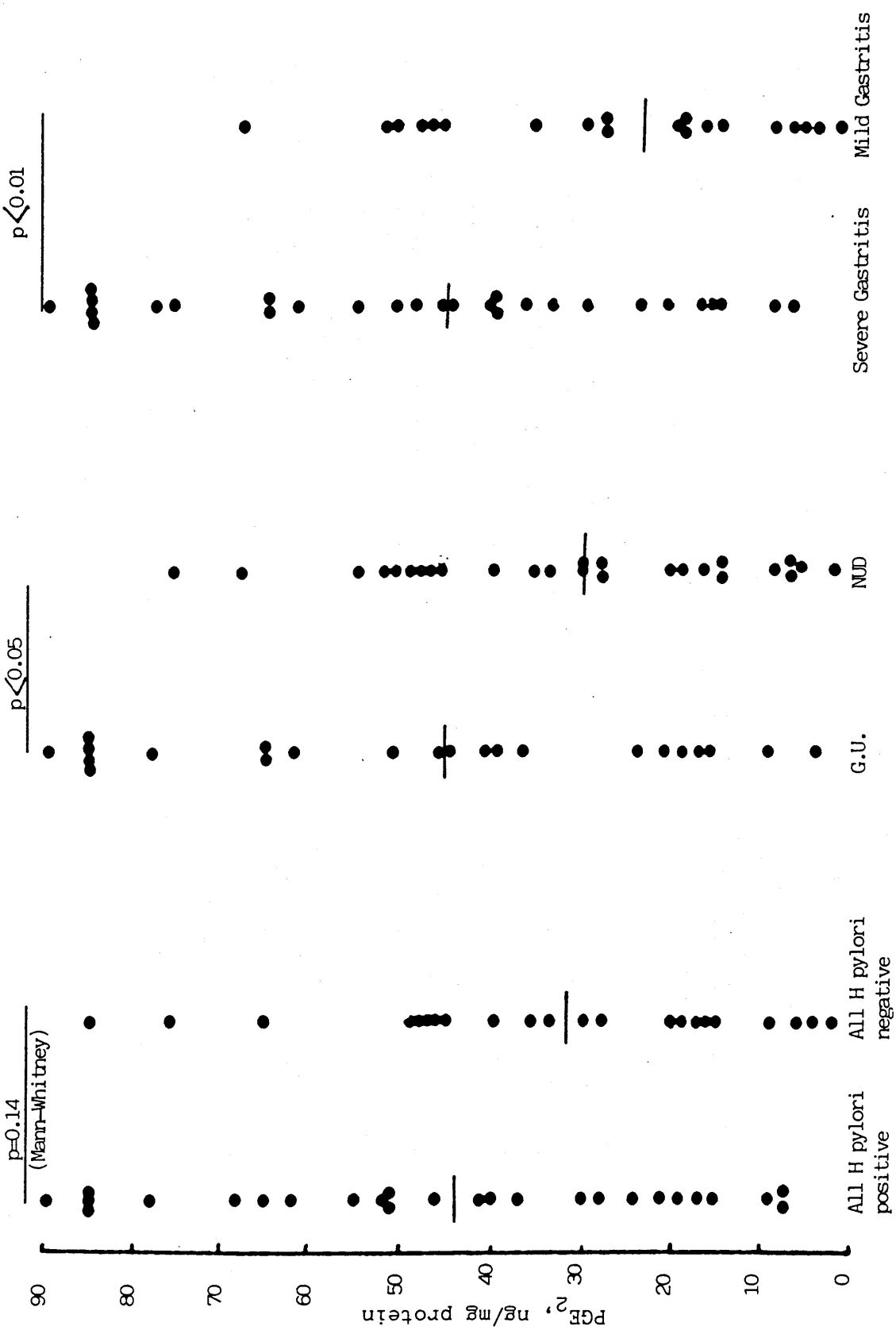


Fig. 18. Gastric PGE_2 in patients with or without H. pylori (H. pylori positive or negative), gastric ulcers (GU), non-ulcer dyspepsia (NUD), and gastritis.

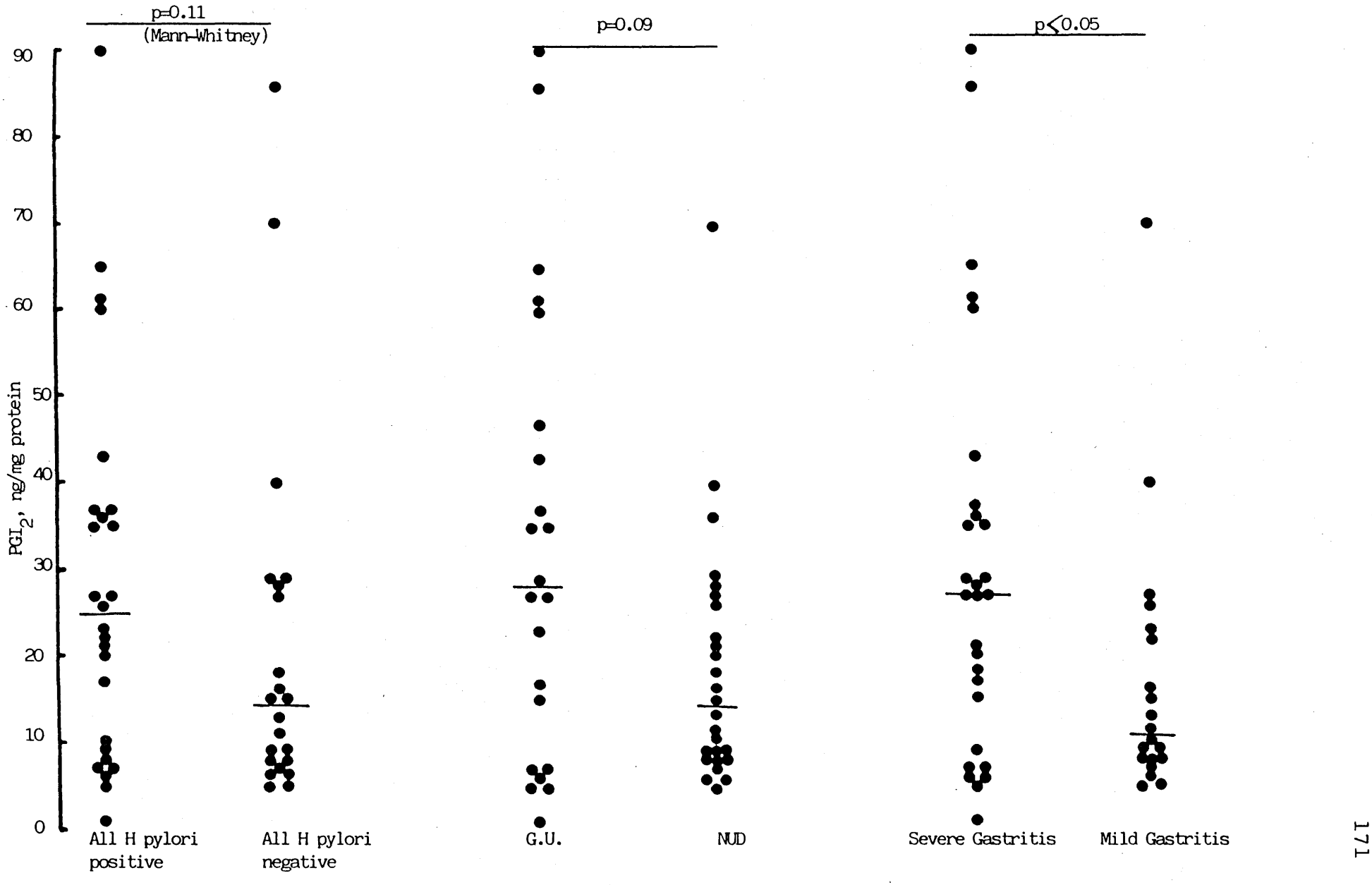


Fig. 19. Gastric PGI_2 in patients with or without H. pylori, gastric ulcers, non-ulcer dyspepsia, and gastritis.

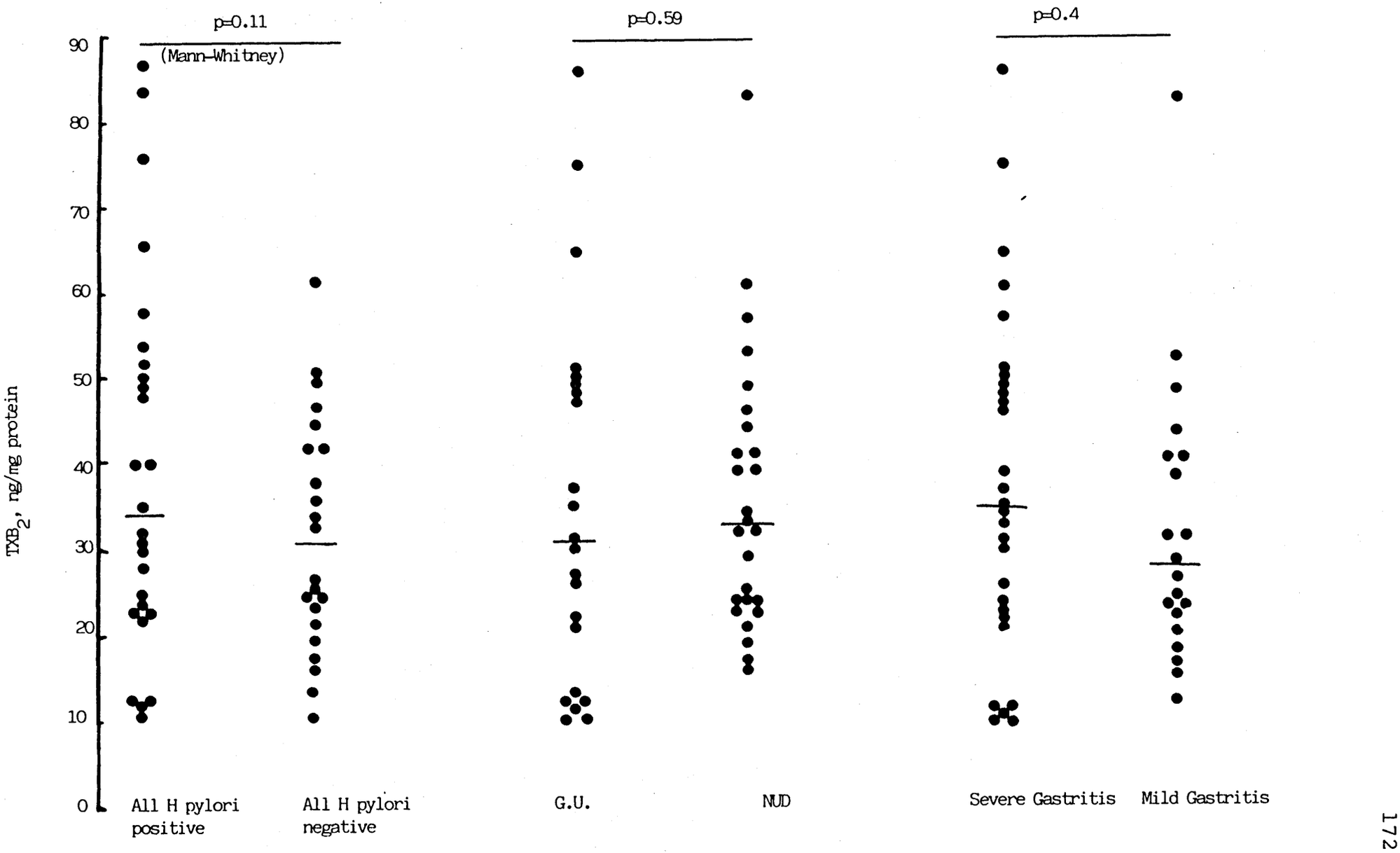


Fig. 20. Gastric TXB_2 in patients with or without H. pylori, gastric ulcers, non- ulcer dyspepsia, and gastritis.

pylori as a whole (77%) but the difference was not statistically significant ($X^2=2.4$, $0.1<p<0.25$).

Gastric mucosal PGs were found to be greater in the presence of severe antral gastritis or ulceration when the presence of H. pylori was disregarded (Figures 18-20). There was a tendency for PG values to be elevated in H. pylori positive group and subgroups (Figures 18-20), but such elevation did not reach the degrees of statistical significance.

11.5. DISCUSSION.

H. pylori was found in 73% of GU patients in this study. This is comparable to previous findings in this field (141,142). Also, 38% of patients with NUD had H. pylori detected in their gastric biopsies. Rokkas et al. (171) isolated H. pylori from 45% of subjects with NUD. Their definition of NUD was similar to the one used in this study. The importance of identifying this condition, NUD, stems from the possibility that it might respond to treatment with bismuth compounds (172).

The mechanism of interaction between H. pylori and local host defences has been the subject of numerous studies because of its importance in understanding the pathogenesis and treatment of H. pylori-related damage. Such interaction is thought to involve a variety of factors including the state of gastric acid secretion (144,173-178), the urease activity (159,179,180), the possibility of cytotoxicity (188), antibody formation and

complement activation (182).

It has not been clear what happens to endogenous gastric mucosal PG synthesis in the presence of H. pylori, which is capable of producing the interactions involving the above-mentioned factors. The findings of this study are in agreement with the results of previous studies that showed increased PG synthesis in the presence of gastritis and GU (87), regardless of H. pylori status. The total amount of PG produced in the presence of gastritis is probably equal to PG synthesized by the mucosal cells plus PG produced by the inflammatory cells: human neutrophils and macrophages both have the capacity to synthesize PG (84). Since H. pylori is associated with a higher prevalence of severe gastritis, one would expect, at least in theory, that PG values would be significantly greater in the H. pylori positive groups and subgroups. This, however, was not observed in this study, and it is somewhat surprising. In these circumstances, it might be tempting to speculate on the possible presence of some form of defective PG production, but such defect is not confirmed by these findings.

Alternatively, it could be argued that the differences in PG values in patients with or without H. pylori might become more discernible if greater numbers of patients were studied. It is also worth noting that this study investigated PG synthesis in relation to the presence or absence of H. pylori, and not in relation to the density of the organisms. This is because I share

the belief that H. pylori infection is patchy within the stomach, and accept that the density of H. pylori could have influenced the results. In addition, it might be necessary to assess PG synthesis in the presence of H. pylori in patients with duodenal ulcers; however, such an assessment could become complicated by the difficulty in finding duodenal ulcer patients who are H. pylori negative, and by gastric metaplasia.

These findings might have some therapeutic implications. Recent studies have demonstrated the beneficial effects of colloidal bismuth subcitrate and antibiotics (162,172,247) in the treatment of H. pylori-related conditions. Colloidal bismuth subcitrate was also shown to stimulate gastric and duodenal alkaline secretion through a PG- dependent mechanism (248). The same agent was later found to have a protective action on aspirin-induced gastric microbleeding and this protection occurred despite a marked suppression of mucosal PG production (249). In the light of these findings, the results of this study would suggest that for an agent to be effective in the treatment of H. pylori related gastritis and GU, its bactericidal activity is more critical than its effect on endogenous PG synthesis. In other words, the primary aim of treatment should continue to be that of eradicating the organism, and not necessarily to increase PG synthesis, since this is not significantly affected, as shown by the above results.

CHAPTER 12

12. THE INTERACTION BETWEEN HELICOBACTER PYLORI CULTURE FILTRATE AND INDOMETHACIN. EFFECTS ON THE INTEGRITY OF THE HUMAN GASTRIC ANTRAL MUCOSA AND ITS PROSTAGLANDIN E_2 PRODUCTION IN VITRO.

12.1. SUMMARY.

Histopathological methods and radioimmunoassay were used to assess the microstructure and prostaglandin E_2 production by paired specimens of human gastric antral mucosa; the specimens were studied after 48 hours of incubation in base-line tissue culture medium, Helicobacter pylori culture filtrate, Helicobacter pylori culture control fluid, indomethacin, and Helicobacter pylori culture filtrate plus indomethacin. When applied alone, the filtrate did not affect the structure of the mucosal tissue or its PGE_2 synthesis. In the overall group (n=21), specimens incubated with the mixture of Helicobacter pylori filtrate and indomethacin had a median histological grade of 1 and prostaglandin E_2 of 29 pg/mg tissue, compared to 2 (p=0.04) and 60 (p=0.0007) respectively, in specimens incubated with indomethacin alone.

These results indicate that an interaction may exist between indomethacin and a factor contained in Helicobacter pylori culture filtrate. Such interaction is damaging to the human gastric antral mucosa, and its

understanding might have therapeutic implications.

12.2. INTRODUCTION.

The evidence for the pathogenicity of Helicobacter pylori (H. pylori) has in most cases been epidemiological in nature and was based on the association between the presence of bacteria and various types of peptic disease (137,162). Experiments involving self-inoculation with the organisms (144,176) provided very useful information, but their use is severely limited by the understandable ethical objections to giving any form of bacteria to human volunteers. Recently, it has been reported that H. pylori can produce a factor capable of causing cytopathic changes in mammalian cell lines (188), and inhibiting acid secretion by rabbit parietal cells (189). The effect of this factor on the human gastric mucosa has not been studied, and its possible interaction with another mucosal damaging agent in common use, indomethacin, is not known. Therefore, this study aimed at investigating the possibility of such interaction using mucosal histology and PGE_2 production as end points in biopsy specimens obtained from subjects presenting with dyspepsia.

12.3. PATIENTS, MATERIALS AND METHODS.

12.3.1. Patients:

Patients were recruited from the Gastroenterology outpatient clinic after presenting with upper abdominal pain, nausea, vomiting or heartburn, if they were 18-70

years old, and after giving informed consent. They were excluded if they took non-steroidal anti-inflammatory drugs or ulcer healing agents within a week, or antibiotics within a month of performing the endoscopy. They were also excluded if they had a history of previous gastric surgery, systemic illness or infection.

Twenty two subjects were studied: 11 positive and 11 negative for H. pylori. In each sub-group, the first 11 patients found to satisfy the inclusion criteria were accepted.

12.3.2. Gastric Biopsies:

These were taken from healthy looking mucosa in the gastric antrum, about 3-5 cm proximal to the pyloric ring. The antrum was chosen because it is considered the commonest site for both H. pylori colonization (137,162) and the side effects of non-steroidal anti-inflammatory drugs (227, Chapter 9). A total of seven biopsies weighing 7-10 mg each, were taken from each subject. Two were used for detecting the presence of H. pylori by both bacteriology and histology as described in Chapter 4. The remaining five were incubated in 24-well plates (1 biopsy/well) in order to study their response to the test reagents (see below). Biopsies were allocated to various procedures at random.

12.3.3. Collection and concentration of H. pylori filtrate, control fluid, and gastric tissue

culture fluid.

These were all prepared as described in Chapter 4.

12.3.4. Incubation of gastric tissue with test agents:

Seven antral biopsies were taken,at endoscopy, from each patient as mentioned above. Those allocated for incubation (5 specimens) were immediately immersed in 2 ml of gastric culture fluid. The fluid was discarded to remove the debris, and the biopsies were weighed and transferred to 24-well plates that had small pieces of capillary matting at their bases to reduce evaporation of the culture fluid. Each well contained one single biopsy, 500 ul of gastric culture fluid and 50 ul of homologous citrated plasma that was freshly prepared to act as a source of growth factors. One well was left with these ingredients only, to act as a base-line. In addition, the other 4 wells contained one of the following per well: 50 ul filtrate (1.6 Units as defined above), 50 ul H.pylori culture control fluid, 7.4 ug indomethacin in 50 ul buffer, and 50 ul filtrate plus 7.4 ug indomethacin. An extra 50 ul gastric culture fluid was added to the base-line well to make a final volume of 600 ul per well. The amount of indomethacin used was previously found to be capable of inhibiting PG synthesis in gastric biopsies. After the addition of various agents the wells were covered, sealed with micropore and placed in an incubator set at 37^{o}C and 5.6% CO_2. After 24 hours the medium was

removed and fresh culture fluid and agents added. Incubation was continued for another 24 hours (total of 48 hours); this is because of the previous finding (188) that the cytotoxic activity was maximal by 48 hours. At the completion of incubation the medium was removed and mixed with an equal volume of methyloximating agent (MOX)(96) to stabilise PG compounds till the time of PG measurements. The biopsies were weighed, given code numbers to facilitate randomization, and fixed in formalin-saline in order to grade their histology.

12.3.5. Assessment of post-incubation tissue histology:

Histopathological methods were used to monitor tissue viability. This approach was used in preference to other techniques such as tritiated leucine incorporation because this study dealt with biopsies composed of numerous cell layers and not with single cells or monolayers. In the latter method, the label would be taken up only by the superficial layers. Sections were made at 3 levels in each biopsy. Histological scoring was performed by noting the proportion of intact or regenerative tissue in comparison to a base-line specimen that did not undergo incubation. It was assumed that cells that looked microscopically intact were viable. Grading was as follows: Grade 0 meant that no intact epithelium was seen, Grade 1: 1-10% of the epithelium was intact, Grade 2: 11-30%, Grade 3: 31-60%, Grade 4: 61-90%, and Grade 5: 91-100% of the epithelium was maintained. The histologist was blinded to the study specimens by the

use of code numbers.

12.3.6. Prostaglandin Measurements:

PGE_2 was measured by radioimmunoassay in the methyloximated medium obtained after the second 24-hour incubation period; this is because the medium from the first period was thought to contain large amounts of PG compounds induced by trauma. All samples were assessed in duplicate and had code numbers to facilitate randomization. The radioimmunoassay for PGE_2 is described in Chapter 2. The results were expressed in pg PGE_2/mg of post-incubation tissue weight.

The study was approved by the local Ethics Committee.

12.3.7. Statistical Analyses:

The Wilcoxon's, Mann-Whitney, Kruskall-Wallis and X^2 tests were used where appropriate.

12.4. RESULTS.

One subject was dropped from the final analysis because of mild impairment in his liver function tests. The characteristics of the remaining 21 subjects are shown in Table 9. Patients described as being positive for H. pylori were found to have the organisms in specimens assessed by both histology and bacteriology. They were comparable to the negative group as far as age and sex are concerned. The positive group included a greater proportion of smokers and of cases with active chronic superficial gastritis. PGE_2 and viability histological

TABLE 9.

GENERAL CHARACTERISTICS OF THE STUDY SUBJECTS.

	Total (n=21)	H. pylori positive (n=10)	H. pylori negative (n=11)
Median age (years)	41	41	42
Sex: Males	9	4	5
Females	12	6	6
Smokers	12	8	4
Endoscopic findings:			
a. Normal	13	4	9
b. Oesophagitis	2	1	1
c. Hiatus Hernia	2	2	0
d. Gastric Ulcer	1	1	0
e. Duodenal Ulcer	3	2	1
Base-line Histology:			
a. Normal	6	0	6
b. Active chronic superficial gastritis	14	10*	4
c. Chronic atrophic gastritis	1	0	1

* significant rise compared to subjects negative for H. pylori:
$X^2=9.3$, df=1, $0.001<p<0.01$

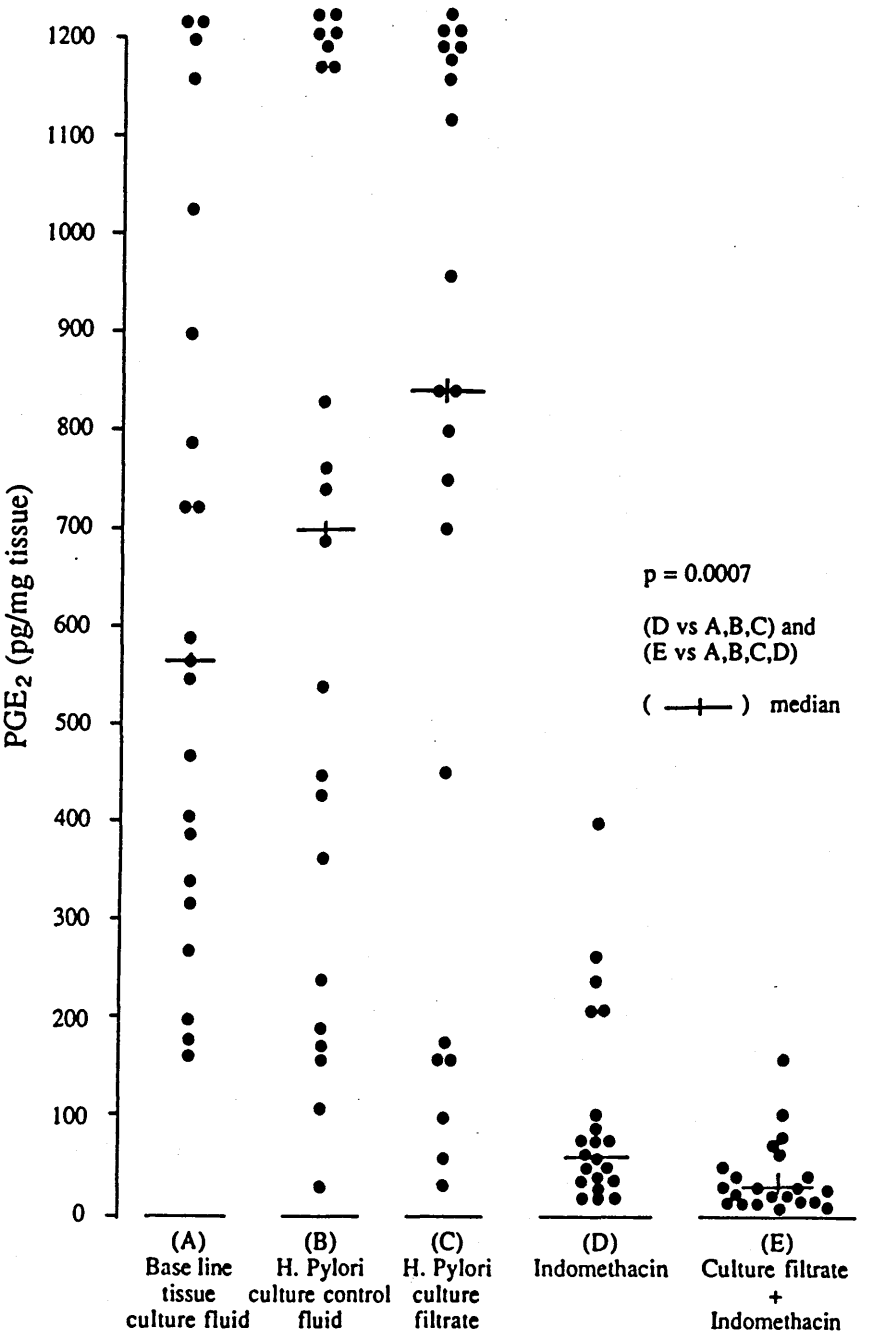


Fig. 21. PGE_2 produced by gastric biopsies incubated with H. pylori culture filtrate, indomethacin, mixture of H. pylori culture filtrate and indomethacin, and the control fluids.

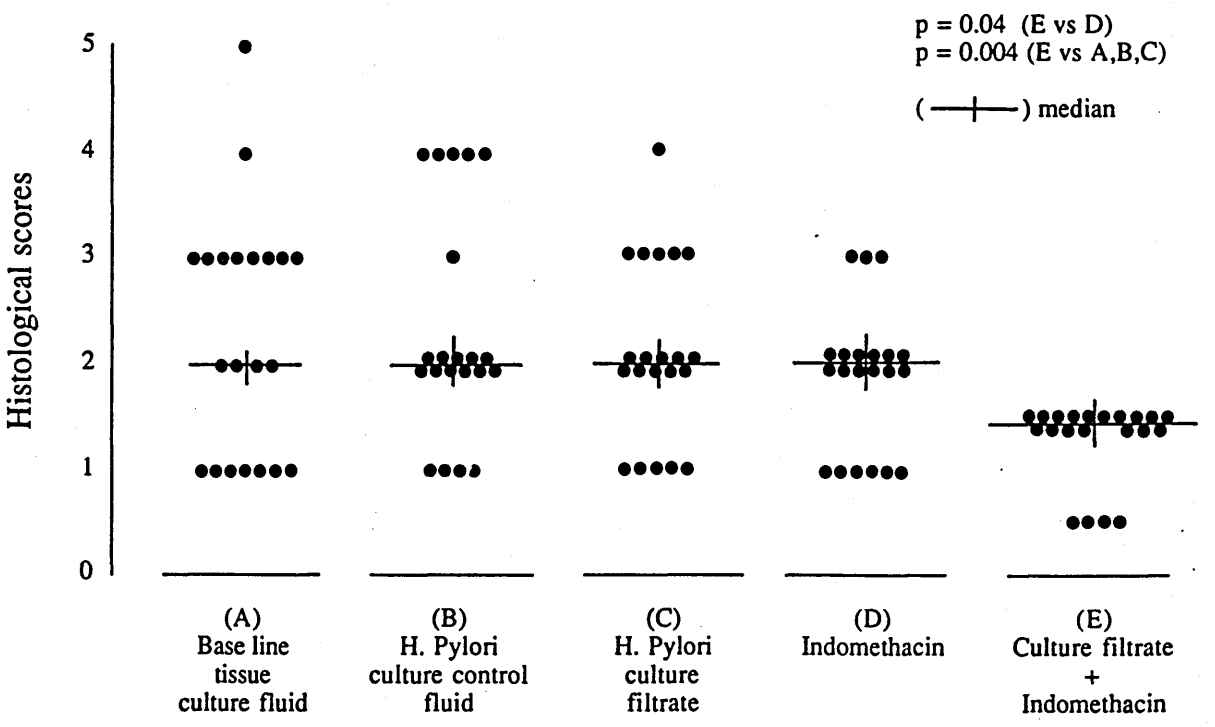


Fig. 22. Gastric mucosal microstructural (viability) scores after incubation with H. pylori culture filtrate, indomethacin, mixture of H. pylori filtrate and indomethacin, and the control fluids.

grades are shown in Figures 21 and 22. Specimens incubated in the presence of a mixture of indomethacin and H. pylori filtrate had the lowest histological scores, and their PGE_2 values were even lower than those produced by specimens incubated with indomethacin alone. Similar findings were noted after subgroup analysis of the data on subjects who were found to be positive or negative for H. pylori at base-line. In addition, it was found that base-line values of PGE_2 in patients with active chronic gastritis, 895 (658-1182) pg/mg tissue, median (interquartile ranges), were greater than those of patients without gastritis, 353 (237-440) pg/mg tissue, $p=0.003$ (Mann-Whitney U-test). The differences in PGE_2 values and viability grades between smokers and non-smokers, or between patients positive or negative for H. pylori did not reach the degrees of statistical significance.

12.5. DISCUSSION.

This study demonstrates that biopsies incubated in the presence of a mixture of H. pylori culture filtrate and indomethacin had lower PGE_2 values than those exposed to indomethacin only. This could be due to a combination of cyclo-oxygenase enzyme inhibition by indomethacin and the reduction in the mass of viable cells in gastric tissue subjected to the above mixture, as indicated by the histological grading. On the other hand, the inhibition of PGE_2 by indomethacin alone is largely due to its direct

effect on the enzymatic system involved in PG synthesis.

The fact that indomethacin suppressed PGE_2 formation is not surprising but it confirms that PGE_2 synthesis was permitted in the other specimens that were not subjected to this NSAID.

The results of this study could have been influenced by patients age, sex, smoking, the presence or absence of gastritis or H. pylori in their base-line specimens. It is believed that correction for the effects of these factors was achieved by studying multiple specimens taken from each patient and by the use of paired analysis. The finding that H. pylori was associated with a significant number of cases with active chronic gastritis is in line with previous studies in this field (137,162). Also, the association between gastritis and the rise in PGE_2, regardless of H. pylori status, is in agreement with the results of investigations performed in this Thesis (Chapter 11) and elsewhere (86,87).

In general, higher grades of viability could not be achieved in the study groups and this highlights the difficulties encountered in trying to keep multiple layers of gastric tissue alive for a relatively long time. It was aimed to culture the tissue, as far as possible under physiological circumstances, for a period of 48 hours, the optimal time for cytotoxic activity (188,250). To incubate for shorter periods may ensure a greater degree of viability, but this would not be representative.

The relationship between bacterial toxins and PG synthesis is still poorly understood. H. pylori culture filtrate alone did not have any significant effect on the integrity of gastric antral tissue or its PGE_2 production over the incubation period used in this study. Other bacterial toxins, the lipopolysaccharides, were previously found to increase intestinal PG synthesis in the laboratory animal even after their application for short periods (251,252). This could be due to species-specific responses and to the structural differences between these agents. The precise structure of the "toxic" factor in H. pylori culture filtrate is not known, although it is thought to be a protein as suggested by a recent study (189) and Chapter 13.

The amount of "toxic" factor contained in the culture filtrate used in this study was determined by titrating its cytopathic activity against Vero cells. It is not known whether such concentration is comparable to that found in the gastric mucosa in vivo. It did not affect the viability of gastric tissue in this study, and this confirms previous results in mammalian cells (188). Such a finding could be attributed to the possibility that this study used a relatively low concentration of the filtrate. It is, however, interesting to find that, even at this concentration, it was possible to detect evidence of an interaction with indomethacin.

The understanding of this interaction between indomethacin and H. pylori culture filtrate might have

therapeutic implications if its presence could be confirmed by in vivo studies. It could be justifiable to ask whether the results of this study could explain, at least in part, the higher incidence of complications related to non-steroidal anti-inflammatory drugs (NSAIDs) in the elderly patient (100,108,116) who, at the same time, are believed to have a higher prevalence of H. pylori colonization (196,253). The role of H. pylori is not clear in such cases, and other factors have been suspected. These include the continuing increase in NSAID prescribing (100), the possible role of diet (117), and the effect of the cohort phenomenon (118).

The possible role of bacteria in increasing NSAID toxicity was previously described in experiments involving the intestine of laboratory animals. It was observed that the ulcerogenic effect of NSAIDs could be reduced by antibiotic treatment (135). Germ-free animals were also found to be resistant to indomethacin-induced lesions (136). It remains to be seen whether the eradication of H. pylori would reduce the frequency, or at least the degree of NSAID-induced gastric damage.

CHAPTER 13.

13. INHIBITION OF GASTRIC CYCLIC AMP PRODUCTION BY HELICOBACTER PYLORI PROTEIN. POSSIBLE INVOLVEMENT OF MUCOSAL PROSTAGLANDIN E_2.

13.1. SUMMARY.

Although H. pylori has been linked to the development of peptic ulceration, its effect on gastric physiology is not clear. H. pylori protein has been shown to inhibit gastric acid secretion in rabbit parietal cells. Cyclic AMP acts as a second messenger in several physiological activities in the stomach, including acid secretion. Cyclic AMP is also known to interact with PGE_2 in various tissues including the gastric mucosa. This study investigated the effect of H. pylori protein on c.AMP and PGE_2 production by incubates of human gastric fundic mucosa. Biopsies were taken from 12 subjects: 5 males and 7 females, median age 52 years. At 24 hours, specimens incubated in the control fluid had a median c.AMP value of 81 pmol/mg protein, compared to 28 ($p<0.05$) in H. pylori protein, 155 ($p<0.006$) in histamine, and 23 ($p<0.05$) in histamine plus H. pylori protein. A similar trend was observed at 48 hours. Although H. pylori protein had no direct effect on mucosal PGE_2, it intensified the inhibitory effect of indomethacin and prevented the stimulatory effect of histamine on both PGE_2 and c.AMP production. Given the role of c.AMP in various physiological responses, these results suggest that acute

exposure to H. pylori protein might alter those functional aspects of the human gastric mucosa mediated by c.AMP. Assuming that PGE_2 mediates such effect, its role would appear to be either partial or indirect.

13.2. INTRODUCTION.

The precise mechanism of interaction between Helicobacter (H.) pylori and the gastric mucosa is not fully understood. It is increasingly accepted that H. pylori produces a factor which might be comparable to other bacterial toxins. The nature of this factor and the range of its activities have been the subject of several studies in recent years. Leunk et al. (188) demonstrated that H. pylori culture filtrates induced cytopathic changes in Vero (mammalian) cells. Cave and Vargas (189) later showed that a protein isolated from H. pylori culture filtrates could reduce acid secretion by rabbit parietal cells. As in many other tissues, receptors for the control of gastrointestinal mucosal function can be divided into two major classes: those which trigger the production of c.AMP and those which initiate inositol phospholipid turnover, calcium mobilisation, and frequently arachidonic acid release and cyclic GMP production (254,255). Cyclic AMP appears to have a major role in controlling gastric acid secretion (256-259). This role can be influenced by the interaction thought to exist between c.AMP, prostaglandins (PGs), and histamine (260-264). Reduced acid secretion was noted when H. pylori organisms were ingested (144,176)

and when H. pylori protein was applied to parietal cells (189). It is not known whether an interaction exists between H. pylori protein, c.AMP, histamine, or mucosal PGE_2. This study aimed at investigating the possibility of such an interaction by measuring the generation of c.AMP and PGE_2 in response to H. pylori protein with or without histamine, *in vitro*.

13.3. PATIENTS AND METHODS.

13.3.1. Patients:

Twelve subjects were studied. They included 5 males and 7 females, median age 52 years. Five were smokers. They all presented with upper abdominal pain, nausea, vomiting, or heartburn. Patients were excluded if they took any medications within a week of performing the endoscopy, or if they had any other illness.

13.3.2. Gastric Biopsies:

Twelve biopsies, weighing 3-5 mg each, were taken from the gastric fundus of each patient, within 2-5 cm. of the gastro-oesophageal junction. They were grouped in pairs, and each pair was kept fresh at $4^{o}C$ in 4 ml of tissue culture fluid (see below), until the time of incubation 3 hours later. The fluid used during this period was discarded to minimise measurement of PGs induced by trauma. Two more biopsies were taken to detect the presence of H. pylori in each patient by both histology and bacteriology, as described in Chapter 4.

13.3.3. Isolation of H. pylori protein, preparation of H. pylori culture control fluid and gastric tissue culture medium:

As described in Chapter 4.

13.3.4. Incubation of gastric biopsies:

Six pairs of fundic biopsies, taken from each subject, were incubated inside 24-well plates containing small pieces of capillary matting to reduce evaporation of the culture media. Each well contained one pair of biopsies, 800 ul of gastric tissue culture medium, and 100 ul of homologous citrated plasma which was freshly prepared to act as a source of growth factors. In addition, one of the following was added per well: (A) 100 ul H. pylori protein, (B) 100 ul H. pylori control fluid, (C) 7.4 ug indomethacin, (D) 100 ul H. pylori protein plus 7.4 ug indomethacin, (E) 10×10^{-7} molar histamine, (F) 10×10^{-7} molar histamine plus 100 ul H. pylori protein. The wells were then sealed and incubated at 37°C with 5.6% CO_2. Indomethacin was used in order to inhibit PGE_2 production, and as a result to prove that PGE_2 synthesis was permitted in specimens not exposed to indomethacin. After 24 hours the supernatants were collected and fresh fluids and materials added. Incubation was continued for another 24 hours. The two biopsies within each well were then removed: one was immediately frozen at -20°C for intracellular c.AMP measurements, and the other fixed in formalin/saline for

post-incubation histological assessment. The supernatants yielded from individual wells at the end of each 24 hour period were divided into 2 halves. The first was frozen at -20°C for c.AMP measurement. The other half was added to an equal volume of methyl-oximating (MOX) agent (96) to stabilise PG compounds till the time of PG measurement.

13.3.5. Measurement of Cyclic AMP:

Cyclic AMP was measured by radioimmunoassay in both supernatants and the corresponding biopsy specimens of each well. Single biopsies were homogenized in 1.5 ml of a buffer composed of the following: Tris HCl 50 mmol/l, mercaptoethanol 16 mmol/l, isobutylmethylxanthine 1 mmol/l and HCl 0.1 mmol/l. The homogenate was sonicated for 3 minutes and then mixed with 50 ul of 1M NaOH. 500 ul of the resulting solution was taken for protein measurement as described by Lowry (98). Another 500 ul of the same solution was lyophilised and dissolved in 50 mM acetate buffer, pH 4.8, prior to acetylation with triethylamine/acetic anhydride (2:1) and estimation of c.AMP by radioimmunoassay. Results were expressed as pmol c.AMP/mg protein. Values obtained from the supernatant of the first incubation period were identified as 24 hour c.AMP. Values related to the second incubation period in supernatant plus biopsy homogenates were identified as 48 hour c.AMP. All specimens were assayed in duplicate, and at random, using code numbers. The c.AMP antisera have been described previously (265). The assay has a minimum detection limit of 30 pmol/l and a

between batch coefficient of variation of 10-15% over the working range.

13.3.6. PGE_2 measurement:

PGE_2 was measured by radioimmunoassay in the methyloximated supernatant as described in Chapter 2. The results were expressed in pg PGE_2/mg of post-incubation tissue weight. Code numbers were used to facilitate randomization.

13.3.7. Statistical Analyses:

Paired analysis was carried out using the Wilcoxon's signed ranks and the Kruskall-Wallis tests where appropriate.

The study was approved by the Local Ethics Committee.

13.4. RESULTS.

Endoscopic findings in the study subjects included 1 gastric ulcer, 5 duodenal ulcers and 6 normal findings. Histological examination of un-incubated baseline specimens showed that 11 subjects had features of chronic superficial active gastritis. These all had H. pylori detected in their biopsies by both histology and bacteriology. The remaining subject did not have H. pylori, and the histology of his baseline biopsy was normal. Comparing the histological appearances of gastric biopsies after 48 hours of incubation to baseline un-incubated specimens, it was found that 30-60% of epithelial cells were maintained, regardless of the

contents of the culture media in 5 out of 6 wells. However, only 10-30% of cells within specimens incubated in the mixture of H. pylori protein and indomethacin were maintained ($p<0.05$). This histological comparison between biopsies before and after incubation was taken as a rough guide to tissue viability during incubation, as discussed in Chapter 12.

Gastric c.AMP and PGE_2 at 24 and 48 hours are shown in Figures 23-26. When applied alone, H. pylori protein suppressed c.AMP generation but did not seem to affect that of PGE_2 . Also, the synthesis of both c.AMP and PGE_2 was inhibited by indomethacin and stimulated by histamine. The addition of H. pylori protein appeared to intensify the effect of indomethacin and inhibit that of histamine.

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Cyclic AMP measured in this study probably represents the total amount released by all cellular elements contained in the fundic mucosal specimens. The duration of the incubation period was intended to allow H. pylori protein to permeate through the superficial and inner layers of gastric tissue. The values of c.AMP and PGE_2 reported do not necessarily equal the overall amounts synthesised during 24 or 48 hours, because of the instability of these compounds. They probably represent c.AMP and PGE_2 produced within minutes before incubation was terminated.

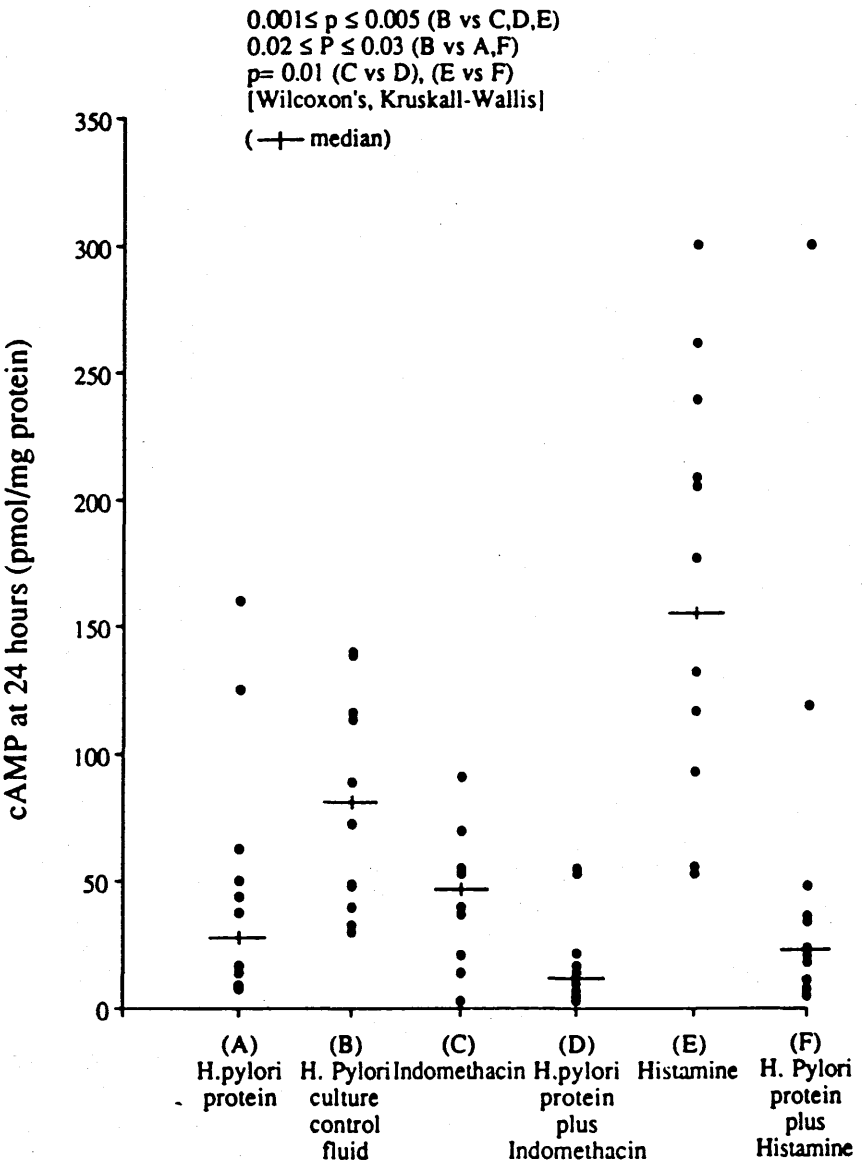

Fig. 23. Gastric c.AMP produced at 24 hours of incubation with H. pylori, indomethacin, histamine, mixture of H. pylori protein and indomethacin, mixture of H. pylori protein and histamine, and control fluid.

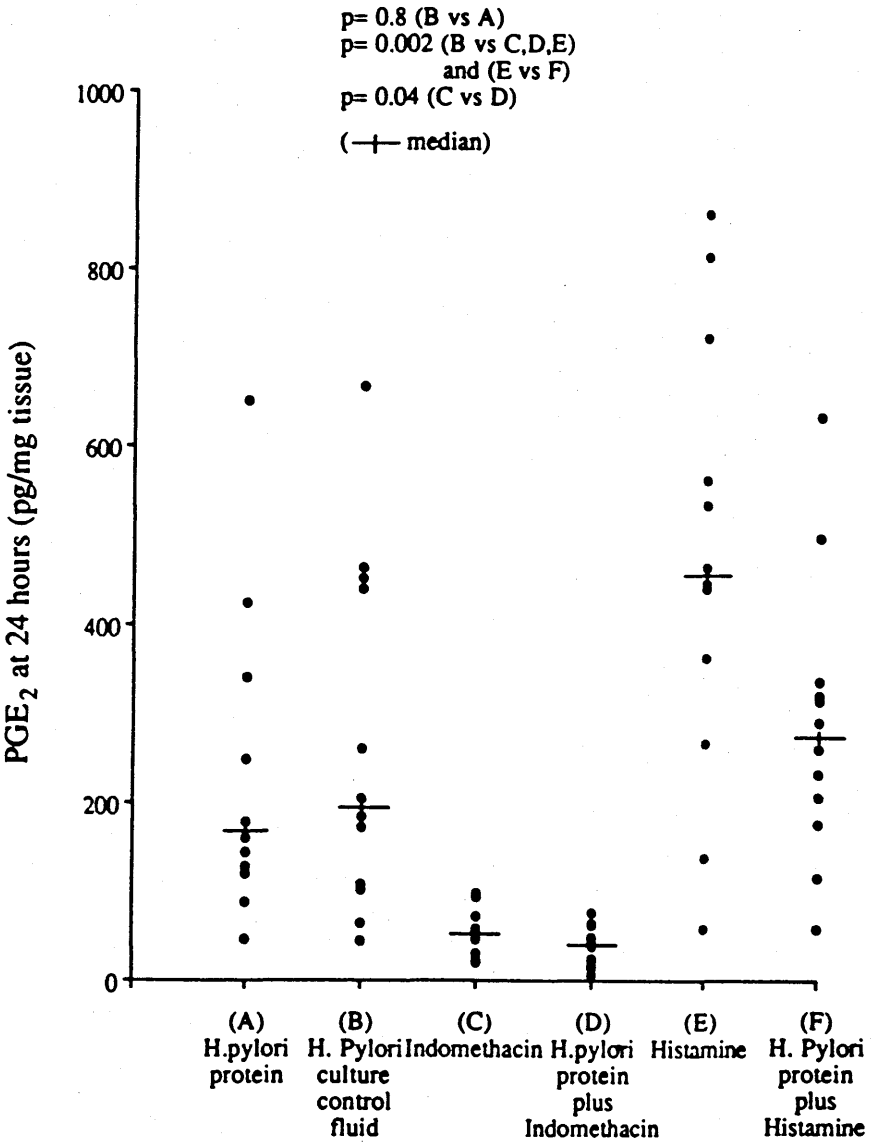

Fig. 24. Gastric PGE_2 produced at 24 hours of incubation with H. pylori, indomethacin, histamine, mixture of H. pylori protein and indomethacin, mixture of H. pylori protein and histamine, and control fluid.

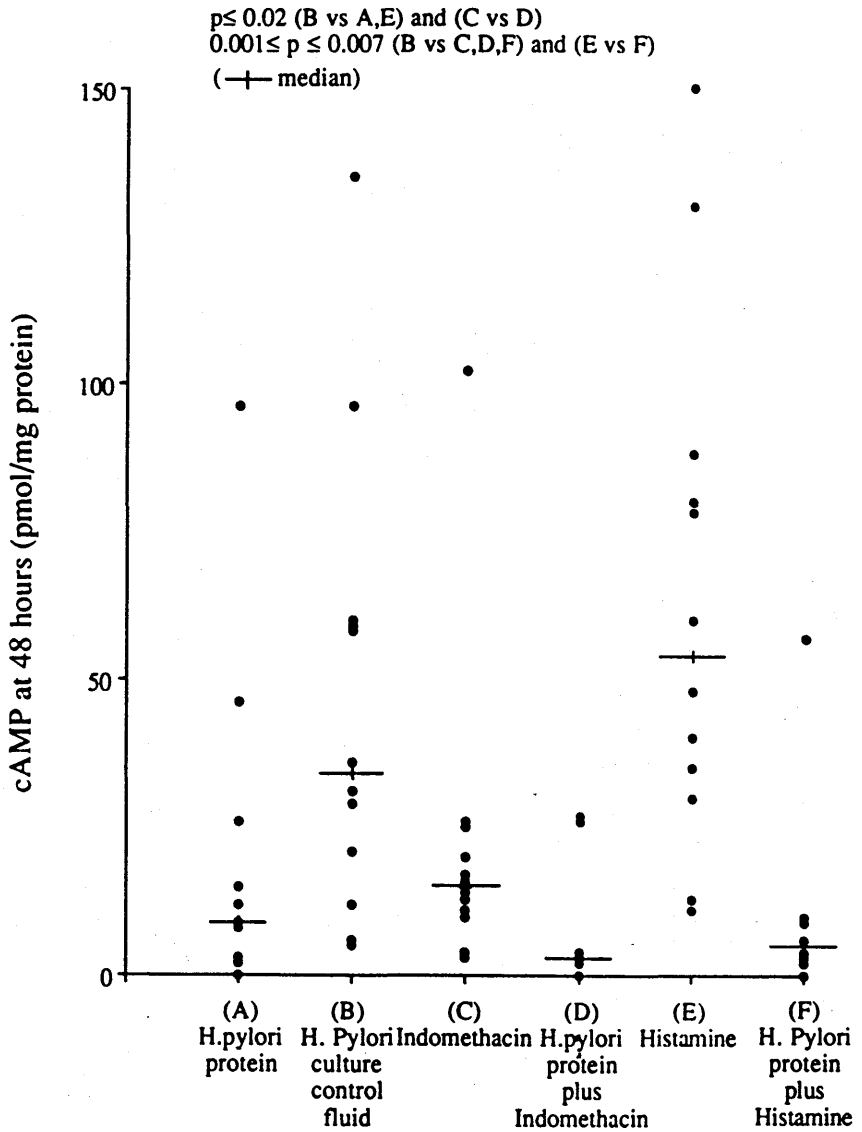

Fig. 25. Gastric c.AMP produced at 48 hours of incubation with H. pylori, indomethacin, histamine, mixture of H. pylori protein and indomethacin, mixture of H. pylori protein and histamine, and control fluid.

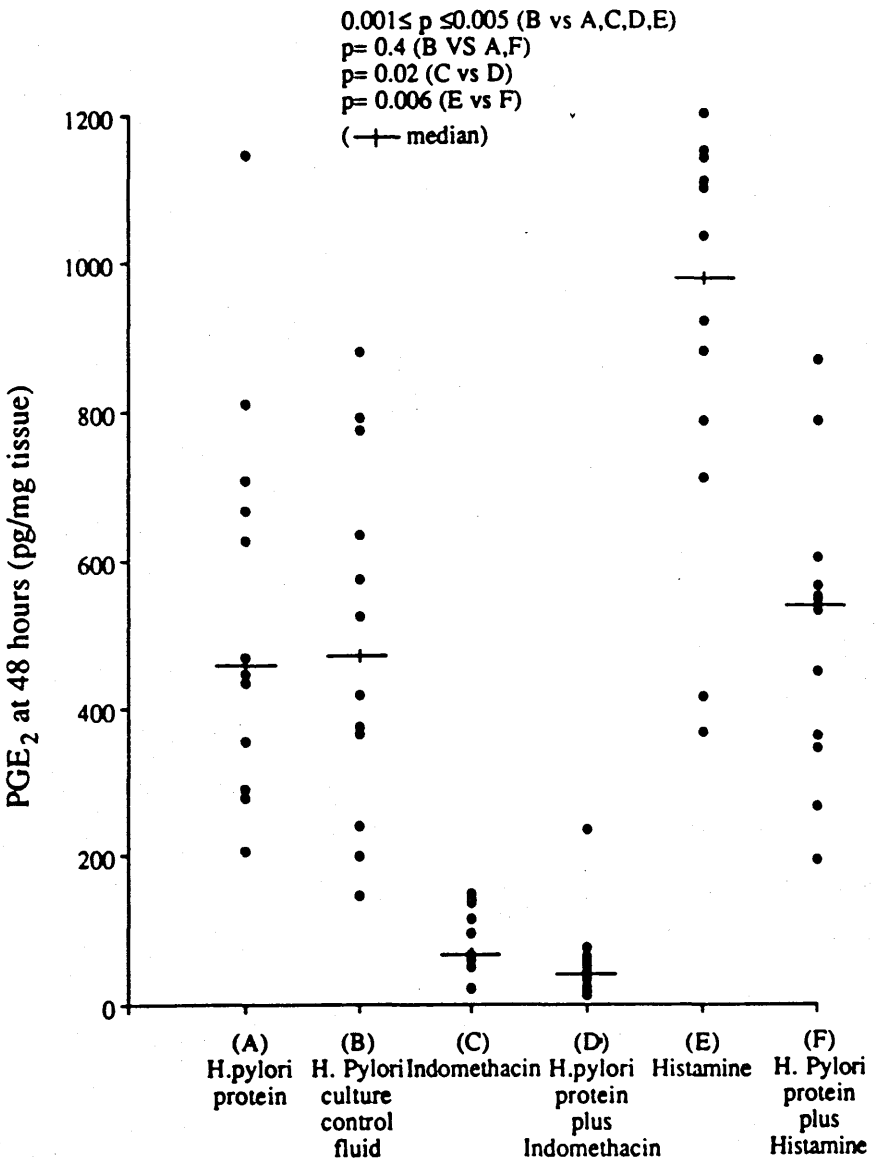

Fig. 26. Gastric PGE_2 produced at 48 hours of incubation with H. pylori, indomethacin, histamine, mixture of H. pylori protein and indomethacin, mixture of H. pylori protein and histamine, and control fluid.

In addition to its role in gastric acid secretion (256-259), c.AMP is thought to be involved in many other activities of the gastrointestinal tract. Several in vitro studies have demonstrated that c.AMP or its derivatives exerted a stimulatory effect on pepsinogen secretion (266-268), bicarbonate transport (269), and mucus glycoprotein synthesis (270). Elevation of tissue c.AMP content was associated with vasodilator activity induced by a variety of agents; exogenously applied c.AMP dilated the canine mesenteric circulation (271). By interfering with c.AMP production, it is possible to speculate that H. pylori protein might alter at least some functional aspects of the human fundic mucosa.

Assuming that the parietal cell c.AMP was also suppressed, our results might explain the initial hypochlorhydria observed following acute exposure to H. pylori (144,176). In support of this assumption is the recent observation that stimulation of histamine H_2 receptors activates adenylate cyclase in the cell, thereby increasing its c.AMP content, while stimulation of histamine H_1 receptors activates phosphinositidase C to produce diacylglycerol and inositolphosphates (272). This study showed that H. pylori protein suppressed the synthesis of c.AMP and the stimulatory effect of histamine on its generation. These two activities are thought to involve histamine H_2 receptors, which in turn mediate acid secretion (258,259,272).

Little is known about the effects of bacterial toxins

on the human gastric mucosal c.AMP. Most studies have investigated their effects on the intestinal mucosa of laboratory animals. Unlike H. pylori protein, the enterotoxins of Salmonella and Cholera were shown to increase mucosal c.AMP production (273,274). This might help explain the difference in the degree and type of toxicity between these organisms.

When applied alone, H. pylori protein did not seem to affect mucosal PGE_2 values, which is consistent with the findings of Chapter 12 using H. pylori culture filtrate. It, however, intensified the inhibitory effect of indomethacin and prevented the stimulatory effect of histamine on PGE_2 formation. PGE_2 was previously found to increase gastric c.AMP synthesis (275,276), although some workers (260,261) believe that histamine-stimulated production of c.AMP was suppressed by PGE_2 or its analogues in isolated canine parietal cells. The mechanism by which PGs increase c.AMP formation is thought to involve their receptor interaction with stimulatory G proteins (receptor associated GTP-binding proteins) and adenylcyclase; under some circumstances PGs and other eicosanoids may interact with alternate receptors and activate inhibitory G proteins to prevent c.AMP production (277). The simultaneous inhibition by indomethacin of both PGE_2 and c.AMP production in this study and the parallel intensification of this by H. pylori protein might suggest some role for PGE_2 in mediating the interaction between H. pylori protein and c.AMP, given the

known effect of PGE_2 on c.AMP synthesis. This is further supported by the finding that H. pylori protein interfered with the stimulatory effect of histamine on both PGE_2 and c.AMP production. Since PGE_2 synthesis was not directly inhibited by H. pylori protein, any role it may have in mediating the effect of H. pylori protein on c.AMP generation would appear to be either partial or indirect.

In conclusion, acute exposure to H. pylori protein lowers gastric mucosal c.AMP and prevents its stimulation by histamine. This may be mediated partially or indirectly by mucosal PGE_2. These findings might help clarify further the interaction between H. pylori protein and the human gastric mucosa.

SECTION 5.

GENERAL DISCUSSION AND CONCLUSIONS.

CHAPTER 14.

14. GENERAL DISCUSSION AND CONCLUSIONS.

The studies included in this Thesis relate to the ongoing debate about the importance of three major factors: PGs, NSAIDs, and H. pylori in the pathogenesis of peptic ulcer disease. The background of this Thesis, discussed in Section 1, reviews the current evidence of the involvement of these factors in peptic ulceration. It also covers the contribution made by this Thesis to the understanding of some aspects of peptic ulcer disease and the methodology used in its research.

Some of the major methodological considerations in PG research are reviewed in Chapter 2. Another important issue in this context, which received little attention in the past, is investigated in Chapter 6. It relates to the importance of tissue handling and how to care for biopsies prior to carrying out the PG assays.The basic question of whether to incubate biopsies fresh or after freezing them and the consequences of either option are explained. It is obvious therefore that fresh specimens follow a different behaviour from frozen ones, probably because of the ultrastructural changes caused by freezing. The effect of low temperature on tissue functions is not new; unlike other functions which are adversely affected,

PG production seems to be enhanced because of the disintegration of cell membranes.

Subsequent sections investigate PG synthesis in response to a variety of factors including diet, duodenal ulceration, naproxen, etodolac, gastric ulcers, gastritis, non-ulcer dyspepsia, H. pylori, H. pylori culture filtrate and H. pylori protein. In addition, the interaction between H. pylori culture filtrate and indomethacin, and the effect of H. pylori protein on gastric c.AMP production are investigated.

The important points can be summarized as follows:

1. As mentioned above, gastric and duodenal biopsies incubated after being frozen can produce more PGE_2 and PGI_2 than fresh specimens. Also, for workers wishing to homogenize their tissues, freezing seems to be both convenient and effective in homogenizing the tissues before PG assays are carried out. Tissue sampling should be standardized within the individual experiment in order to minimize the possible sources of variation in PG results.

2. Diets rich in fish oil, evening primrose oil and olive oil can increase gastric PGE_2 synthesis as shown in rats. Animals fed on fish oil diet tend to have lower PG values than the other diets, possibly due to the competition between the individual fatty acid components of each oil. Human studies in this field may be worthwhile, as this may help understand the nutritional

values of some commonly used oils, olive oil in particular, and their effect on the incidence of peptic ulceration.

3. There is no evidence that duodenal ulceration is associated with defective synthesis in PGE_2, PGI_2, or TXB_2. No such defect is found in gastric ulcers either. (Chapter 11). This might help explain, at least in part, the disappointing results of using so called "cytoprotective" doses of PG analogues in the treatment of peptic ulcers, especially those unrelated to NSAIDs.

4. One of the new NSAIDs, etodolac, seems to have a similar antiarthritic activity to the well established naproxen. The gastroduodenal side effects of these two agents are different, however, since naproxen resulted in worse endoscopic scores than etodolac. The endoscopic abnormalities detected after 4 weeks of treatment are mostly asymptomatic. This highlights the unreliability of patients symptoms as a means of monitoring NSAID side effects on the gastric mucosa.

5. Unlike most previous studies that investigated the effect of NSAIDs on gastric and duodenal PG synthesis, evidence is shown in this Thesis that not all NSAIDs suppress PG production. Etodolac appears to have no effect on gastric or duodenal PGs, while naproxen suppresses gastric PGE_2 and duodenal PGE_2 and PGI_2. These differences between the two agents become clear only after patients are grouped according to the drug they

received and with the advantage of knowing base-line PG values. These aspects of the study are recommended for the proper evaluation of the rest of NSAIDs.

6. In line with findings of other studies, H. pylori was associated with a high prevalence of active chronic gastritis and gastric ulcers. In patients with gastric ulcers and non-ulcer dyspepsia, the presence of H. pylori in gastric tissue is not associated with a significant rise in PG values, which would normally take place in cases of gastritis regardless of H. pylori. The total amount of PGs measured is probably equivalent to PGs produced by the gastric mucosa and those synthesised by the inflammatory cells. The neutrophils are usually present in abundance in cases of H. pylori-induced gastritis, as shown by the histological part of the study in Chapter 11. The failure of PGs to rise in the presence of H. pylori might encourage speculation about the possible presence of a defect in PG synthesis at the level of the mucosal cells, the neutrophils, or both.

7. This Thesis presents evidence showing that an interaction may exist between H. pylori culture filtrate and indomethacin, in vitro. Such interaction appears to be damaging to the gastric antral mucosa. Specimens incubated in a mixture of H. pylori culture filtrate and indomethacin produce less PGE_2 and have lower histological scores than those incubated with indomethacin alone.

8. A protein isolated from H. pylori culture filtrate (H. pylori protein) is shown to reduce c.AMP production by the gastric fundic mucosa *in vitro*. It also prevents the stimulatory effect of histamine on both PGE_2 and c.AMP formation, and interacts with indomethacin in a manner similar to that of H. pylori culture filtrate. This might suggest that H. pylori protein could influence at least some gastric activities in which c.AMP acts as a second messenger.

REFERENCES.

REFERENCES.

1. Kurzok, R., Lieb, C.
Biochemical studies of human semen: II. The action of semen on human uterus.
Proc. Soc. Exp. Biol. Med. 1930; 28: 268-72.

2. von Euler, U.S.
A depressor substance in the vesicular gland.
J. Physiol. 1935; 88: 213-34.

3. Goldblatt, M.W.
Properties of human seminal plasma..
J. Physiol. 1935; 84: 208-18.

4. Bergstrom, S., Sjovall, J.
The isolation of prostaglandin E from sheep prostate glands.
Acta. Chem. Scand. 1960; 14: 1701-5.

5. Hamberg, M., Svensson, J., Wakabayashi, T., Samuelsson, B.
Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation.
Proc. Natl. Acad. Sci. USA. 1974; 71: 345-9.

6. Nugteren, D.H., Hazelhof, E.
Isolation and properties of intermediates in prostaglandin biosynthesis.
Biochim. Biophys. Acta. 1973; 326: 448-61.

7. Hamberg, M., Svensson, J., Samuelsson, B.
Thromboxanes. A new group of biologically active compounds derived from prostaglandin endoperoxides.

Proc. Natl. Acad. Sci. USA. 1975; 72: 2994-8.

8. Bunting, S., Gryglewski, R., Moncada, S., Vane, J.R.
Arterial wall generates from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation.
Prostaglandins 1976; 12: 897-913.

9. Bray, M.A.
The pharmacology and pathophysiology of leukotriene B4.
Br. Med. Bull. 1983; 39: 249-54.

10. Borgent, P., Samuelsson, B.
Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structural analysis of novel hydroxylated compounds.
J. Biol. Chem. 1979; 254: 7865-9.

11. Caton, M.P.L.
Chemistry structure and availability.
In: The Prostaglandins. M.F. Cuthbert (ed).
London, William Heinemann Medical Books, 1973, pp 1-22.

12. Dyeberg, J., Bang, H.O., Stoffersen, E., Moncada, S., Vane, J.R.
Eicosapentaenoic acid and the prevention of thrombosis and atherosclerosis?
Lancet 1978; 2: 117-9.

13. McGiff, J.C., Crowshow, K., Terragno, N.A., Malik, K.U., Lonigro, A.J.
Differential effect of noreadrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance.

Clin. Sci. 1972; 42: 223-33.

14. Gimbrone, M.A., Alexander, B.W.
Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium.
Science 1975; 184: 219-20.

15. Ramwell, P.W., Shaw, J.E., Douglas, W.W., Poisner, A.A.
Efflux of prostaglandin from adrenal glands stimulated with acetyl-choline.
Nature 1966; 210: 273-4.

16. Granstrom, E., Diczfalusy, U., Mamberg, M., Hansson, G., Malmsten, C., Samuelsson, B.
Thromboxane A_2: Biosynthesis and effects on platelets.
In: Prostaglandins and the Cardiovascular System.
J.A. Oates (ed.). New York, Raven Press, 1982, pp 15-58.

17. William, T.J.
Interactions between prostaglandins, leukotrienes, and other mediators of inflammation.
Br. Med. Bull. 1983; 39: 239-42.

18. Mehta, J., Mehta, P., Ostrowski, N.
Stimulation of vessel wall prostacyclin by selective thromboxane synthetase inhibitor OKY 1581.
Prostaglandins Leukotrienes Med. 1983; 12: 49-52.

19. Bennet, A., Stamford, I.F., Stockley, H.L.
Estimation and characterization of prostaglandins in the human gastrointestinal tract.
Br. J. Pharmacol. 1977; 61: 579-86.

20. Ahlquist, D.A., Duenes, J.A., Madson, T.H., Romero, J.C., Dozois, R.R., Malegalada, J.R.
Prostaglandin generation from gastroduodenal mucosa: Regional and species differences.
Prostaglandins 1982; 24: 115-25.

21. Bennet, A., Murray, J.G., Wyllie, J.H.
Occurrence of prostaglandin E_2 in the human stomach, and a study of its effects on human isolated gastric muscle.
Br. J. Pharmacol. 1968; 32: 339-49.

22. Gallavan, R.H. Jr., Jacobson, E.D.
Prostaglandins and the splanchnic circulation.
Proc. Soc. Exp. Biol. Med. 1982; 170: 391-7.

23. Robert, A.
Effects of prostaglandins on the stomach and intestine.
Prostaglandins 1974; 6: 523-32.

24. Jacobson, E.D.
Comparison of prostaglandin E_1 and norepinephrine on the gastric mucosal circulation.
Proc. Soc. Exp. Biol. Med. 1970; 113: 516-9.

25. Walus, K.M., Pawlik, W., Konturek, S.J.
Prostacyclin-induced gastric mucosal vasodilatation and inhibition of acid secretion in the dog.
Proc. Soc. Exp. Biol. Med. 1980; 163: 228-32.

26. Konturek, S.J., Robert, A., Hauchar, A.J., Nezamis, J.E.
Comparison of prostacyclin and prostaglandin E_2 on gastric secretion, gastrin release and mucosal blood flow in dogs.

Dig. Dis. Sci. 1980; 25: 673-9.

27. Classen, M., Koch, H., Bickhardt, J., Topf, G., Demling, L.
The effect of prostaglandin E_1 on the pentagastrin-stimulated gastric secretion in man.
Digestion 1971; 4: 333-44.

28. Horton, E.W., Main, I.H.M., Thomson, C.J., Wright, P.M.
Effect of orally administered prostaglandin E_1 on gastric secretion and gastro-intestinal motility in man.
Gut 1968; 9: 655-8.

29. Johansson, B.R.
Oral PGE_2 inhibits gastric acid secretion in man.
Prostaglandins 1985; 298: 143-52.

30. Ippoliti, A.F., Isenberg, J., Hagiel, L.
Effect of oral and intravenous 16, 16-dimethyl PGE_2 on duodenal ulcer and Zollinger Ellison syndrome patients.
Gastroenterology 1981; 80: 55-60.

31. Robert, A.
Prostaglandins and the gastrointestinal tract.
In: Physiology of the Gastrointestinal Tract.
L.R. Johnson (ed.) New York, Raven Press, 1981, pp 1407-34.

32. Nylander, B., Andersson, S.
Gastric secretory inhibition induced by three methyl analogues of prostaglandin E_2 administered intragastrically to man.
Scand. J. Gastroenterol. 1974; 9: 751-8.

33. Levin, R.A., Kohen, K.R., Schwartzek, E.H., Ramsay, C.E.
Prostaglandin E_2-histamine interactions on c.AMP, c.GMP and acid

production in isolated fundic glands.

Am. J. Physiol. 1982; 242: G21-G29.

34. Soll, A.H.

Specific inhibition by prostaglandins E_2 and I_2 of histamine-stimulated [^{14}C] aminopyrine accumulation and cyclic AMP generation by isolated canine parietal cells.

J. Clin. Invest. 1980; 65: 1222-9.

35. Bickel, M., Kauffman, G.L.

Gastric gel mucus thickness: Effect of distension, 16, 16-dimethyl prostaglandin E_2, and carbenoxolone.

Gastroenterology 1981; 80: 770-5.

36. Ruppin, H., Person, B., Robert, A., Domschke, W.

Gastric cytoprotection in man by prostaglandin E_2.

Scand. J. Gastroenterol. 1981; 16: 647-52.

37. Konturek, S.J., Bilski, J., Tasler, J., Kania, J.

Prostaglandins and alkaline secretion from oxyntic antral and duodenal mucosa of the dog.

Am. J. Physiol. 1983; 245: G539-G546.

38. Robert, A.

Cytoprotection by prostaglandins.

Gastroenterology 1979; 77: 761-7.

39. Konturek, S.J., Brzozowski, T., Piastucki, I., Radecki, T., Dembinski, A., Dembinska-kiec, A.

Role of locally generated prostaglandins in adaptive gastric cytoprotection.

Dig. Dis. Sci. 1982; 27: 967-71.

40. Robert, A., Nezamis, J.E., Lancaster, C., Davis, J.P., Field, S.O., Hanchar, A.J.
Mild irritants prevent gastric necrosis through adaptive cytoprotection mediated by prostaglandins.
Am. J. Physiol. 1983; 245: G113-G121.

41. Whittle, B.J.R., Steel, G.
Evaluation of the protection of rat gastric mucosa by prostaglandin analogue using cellular enzyme marker and histologic techniques.
Gastroenterology 1985; 88: 315-27.

42. Lichtenberger, L.M., Richards, J.E., Hills, B.A.
Effect of 16, 16-dimethyl prostaglandin E_2 on the surface hydrophobicity of aspirin-treated canine gastric mucosa.
Gastroenterology 1985; 88: 308-14.

43. Lichtenberger, L.M., Graziani, L.A., Dial, E., Butler, B.D., Hills, B.A.
Role of surface-active phospholipids in gastric cytoprotection.
Science 1983; 219: 1327-9.

44. Thor, P., Konturek, J.W., Konturek, S.J., Anderson, J.H.
Role of prostaglandins in control of intestinal motility.
Am. J. Physiol. 1985; 248: G352-G359.

45. Robert A., Nezamis J.E., Lancaster C., Hanchar A.J, Klepper M.S.
Enteropooling assay: A test for diarrhoea produced by prostaglandins.
Prostaglandins 1976; 11: 809-28.

46. Robert, A.
Cytoprotection and enteropooling by prostaglandins.

In: The Prostaglandin System. F. Beri, G.P. Velo (eds.). New York, Plenum Press, 1981, pp 393-400.

47. Graham, D.Y., Agrawal, N.M., Roth, S.H.
Prevention of NSAID-induced gastric ulcer with misoprostol: multicentre, double-blind, placebo-controlled trial.
Lancet 1988; 2: 1277-80.

48. Main, I.H.M., Whittle, B.J.R.
The effects of E and A prostaglandins on gastric mucosal blood flow and acid secretion in the rat.
Br. J. Pharmacol. 1973; 49: 428-36.

49. Kauffman, G.L.Jr., Aures, D., Grossman, M.I.
Indomethacin decreases basal gastric mucosal blood flow.
Gastroenterology 1979; 76: 1165(A).

50. Pawlik, W., Shepherd, A.P., Jacobson, E.D.
Effects of vasoactive agents on intestinal oxygen consumption and blood flow in dogs.
J. Clin. Invest. 1975; 56: 484-90.

51. Damman, H.G., Walter, Th.A., Muller, P., Simon, B.
Night-time rioprostil versus ranitidine in duodenal ulcer healing
Lancet 1986; 2: 335.

52. Shield, M.J.
Interim results of a multicentre international comparison of misoprostol and cimetidine in the treatment of outpatients with benign gastric ulcers.
Dig. Dis. Sci. 1985; 30: 178(S).

53. Phillipson, B.E., Rothrock, D.W., Connor, W.E., Illingworth, D.R.
Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia.
N. Engl. J. Med. 1985; 312: 1210-6.

54. Nestel,P.J., Connor, W.E., Reardon, M.F., Connor, S., Wong, S., Boston, R.
Suppression by diets rich in fish oil of very low density lipoprotein production in man.
J. Clin. Invest. 1984; 74: 82-9.

55. Von Schacky, C., Fischer, S., Weber, P.C.
Long-term effects of dietary marine w-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans.
J. Clin. Invest. 1985; 76: 1626-31.

56. Knap, H.R., Reilly, I.A.G., Alessandrini, P., Fitzgerald, G.A.
In-vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis.
N. Engl. J. Med. 1986; 314: 937-42.

57. Lee, T.H., Hoover, R.L., Williams, J.D., Sperling, R.I., Ravalese, J., Spur, B.W., Robinson, D.R., Corey, E.J., Lewis, R.A., Austen, K.F.
Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function.
N. Engl. J. Med. 1985; 312: 1217-24.

58. Weiner, B.H., Ockene, I.S., Levine, P.H., Cuenoud, H.F., Fisher, M., Johnson, B.F., Daoud, A.S., Jarmolych, J., Hosmer D.,

Johnson, M.H., Natale, A., Vaureuil, C., Hoogasian, J.J.
Inhibition of atherosclerosis by codliver oil in a hyperlipidemic swine model.
N. Engl. J. Med. 1986; 315: 841-6.

59. Hollander, D., Krause, W.J., Stachura, J., Zipster, R.D., Gergly, H., Dadufalza, V.
Is linoleic acid (dietary essential fatty acid - EFA) cytoprotective for the gastric mucosa?
Gastroenterology 1985; 88: 1610 (A).

60. Hollander, D., Tarnawski, A., Ivey, K.J., DeZeery, A., Zipster, R.D., McKenzie, W.N.Jr., McFarland, W.D.
Arachidonic acid protection of rat mucosa against ethanol-induced injury.
J. Lab. Clin. Med. 1982; 100: 296-309.

61. Needleman, P., Raz, A., Minkes, M.S., Ferrendelli, J.A., Sprecher, H.
Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties.
Proc. Natl. Acad. Sci. USA 1979; 76: 944-8.

62. Corey, E.J., Shih C., Cashman, J.R.
Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis.
Proc. Natl. Acad. Sci. USA. 1983; 80: 3581-4.

63. Hollander D, Tarnawski A.
Dietary essential fatty acids and the decline in peptic ulcer disease - a hypothesis.
Gut 1986; 27: 239-42.

64. Konturek, S.J., Kwiecien, N., Obtulowicz, W., Oleksy, J., Sito, E., Kopp, B.
Prostaglandins in peptic ulcer disease: Effect of non steroidal anti inflammatory compounds (NOSAC).
Scand. J. Gastroenterol 1984; 19 (Suppl.92): 250-4.

65. Wright, J.P., Young, G.O., Klaff, L.J., Weers, L.A., Price, S.K., Marks, I.N
Gastric mucosal prostaglandin E levels in patients with gastric ulcer disease and carcinoma.
Gastroenterology 1982; 82: 263-7.

66. Hindsale, J.G., Engel, J.J., Wilson, D.E.
Prostaglandin E in peptic ulcer disease.
Prostaglandins 1974; 6: 495-500.

67. Tonnesen, M.G., Jubiz, W., Moore, J.G., Frailey, J.
Circadian variation of prostaglandin E (PGE) production in human gastric juice.
Am. J. Dig. Dis. 1974; 19: 644-8.

68. Baker, R., Jaffe, B.M., Reed, J.D., Shaw, B., Venables, C.W.
Are prostaglandins deficient in peptic ulceration?
Gut 1977; 18: 950-1 (Abstract).

69. Baker, R., Jaffe, B.M., Venables, C.W.
Endogenous prostaglandins in peptic ulcer disease.
Gut 1979; 20: 394-9.

70. Ferreira, S.H., Vane, J.R.
Prostaglandins: their disappearance from and release into the circulation.
Nature 1967; 216: 868-73.

71. Piper, P.J., Vane, J.R., Wyllie, J.H.
Inactivation of prostaglandins by the lungs.
Nature 1970; 225: 600-604.

72. Pace-Asciak, C.
Prostaglandin synthetase activity in the rat stomach fundus. Activation by L-norepinephrine and related compounds.
Biochim. Biophys. Acta. 1972; 280: 161-71.

73. Peskar, B.M., Peskar, B.A.
On the metabolism of prostaglandins by human gastric fundus mucosa.
Biochim. Biophys. Acta 1976; 424: 430-8.

74. Baker, R., Jaffe, B.M., Reed, J.D., Shaw, B., Venables, C.W.
Endogenous prostaglandins and gastric secretion in the cat.
J. Physiol. 1978; 278: 451-60.

75. Moore, S.C., Shorter, R.G., Barham, S.S., Duenes, J.A., Zinsmeister, A.R., Malegalada, J.R.
Interrelationships among gastric mucosal morphology and prostaglandin (PG) synthesis, luminal pH, and motility in ulcer disease.
Gastroenterology 1984; 86: 1188(A).

76. Sharon, P., Cohen, F., Zifroni, A., Karmeli, F., Ligumsky, M., Rachmilewitz, D.
Prostanoid synthesis by cultured gastric and duodenal mucosa: Possible role in the pathogenesis of duodenal ulcer.
Scand. J. Gastroenterol. 1983; 18: 1045-9.

77. Hillier, K., Smith, C.L., Jewell, R., Arthur, M.J.P., Ross, G.
Duodenal mucosa synthesis of prostaglandins in duodenal ulcer

disease.

Gut 1985; 26: 237-40.

78. Ahlquist, D.A., Dozois, R.R., Zinsmeister, A.R., Malagelada, J.R.
Duodenal prostaglandin synthesis and acid load in health and in duodenal ulcer disease.

Gastroenterology 1983; 85: 522-8.

79. Ferreira, S.H.
Prostaglandins, aspirin-like drugs, and analgesia.

Nature New Biol. 1972; 240: 200-3.

80. Williams, T.J., Morley, J.
Prostaglandins as potentiators of increased vascular permeability in inflammation.

Nature 1973; 246: 215-7.

81. Moncada, S., Ferreira, S., Vane, J.R.
Prostaglandins, aspirin-like drugs and the oedema of inflammation

Nature 1973; 246: 217-9.

82. Bourne, H.R., Lichenstein, L.M., Melmon, K.L., Henney, C.S., Weinstein, Y., Shearer, G.M.
Modulation of inflammation and immunity by cyclic AMP.

Science 1974; 184: 19-28.

83. Dy, M., Astoin, M., Rigaud, M., Hamburger, J.
Prostaglandin (PG) release in the mixed lymphocyte culture; effect of presensitization by a skin allograft; nature of the PG-producing cell.

Eur. J. Immunol. 1980; 10: 121-6.

84. Morley, J., Bray, M.A., Jones, R.W., Nugteren, D.M. Van Drop D.A.

Prostaglandin and thromboxane production by human and guinea pig macrophages and leucocytes.

Prostaglandins 1979; 17: 730-6.

85. Humes, J.L., Bonney, R.J., Pelus, L., Dahlgren, M.E. Sadowski, S.J., Kuehl, F.A.Jr., Davies, P.
Macrophages synthesise and release prostaglandins in response to inflammatory stimuli.

Nature 1977; 269: 149-51.

86. Schlegel, W., Werk, K., Dollingor, H.C., Raptis, S.
Concentrations of prostaglandin A-, E- and F-like substances in gastric mucosa of normal subjects and of patients with various gastric disease.

Clin. Sci. Mol. Med. 1977; 52:255-8.

87. Hawkey, C.J.
Synthesis of prostaglandin E2, thromboxane B2 and prostaglandin catabolism in gastritis and gastric ulcer.

Gut 1986; 27: 1484-92.

88. Granstrom, E., Samuelsson, B.
Quantitative measurement of prostaglandins and thromboxanes: general considerations.

Prostaglandin Thromboxane Res. 1978; 5: 1-13.

89. Konturek, S.J., Obtulowicz, W., Sito, E., Oleksy, J., Wilkon S., Kiec-Dembinska, A.
Distribution of prostaglandins in gastric and duodenal mucosa of healthy subjects and duodenal ulcer patients: effect of aspirin and paracetamol.

Gut 1981; 22: 283-9.

90. Rachmilewitz, D., Ligumsky, M., Fich, A., Goldin, E., Eliakim, A., Karmeli, F.
Role of endogenous gastric prostanoids in the pathogenesis and therapy of duodenal ulcer.
Gastroenterology 1986; 90: 963-9.

91. Redfern, J.S., Lee, E., Feldman, M.
Effects of indomethacin on gastric mucosal prostaglandins in humans, correlation with mucosal damage.
Gastroenterology 1987; 92: 969-77.

92. Shea-Donohue, T., Steel, L., Montcalm, E., Dobois, A.
Gastric protection by sucralfate. Role of mucus and prostaglandins.
Gastroenterology 1986; 91: 660-6.

93. Andreone, P., Baraldini, M., Micaletti, E., Cursaro, C., Saggioro, A., Della Monica, A., Bortoluzzi, F., Miglio, F., Gasbarrini, G.
Influence of cimetidine in low doses ($<ED_{50}$) on prostanoid production by human gastric mucosa in vitro.
Drugs Exptl. Clin. Res. 1989; 15: 91-6.

94. Aly, A., Green, K., Johansson, G.
Prostaglandin synthesis in the human gastrointestinal mucosa.
Scand. J. Gastroenterol. 1987; 22 [suppl. 127]: 35-38.

95. Needleman, P.
Experimental criteria for evaluating prostaglandin biosythesis and intrinsic function.
Biochem. Pharmacol. 1978; 27: 1515-8.

96. Kelly, R.W., Deam, S., Cameron, S.J., Seamark, R.F.

Measurement by radioimmunoassay of prostaglandins as their methyloximes. Prostaglandins Leukotrienes Med. 1986; 24: 1-14.

97. Kelly, R.W., Healy, D.L., Cameron, M.J., Cameron. I.T. Baird D.T. The stimulation of prostaglandin production by two anti-progesterone steroids in human endometrial cells.
J. Clin. Endocrinol. Metab. 1986; 62: 1116-23.

98. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. Protein measurements with the folin phenol reagent.
J. Biol. Chem. 1951; 193: 265-75.

99. Baum, C., Kennedy, D.L., Forbes, M.B. Utilization of non-steroidal anti-inflammatory drugs.
Arthritis Rheum. 1985; 28: 686-92.

100. Walt, R., Katchinski, B., Logan, R., Ashley, J., Langman, M. Rising frequency of ulcer perforation in elderly people in the United Kingdom.
Lancet 1986; 1: 489-92.

101. Rossi, A.C., Hsu, J.P., Faich, G.A. Ulcerogenicity of piroxicam: an analysis of spontaneously reported data.
Br. Med. J. 1987; 294: 147-50.

102. Committee on Safety of Medicines Update. Non-steroidal anti-inflammatory drugs and serious gastro-intestinal adverse reactions - 1.
Br. Med. J. 1986; 292: 614.

103. Committee on Safety of Medicines Update. Non-steroidal anti-inflammatory drugs and serious gastro-

intestinal adverse reactions - 2.

Br. Med. J. 1986; 292: 1190-1.

104. O'Brien, W.M.

Pharmacology of non-steroidal anti-inflammatory drugs - practical review for clinicians.

Am. J. Med. 1983; 75(4B): 32-9.

105. Caruso, I., Bianchi Porro, G.B.

Gastroscopic evaluation of anti-inflammatory agents.

Br. Med. J. 1980; 280: 75-8.

106. Coggon, D., Langman, M.J.S., Spiegelhalter, D.

Aspirin, paracetamol and haematemesis and melaena.

Gut 1982; 23: 340-4.

107. Levy, M.

Aspirin use in patients with major upper gastrointestinal bleeding and peptic ulcer disease. A report from the Boston Collaborative Drug Surveillance Program.

N. Engl. J. Med. 1974; 290: 1158-72.

108. Armstrong, C.P., Blower, A.L.

Non-steroidal anti-inflammatory drugs and life threatening complications of peptic ulceration.

Gut 1987; 28: 527-32.

109. Catford, J.C., Simpson, R.J.

Confidential enquiry into deaths from peptic ulcer.

Health Trends 1986; 18: 37-40.

110. Collier, D.St.J., Pain, J.A.

Non-steroidal anti-inflammatory drugs and peptic ulcer

perforation.

Gut 1985; 26: 359-63.

111. O'Brien, J.D., Burnham, W.R.
Bleeding from peptic ulcers and use of non-steroidal anti-inflammatory drugs in Romford area.
Br. Med. J. 1985; 291: 1609-10.

112. Carson, J.L., Strum, B.L., Soper, K.A., West, S.L., Morse, L.
The association of non-steroidal anti-inflammatory drugs with upper gastro-intestinal tract bleeding.
Arch. Intern. Med. 1987; 147: 85-8.

113. Jick, S.S., Perera, D.R., Walker, A.M., Jick, H.
Non-steroidal anti-inflammatory drugs and hospital admission for perforated peptic ulcer.
Lancet 1987; ii: 380-2.

114. Inman, W.H.W., Rawson, N.S.B.
Peptic ulcer and piroxicam.
Br. Med. J. 1985; 290: 932-3.

115. Inman, W.H.W.
Comparative study of five NSAIDs.
PEM News 1985; 3: 3-13.

116. Sommerville, K., Faulkner, G., Langman, M.
Non-steroidal anti-inflammatory drugs and bleeding peptic ulcer.
Lancet 1986; 1: 462-4.

117. Sonnenberg, A.
Dietary salt and gastric ulcer.
Gut 1986; 27: 1138-42.

118. Sonnenberg, A.
Geographical and temporal variations in the occurrence of peptic ulcer disease.
Scand. J. Gastroenterol. 1985; 20[suppl.110]: 11-24.

119. Flower, R.J.
Drugs which inhibit prostaglandin biosynthesis.
Pharmacol. Rev. 1974; 26: 33-67.

120. Shen, T.Y.
The chemical and pharmacologic characterizations of suldinac.
In: Current Concepts on Anti-Inflammatory Drugs. C.M. Plotz (ed.)
New York, Biomedical Information Corporation Publications,
1980, p1.

121. Metz, S.A.
Anti-inflammatory agents as inhibitors of prostaglandin synthesis in man.
Med. Clin. North Am. 1981; 65: 713-57.

122. Maddox, I.S.
The role of copper in prostaglandin production.
Biochim. Biophys. Acta 1973; 306: 74-81.

123. Kauffman, G.L., Aures, D., Grossman, M.I.
Intravenous indomethacin and aspirin reduce basal gastric mucosal blood flow in dogs.
Am. J. Physiol. 1980; 238: G131-4.

124. Gerkens, J.F., Shand, D.G., Flexner, C., Nies, A., Oates, J., Data, J.
Effect of indomethacin and aspirin on gastric blood flow and acid secretion.

J. Pharmacol. Exp. Ther. 1977; 203: 646-52.

125. Rainsford, K.D., Willis, C.
Relationship of gastric mucosal damage induced in pigs by anti-inflammatory drugs to their effects on prostaglandin production.
Dig. Dis. Sci. 1982; 27: 624-35.

126. Johnson, L.R., Overholt, B.F.
Release of histamine into gastric venous blood following injury by acetic or salicylic acid.
Gastroenterology 1977; 52: 505-9.

127. Baskin, W., Ivey, K.J., Krause, W.J., Jeffrey, G.E. Gemmell, R.T.
Aspirin-induced ultrastructural changes in human gastric mucosa: correlation with mucosal difference.
Ann. Intern. Med. 1976; 85: 299-303.

128. Cooke, A.R.
The role of the mucosal barrier in drug-induced gastric ulceration and erosions.
Dig. Dis. Sci. 1976; 21: 155-64.

129. Feldman, M., Colturi, T.J.
Effect of indomethacin on gastric acid and bicarbonate secretion in humans.
Gastroenterology 1984; 87: 1339-43.

130. Levine, R.A., Schwartzel, E.H.
Effect of indomethacin on basal and histamine stimulated human gastric acid secretion.
Gut 1984; 25: 718-22.

131. Menguy, R., Masters, Y.F.,
Effects of aspirin on gastric mucous secretion.
Surg. Gynecol. Obstet. 1965; 120: 92-8.

132. Menguy, R., Desbaillets, L.
Role of inhibition of gastric mucous secretion in the phenomenon of gastric mucosal injury by indomethacin.
Am. J. Dig. Dis. 1967; 12: 862-6.

133. Sarosiek, J., Mizuta, K., Slomiany, A., Slomiany, B.
Effect of acetylsalicylic acid on gastric mucin viscosity, permeability to hydrogen ion, and susceptibility to pepsin.
Biochem. Pharmacol. 1986; 35: 4291-5.

134. Rees, W.D.W., Gribbons, L.C., Turnberg, LA.
Effects of non-steroidal anti-inflammatory drugs and prostaglandins on alkali secretion by rabbit gastric fundus in vitro.
Gut 1983; 24: 784-9.

135. Kent, T.H., Cardelli, R.M., Stamler, F.W.
Small intestinal ulcers and intestinal flora in rats given indomethacin.
Am. J. Pathol. 1969; 54: 237-49.

136. Robert, A., Asano, T.
Resistance of germ-free rats to indomethacin-induced intestinal lesions.
Prostaglandins 1977; 14: 333-41.

137. Warren, J.R., Marshall, B.
Unidentified curved bacilli on gastric epithelium in active chronic gastritis.

Lancet 1983; 1: 1273-5.

138. Rathbone, B.J., Wyatt, J.I., Heatley, R.V.
Campylobacter pyloridis. A new factor in peptic ulcer disease?
Gut 1986; 27: 635-41.

139. Marshall, B.J., McGechie, O.B., Rogers, P.A., Glancy, R.J.
Pyloric Campylobacter infection and gastroduodenal disease.
Med. J. Austr. 1985; 142: 439-44.

140. Price, A.B., Levi, J., Dolby, J.M., Dunscombe, P.L., Smith, A., Clark, J., Stephenson, M.
Campylobacter pyloridis in peptic ulcer disease: microbiology, pathology, and scanning electron microscopy.
Gut 1985; 26: 1183-8.

141. Marshall, B.J., Warren, J.R.
Unidentified curve bacilli in the stomach of patients with gastritis and peptic ulceration.
Lancet 1984; 1: 1311-5.

142. McNulty, C.A.M., Watson, D.M.
Spiral bacteria of the gastric antrum.
Lancet 1984; 1: 1068-9.

143. Jones, D.M., Lessells, A.M., Eldridge, J.
Campylobacter-like organisms on the gastric mucosa: culture, histological, and serological studies.
J. Clin. Pathol. 1984; 37: 1002-6.

144. Marshall, B.J., Armstrong, J.A., McGechie, D.B., Glancy, R.J.
Attempt to fulfil Koch's postulates for pyloric Campylobacter.
Med. J. Austr. 1985; 142: 436-9.

145. Ramsey, E.J., Carey, K.V., Paterson, W.L., Jackson, J.J., Murphy, F.K., Read, N.W., Taylor K.B., Trier, J.S., Fordtran J.S. Epidemic gastritis with hypochlorhydria.
Gastroenterology 1979; 76: 1449-57.

146. Wiersinga, W.M., Tytgat, G.N.J.
Clinical recovery owing to target parietal cell failure in a patient with Zollinger-Ellison syndrome.
Gastroenterology 1977; 73: 1413-7.

147. Tytgat, G.N.J., Rauws, E.A.J.
"Significance of Campylobacter pylori", a lecture given at an international symposium covering new advances in peptic ulcer disease, and held in Amsterdam, The Netherlands, 7 March 1987.

148. Blaser, M.J.,
Gastric Campylobacter-like organisms, gastritis, and peptic ulcer disease.
Gastroenterology 1987; 93: 371-83.

149. Whitehead, R., Truelove, S.C., Gear, M.W.
The histological diagnosis of chronic gastritis in fiberoptic gastroscope biopsy specimens.
J. Clin. Pathol. 1972; 25: 1-11.

150. Strickland, R.G., Mackay, I.R.
A reappraisal of the nature and significance of chronic atrophic gastritis.
Am. J. Dig. Dis. 1973; 18: 426-40.

151. Glass, G.B.J., Pitchumoni, C.S.
Atrophic gastritis.
Human Pathol. 1975; 6: 219-50.

152. Dixon, M.F., O'Connor, H.J., Axon, A.T.R., King, R.F.J.G., Johnston, D.
Reflux gastritis: distinct histopathological entity?
J. Clin. Pathol. 1986; 39: 524-30.

153. Laine, L., Marin-Sorensen, M., Weinstein, W.M.
The histology of gastric erosions in patients taking non-steroidal anti-inflammatory drugs (NSAIDs): a prospective study.
Gastroenterology 1988; 94: A247.

154. Correa, P.
Chronic gastritis: a clinico-pathological classification.
Am. J. Gastroenterol. 1988; 83: 504-9.

155. Wyatt, J.I., Rathbone, B.J.
Immune response of the gastric mucosa to Campylobacter pylori.
Scand. J. Gastroenterol. 1988; 23 [Suppl. 142]: 44-9.

156. Filipe, M.I., Potet, F., Bogomoletz, W.V., Dawson, P.A., Fabiani, B., Chauveinc, P., Fenzy, A., Gazzard, B., Goldfain, D., Zeegen, R.
Incomplete sulphomucin-secreting intestinal metaplasia for gastric cancer. Preliminary data from a prospective study from three centres.
Gut 1985; 26: 1319-26.

157. Thomas, J.M.
Campylobacter-like organisms in gastritis.
Lancet 1984; ii: 1217.

158. Rawles, J.W., Paull, G., Yardley, J.H., Hendrix, T.R.,

Rayich, W.J., Walters, L.L., Dick, J.D., Margolis, A.
Gastric Campylobacter-like organisms in a U.S. hospital population.
Gastroenterology 1986; 91: 1599A.

159. Goodwin, C.S., Armstrong, J.A., Marshall, B.J.
Campylobacter pyloridis, gastritis and peptic ulceration.
J. Clin. Pathol. 1986; 39: 353-65.

160. Andersen, L.P., Holck, S., Povlsen, C.O., Elsborg, L., Justesen, T.
Campylobacter pyloridis in peptic ulcer disease.
Scand. J. Gastroenterol. 1987; 22: 219-24.

161. Feng, Y.Y., Wang, Y.
Campylobacter pylori in patients with gastritis, peptic ulcer, and carcinoma of the stomach in Lanzhou, China.
Lancet 1988; i: 1055-6.

162. Rauws, E.A., Langenberg, W., Houthoff, H.J., Zanen, H.C., Tytgat, G.N.J.
Campylobacter pyloridis - associated chronic active antral gastritis. A prospective study of its prevalence and the effect of antibacterial and antiulcer treatment.
Gastroenterology 1988; 94: 33-40.

163. Gear, M.W.L., Truelove, S.C., Whitehead, R.
Gastric ulcer and gastritis.
Gut 1972; 12: 639-45.

164. Greenlaw, E., Sheahan, D.G., DeLuca V., Millder, D., Myerson, D., Myerson, P.
Gastroduodenitis - broader concept of peptic ulcer disease.

Dig. Dis. Sc. 1980; 25: 660-72.

165. Sipponen, P., Seppala, K., Aarynen, M., Helske, T., Kettunen, P.
Chronic gastritis and gastroduodenal ulcer: a case control study on risk of coexisting duodenal or gastric ulcer in patients with gastritis.
Gut 1989; 30: 922-9.

166. O'Connor, H.J., Dixon, M.F., Wyatt, J.I., Axon, A.T.R., Dewar, E.P., Johnston, D.
Campylobacter pylori and peptic ulcer disease.
Lancet 1987; ii: 633-4.

167. Kreuning, J., Bosman, F.T., Kuiper, G., Wal, A.M.vd., Lindeman, J.
Gastric and duodenal mucosa in "healthy" individuals.
An endoscopic and histopathological study of 50 volunteers.
J. Clin. Pathol. 1978; 31: 69-77.

168. Shousha, S., Spiller, R.C., Parkins, R.A.
The endoscopically abnormal duodenum in patients with dyspepsia: biopsy findings in 60 cases.
Histopathology 1983; 7: 23-34.

169. Morrissey, S.M., Ward, P.M., Jayaraj, A.P., Tovey, F.I., Clark, C.G.
Histochemical changes in mucosa in duodenal ulceration.
Gut 1983; 24: 909-13.

170. Wyatt, J.I., Rathbone, B.J., Dixon, M.F., Heatley, R.V.
Campylobacter pyloridis in acid-induced gastric metaplasia in the pathogenesis of duodenitis.
J. Clin. Pathol. 1987; 40: 841-8.

171. Rokkas, T., Pursey, C., Uzoechina, E., Dorrington, L., Simmons, N.A., Filipe, M.I., Sladen, G.E.
Campylobacter pylori and non-ulcer dyspepsia.
Am. J. Gastroenterol. 1987; 82: 1149-52.

172. Rokkas, T., Pursey, C., Uzoechina, E., Dorrington, L., Simmons, N.A., Filipe, M.I., Sladen, G.E.
Non-ulcer dyspepsia and short term De-Nol therapy: placebo controlled trial with particular reference to the role of Campylobacter pylori.
Gut 1988; 29: 1386-91.

173. Peterson, W., Lee, E., Skoghund, M.
The role of Campylobacter pyloridis in epidemic gastritis with hypochlorhydria.
Gastroenterology 1987; 92: 1575 (A).

174. Graham, D.Y., Smith, J.L. Alpert, L.C., Yoshimura, H.H.
Epidemic achlorhydria is not viral but caused by Campylobacter pyloridis.
Gastroenterology 1987; 92: 1412 (A).

175. Lambert, J.F., Borromeo, M., Turner, H., Korman, M.G., Hansky, J.
Colonization of gnotobiotic piglets with Campylobacter pyloridis.
Gastroenterology 1987; 92: 1489 (A).

176. Morris, A., Nicholson, G.
Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH.
Am. J. Gastroenterol. 1987; 82: 192-9.

177. Brady, C.E., Hadfield, T.L., Hyatt, J.R., Utts, S.J.
Acid secretion and serum gastrin levels in individuals with

Campylobacter pylori.

Gastroenterology 1988; 94: 923-7.

178. Levi, S., Haddad, G., Ghosh, P., Beardshall, K., Playford, R., Calam, J.
Campylobacter pylori and duodenal ulcers: the gastrin link.
Lancet 1989; i: 1167-8.

179. Hazell, S.L., Lee, A.
Campylobacter pyloridis, urease, hydrogen ion back diffusion, and gastric ulcers.
Lancet 1986; 2: 15-17.

180. Ferrero, R.L., Hazell, S.L., Lee, A.
The urease enzymes of Campylobacter pylori and a related bacterium.
J. Med. Microbiol. 1988; 27: 33-40.

181. Sarosiek, J., Slomiany, A., Slomiany, B.L.
Evidence for weakening of gastric mucus integrity by Campylobacter pylori.
Scand. J. Gastroenterol. 1988; 23: 585-90.

182. Das, S.S., Karim, Q.N., Easmon, C.S.F.
Opsonophagocytosis of Campylobacter pylori.
J. Med. Microbiol. 1988; 27: 125-30.

183. Taha, A.S., Boothman, P., Holland, P., McKinlay, A.W., Upadhyay, R., Kelly, R.W., Lee, F.D., Russell, R.I.
Duodenal ulcer and Campylobacter pylori.
Lancet 1989; i: 333-4.

184. Taha, A.S., Boothman, P., Holland, P., McKinlay, A.,

Upadhyay, R., Kelly, R.W., Lee, F., Russell, R.I.
Effects of C. pylori on gastric prostaglandins in patients with gastric ulcers and non-ulcer dyspepsia.
Gut 1989; 30: A732.

185. Taha, A.S., Boothman, P., Holland, P., McKinlay, A., Upadhyay, R., Kelly, R.W., Lee, F., Russell, R.I.
Gastric mucosal prostaglandin synthesis in the presence of Campylobacter pylori in patients with gastric ulcers and non-ulcer dyspepsia.
Am. J. Gastroenterol. 1990; 85: 47-50.

186. Goren, A., Fotherby, K.J., Shorthouse, M., Wight, D.G.D., Hunter, J.O.
Campylobacter pylori and acid secretion.
Lancet 1989; ii: 212-3.

187. Taha, A.S., McKinlay, A.W., Upadhyay, R., Kelly, R., Russell, R.I.
Prostaglandins and Campylobacter pylori.
Lancet 1989; ii: 800-801.

188. Leunk, R.D., Johnston, P.T., David, B.C., Kraft, W.G. Morgan, D.R
Cytotoxic activity in broth-culture filtrates of Campylobacter pylori.
J. Med. Microbiol. 1988; 26: 93-9.

189. Cave, D.R., Vargas, M.
Effect of Campylobacter pylori protein on acid secretion by parietal cells.
Lancet 1989; ii: 187-9.

190. Gray, S.F., Wyatt, J.I., Rathbone, B.J.
Simplified techniques for identifying Campylobacter pyloridis.
J. Clin. Pathol. 1986; 39: 1279.

191. McNulty, C.A.M., Wise, R.
Rapid diagnosis of Campylobacter pyloridis gastritis.
Lancet 1986; i: 387.

192. Arvind, A.S., Cook, R.S., Tabaqchali, S., Farthing, M.J.G.
One-minute endoscopy room test for Campylobacter pylori.
Lancet 1988; i: 704.

193. Engstrand, L., Pahlson, C., Gustavsson, S., Schwan, A.
Mono clonal antibodies for rapid identification of
Campylobacter pyloridis.
Lancet 1986; ii: 1402-3.

194. Graham, D.Y., Klein, P.D., Evans, D.J.Jr., Evans, D.G.,
Alpert, L.C., Opekun, A.R., Boutton, T.W.
Campylobacter pylori detected non-invasively by the
^{13}C-urea breath test.
Lancet 1987; i: 1174-7.

195. Bell, G.D., Weil, J., Harrison, G.
C-urea breath analysis, a non-invasive test for
Campylobacter pylori in the stomach.
Lancet 1987; i: 1367.

196. Jones, D.M., Eldridge, J., Fox, A.J., Sethi, P., Whorwell, P.J.
Antibody to the gastric Campylobacter-like organism
(Campylobacter pyloridis) - clinical correlations and
distribution in the normal population.
J. Med. Microbiol. 1986; 22: 57-62.

197. Burnett, R.A., Brown, J.L., Findlay, J.
Cresyl fast violet staining method for Campylobacter-like organisms.
J. Clin. Pathol. 1987; 40: 353.

198. Wyatt, J.I., Dixon, M.F.
Chronic gastritis - a pathogenetic approach.
J. Pathol. 1988; 154: 113-24.

199. Misiewicz, J.J.
What is new in the epidemiology and pathogenesis of peptic ulcer?
Aliment. Pharmacol. Therap. 1987; 1: 510S-17S.

200. Farrant, J., Walter, C.A., Lee, H., Morris, G.J. Clarke, K.J. eds., Low temperature biological microscopy and microanalysis.
Royal Microscopical Society, London, 1978, p.17.

201. Polge, C., Smith, A.U., Parkes, A.S.
Revival of the spermatozoa after vitrification and dehydration at low temperatures.
Nature 1949; 164: 666.

202. Schill, W.B., Wolff, H.H.
Ultrastructure of human sperm acrosome and determination of acrosin activity under conditions of semen preservation.
Int. J. Fertid. 1974; 19(4): 217-23.

203. Ahlquist, D.A., Madson, T.H., Romero, J.C., Dozis, R.R., Malegalada, J.R.
Factors influencing metabolism of arachidonic acid in guinea pig gastric mucosa.
Am. J. Physiol. 1983; 244: G131-G137.

204. Burton, J.L.
Dietary fatty acids and inflammatory skin disease.
Lancet 1989; i: 27-31.

205. Lee, T.H., Arm, J.P.
Benefits from oily fish.
Br. Med. J. 1988; 297: 1421-2.

206. Belch, J.J.F., Ansell, D., Madhok, R., O'Dowd, A. Sturrock R.D..
Effects of altering dietary essential fatty acids on requirements of non-steroidal anti inflammatory drugs in patients with rheumatoid arthritis: a double-blind placebo controlled study.
Ann. Rheum. Dis. 1988; 47: 96-104.

207. Manthrope, R., Petersen, S.H., Prause, J.Y.
Primary Sjogren's syndrome treated with Efamol/Efavit.
Rheumatol. Int. 1984; 4: 165-7.

208. Belch, J.J.F., Shaw, B., O'Dowd, A., Saniabadi, A., Lieberman, P. Sturrock, R.D., Forbes, C.D.
Evening primrose oil (Efamol) in the treatment of Raynaud's phenomenon: a double blind study.
Thrombosis and Haemostasis 1985; 54: 490-4.

209. Jamal, G.A., Carmichael, H., Weir, A.I.
Gamma-linolenic acid in diabetic neuropathy.
Lancet 1986; 1: 1098.

210. Wright, S., Burton, J.L.
Oral evening-primrose-seed oil improves atopic eczema.
Lancet 1982; 2: 1120-2.

211. Robert, A., Nezamis, J.E., Lancaster, C., Hanchar, A.J.

Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury.

Gastroenterology 1979; 77: 433-43.

212. Lauritsen, K., Laursen, L.S., Havelund, T., Bytzer, P., Svendsen, L.B., Rask-Madsen, J.
Enprostil and ranitidine in duodenal ulcer healing: double-blind comparative trial.

Br. Med. J. 1986; 292: 864-6.

213. Jacob, G.B., Hart, K.K., Mullane, J.F., Lutins, S., Lee, T.Y.
Placebo controlled study of etodolac and aspirin in the treatment of rheumatoid arthritis.

Curr. Ther. Res. 1983; 33: 703-13.

214. Vetter, G., Placchi, M., Joubert, L.
Comparative efficacy of etodolac and placebo in rheumatoid arthritic patients.

Int. J. Clin. Pharmacol. Ther. Toxicol. 1982; 20: 240-45.

215. Jaffe, G.
A double-blind, multicentre comparison of naproxen and indomethacin in acute musculo-skeletal disorders.

Curr. Med. Res. Opin. 1976; 4: 373-80.

216. Waltham-Weeks, C.D.
Etodolac versus naproxen in rheumatoid arthritis: a double-blind crossover study.

Curr. Med. Res. Opin. 1987; 10: 540-47.

217. Lanza, F., Rack, Mary F., Lynn, M., Wolf, J., Sanda, Maria.
An endoscopic comparison of the effect of etodolac, indomethacin,

ibuprofen, naproxen and placebo on the gastrointestinal mucosa.
J. Rheumatol. 1987; 14: 338-41.

218. Ritchie, D.M., Boyle,J.A., McInnes, J.M. et al.
Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis.
Quart. J. Med. 1968; 37: 393-406.

219. Lanza, F., Panagides, J., Salom Ira.
Etodolac compared with aspirin: an endoscopic study of the gastrointestinal tract of normal volunteers.
J. Rheumatol. 1986; 13: 299-303.

220. Arnold, J.D., Mullane, J.F., Hayden, D.M., March, L., Mart, K., Perdomo, C.A., Fencik, M., Berger, A.E.
Etodolac, aspirin, and gastrointestinal bleeding.
Clin. Pharmacol. Ther. 1984; 35: 716-21.

221. Jallad, N.S., Sanda, Maria, Salom, Ira, Perdomo, C., Garg, D.C., Mullane, J.F., Weidller, D.J.
Gastrointestinal blood loss in arthritic patients receiving chronic dosing with etodolac and piroxicam.
Am. J. Med. Sci. 1986; 292 (5): 272-6.

222. Salom, Ira, Jacob, Ginette, Jallad, N., Perdomo, C., Mullane, J.F., Weidler, D.J.
Gastrointestinal microbleeding associated with use of etodolac, ibuprofen, indomethacin and naproxen in normal males.
J. Clin. Pharmacol. 1984; 24: 240-6.

223. Lee, D., Dvornik, D.
Etodolac: effect on prostaglandin concentrations in gastric mucosa of rats.

Life Sciences, 1985; 36: 1157-62.

224. Mellem, H., Stave, R., Myren, J., Osnes, M., Hanssen, L.E., Mosvold, J., Hebnes, K.
Symptoms in patients with peptic ulcer and haematemesis and/or melaena related to the use of non-steroidal anti-inflammatory drugs.
Scand. J. Gastroenterol. 1985; 20: 1246-8.

225. Skander, M.P., Ryan, F.P.
Non-steroidal anti-inflammatory drugs and pain free peptic ulceration in the elderly.
Br. Med. J. 1988; 297: 833-4.

226. Duggan, J.M., Dobson, Annette J., Johnson, H., Fahey, P.
Peptic ulcer and non-steroidal anti-inflammatory agents.
Gut 1986; 27: 929-33.

227. Farah, D., Sturrock, R.D., Russell, R.I.
Peptic ulceration in rheumatoid arthritis.
Ann. Rheum. Dis. 1988; 47: 478-80.

228. Domschke, S., Domschke, W.
Gastroduodenal damage due to drugs, alcohol and smoking.
Clin. Gastroenterol. 1984; 13: 405-37.

229. Main, I.H., Whittle, B.J.
Investigation of the vasodilator and antisecretory role of prostaglandins in the rat gastric mucosa by use of non-steroidal anti-inflammatory drugs.
Br. J. Pharmacol. 1975; 53(2): 217-24.

230. Whittle, B.J.R., Higgs, G.A., Eakins, K.E., Moncada, S.,

Vane, J.R.
Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa.
Nature 1980; 284: 271-3.

231. Ligumsky, M., Golanska, E.M. Hansen, D.G., Kauffman, G.L.
Aspirin can inhibit gastric mucosal oxygenase without causing lesions in rat.
Gastroenterology 1983; 84: 756-61.

232. Whittle, B.J.R.
Temporal relationship between cyclo-oxygenase inhibition as measured by prostaglandin biosynthesis and the gastrointestinal damage induced by indomethacin in the rat.
Gastroenterology 1981; 80: 94-8.

233. Konturek, S.J. Obutlowicz, W. Kwiecien, N., Oleksy, J.
Generation of prostaglandins in gastric mucosa of patients with peptic ulcer disease: effect of non-steroidal anti-inflammatory compounds.
Scand. J. Gastroenterol. 1984; 19(suppl.101): 75-7.

234. Tavares, I.A., Collins, P.O., Bennett, A.
Inhibition of prostanoid synthesis by human gastric mucosa.
Aliment. Pharmacol. Ther. 1987; 1: 617-25.

235. Smith, B.M., Skillman, J.J., Edwards, G., Silen W.
Permeability of the human gastric mucosa. Alteration by acetyl-salicylic acid and ethanol.
New Eng. J. Med. 1971; 285: 716-21.

236. Stern, A.I., Hogan, D.L., Isenberg, J.I.
A new method for quantitation of ion fluxes across in vivo

human gastric mucosa: effect of aspirin, ethanol and hyperosmolar solutions.

Gastroenterology 1984; 86: 60-70.

237. Ashley, S.W., Sonnenschein, L.A., Cheung, L.Y.
Focal gastric mucosa blood flow at the site of aspirin-induced ulceration.

Amer. J. Surg. 1985; 149: 53-9.

238. Rosam, A.C., Wallace, J.L. Whittle, B.J.R.
Potent ulcerogenic actions of platelet-activating factor on the stomach.

Nature 1986; 319: 54-6.

239. Turner, N.C., Martin, G.P., Marriott, C.
The influence of native procine gastric mucus gel on hydrogen ion diffusion: the effect of potentially ulcerogenic agents.

J. Pharm. Pharmacol. 1985; 37: 776-80.

240. Whittle, B.R., Kauffman, G.L., Moncada, S.
Vasoconstriction with thromboxane A_2 induces ulceration of the gastric mucosa.

Nature 1981; 292: 472-4.

241. Walt, R.P., Kemp, R.T., Filipowicz, B., Davies, J.G., Bhaskar, N.K., Hawkey, C.J.
Gastric mucosal protection with selective inhibition of thromboxane synthesis.

Gut 1987; 28: 541-4.

242. Robert, A.
Current history of cytoprotection. International workshop on protective actions of prostaglandins on the gastrointestinal

mucosa.

Prostaglandins 1981; 21(suppl): 89-96.

243. Lanza, F.L., Aspinall, R.L., Swabb, E.A., Davis, R.E., Rack, M.F., Rubin, A.
Double-blind, placebo-controlled endoscopic comparison of the mucosal protective effects of misoprostol versus Cimetidine on tolmetin-induced mucosal injury to the stomach and duodenum.
Gastroenterology 1988; 95: 289-94.

244. Reinhart, W.H., Muller, O., Halter, F.
Influence of long-term 16-16-dimethyl prostaglandin E2 treatment on the rat gastrointestinal mucosa.
Gastroenterology 1983; 85: 1003-10.

245. Marshall, B.J.
Campylobacter pyloridis and gastritis.
J. Infect. Dis. 1986; 153: 650-7.

246. Buck, G.E., Gourley, W.K., Lee, W. K., Subramanyam, K., Latimer, J.M., DiNuzzo, A.R.
Relation of Campylobacter pyloridis to gastritis and peptic ulcer.
J. Infect. Dis. 1986; 153: 664-9.

247. Morgan, D., Kraft, I.W., Bender, M., Pearson, A.
Nitrofurans in the treatment of gastritis associated with Campylobacter pylori.
Gastroenterology 1988; 95: 1178-84.

248. Konturek, S.J., Bilski, J., Kwiecien, N., Obtulowicz, W., Kopp, B., Oleksy, J.
De-Nol stimulates gastric and duodenal alkaline secretion

through prostaglandin dependent mechanism.

Gut 1987; 28: 1557-63.

249. Konturek, S.J. Kwiecien, N., Obtulowicz, W., Hebzda, Z., Oleksy, J.
Effects of colloidal bismuth subcitrate on aspirin-induced gastric microbleeding, DNA loss, and prostaglandin formation in humans.

Scand. J. Gastroenterol. 1988; 23: 861-6.

250. Gemmel, C.G., Young, A., McKinlay, A.W., Russell, R.I.
Interaction of C. pylori cytotoxin with polymorpho-nuclear leukocytes in vitro.

J. Med. Microbiol. (In press).

251. Skarnes, R.C., Harper, M.J.K.
Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F.

Prostaglandins 1972; 1: 191-203.

252. Peskar, B.M., Weiler, H., Kroner, E.E., Peskar, B.A.
Release of prostaglandins by small intestinal tissue of man and rat in vitro and the effect of endotoxin in the rat in vivo.

Prostaglandins 1981; (Suppl.21): 9-14.

253. Graham, D.Y., Klein, P.D., Opekun, A.R., Boutton, T.W.
Effect of age on the frequency of active Campylobacter pylori infection diagnosed by the [13C] urea breath test in normal subjects and patients with pepetic ulcer disease.

J. Infect. Dis. 1988; 157:777-80.

254. Berridge, M.J., Irvine, R.F.
Inositol triphosphate, a novel second messenger in cellular signal transduction.
Nature 1984: 312: 315-21.

255. Nishizuka, Y.
The role of protein kinase C in cell surface signal transduction and tumour promotion.
Nature 1984; 308: 693-8.

256. Rees, W.D.W.M., Turnbeg, L.A.
Biochemical aspects of gastric secretion.
Clin. Gastroenterol. 1981; 10: 521-54.

257. Holian, O., Ruiz, C., Bombeck, C.T., Nyhus, L.M.
Comparison of c.AMP system in parietal cells from rat and guinea pig.
Scand. J. Gastroenterol. 1983; 16: 819-24.

258. Mardh, S., Song, Y.H., Wallmark, B.
Effects of some antisecretory drugs on acid production, intracellular free calcium, and cyclic AMP production in isolated pig parietal cells.
Scand. J. Gastroenterol. 1988; 23:977-82.

259. Varis, K., Raij, K., Harkonen, M., Miethinen, T.A.
Cyclic AMP and gastric secretion in man. An in vivo study in healthy volunteers, duodenal ulcer patients, and pernicious anaemia patients.
Scand. J. Gastroenterol. 1988; 23: 1025-34.

260. Wollin, A., Soll, A.H., Samloff, I.M.
Actions of histamine, secretin and PGE_2 on cyclic AMP

production by isolated canine fundic mucosal cells.
Am. J. Physiol. 1979; 65: E437-43.

261. Chen, M.C.Y., Amirian, D.A., Toomey, M., Sanders, M.J., Soll, A.H.
Prostanoid inhibition of canine parietal cells: mediation by the inhibitory guanosine triphosphate-binding protein of adenylate cyclase.
Gastroenterology 1988; 94: 1121-9.

262. Soll, A.H., Toomey, M.
Beta-adrenergic and prostanoid inhibition of canine fundic mucosal mast cells.
Am. J. Physiol. 1989; 256: G727-32.

263. Tsai, B.S., Kessler, L.K., Butchko, G.M., Bauer, R.F.
Effect of misoprostol on histamine-stimulated acid secretion and cyclic AMP formation in isolated canine parietal cells.
Dig. Dis. Sci. 1987; 32: 1010-6.

264. Zucker, K.A., Zdon, M.J., Adrian, T.E., Ballantyne, G.H., Modlin, I.M.
Prostaglandin inhibition of acid is c.AMP dependent.
J. Surg. Res. 1987; 42: 513-20.

265. O'Reilly, D.St.J., Fraser, W.D., Penny, M.D., Logue, F.C., Cowan, R.A., Williams, B.C., Walters, G.
Arginine infusion blocks the action of parathyroid hormone but not arginine vasopressin on the renal tubule of man.
J. Endocrin. 1986; 111: 501-6.

266. Fimmel, C.J., Berger, M.M., Blum, A.L.
Dissociated response of acid and pepsin secretion to omeprazole in an in vitro perfused mouse stomach.
Am. J. Physiol. 1984; 247: G240-G247.

267. Hersey, S.J., Miller, M., May, D., Norris, S.H.
Lack of interaction between acid and pepsinogen secretion in isolated gastric glands.
Am. J. Physiol. 1983; 245: G775-G779.

268. Raufman, J.P., Kasbekar, D.K., Jensen, R.T., Gardner, J.D.
Potentiation of pepsinogen secretioin from dispersed glands from rat stomach.
Am. J. Physiol. 1983; 245: G525-G530.

269. Flemstrom, G., Garner, A.
Gastroduodenal HCO_3 transport: characteristics and proposed role in acidity regulation and mucosal protection.
Am. J. Physiol. 1982; 242: G183-G193.

270. La Mont, J.T., Ventola, A.S.
Stimulation of colonic glycoprotein synthesis by dibutyryl cyclic AMP and theophylline.
Gastroenterology 1977; 72: 82-86.

271. Mailman, D., Pawlik, W., Shepherd, A.P., Tague, L.L., Jacobson, E.D.
Cyclic nucleotide metabolism and vasodilation in canine mesenteric artery.
Am. J. Physiol. 1977; 232: H191-H196.

272. Hill, S.J.

Histamine receptors branch out.

Nature 1987; 327: 104-5.

273. Gill, D.M.

Bacterial toxins. In: CRC Handbook of Microbiology IX.

1985.

274. Beubler, E., Killar, G., Saria, A., Bukhave, K., Rask-Madsen, J.

Involvement of 5-Hydroxytryptamine, prostaglandin E_2,

and cyclic adenosine monophosphate in Cholera toxin-

induced fluid secretion in the small intestine of the

rat in vivo.

Gastroenterology 1989; 96: 368-76.

275. Terano, A., Ivey, K.J., Stachura, J., Sekhon, S., Hosojima, H.,

McKenzie, W.N., Krause, W.J., Wyche, J.H.

Cell culture of rat gastric fundic mucosa.

Gastroenterology 1982; 83: 1280-91.

276. Hiraishi, H., Terano, A., Ota, S., Ivey, K.J., Sugimoto, T.

Regulation of prostaglandin production in cultured

gastric mucosal cells.

Prostaglandins 1989; 38: 65-78.

277. Smith W.L.

The eicosanoids and their biochemical mechanisms of

action.

Biochem. J. 1989; 259: 315-24.

APPENDIX 1.

Publications Arising Out of This Thesis.

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Publications Arising Out of This Work.

Original Articles.

1. Taha A.S., McLaughlin S., Sturrock R.D., Russell R.I.
Evaluation of the efficacy and comparative effect on gastric and duodenal mucosa of etodolac and naproxen in patients with rheumatoid arthritis using endoscopy.
British Journal of Rheumatology 1989; 28: 329-32.

2. Taha A.S., Boothman P., Holland P., McKinlay A., Upadhyay R., Kelly, R.W, Lee F., Russell R.I.
Gastric mucosal prostaglandin synthesis in the presence of Campylobacter pylori in patients with gastric ulcers and non-ulcer dyspepsia.
Am. J. Gastroenterol. 1990; 85: 47-50.

3. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell R.I.
The effect on gastric and duodenal mucosal prostaglandins of repeated intake of therapeutic doses of naproxen and etodolac in rheumatoid arthritis.
Ann. Rheum. Dis. 1990; 49: 354-8.

4. Taha A.S., Tulloch I., Sutherland C., Anderson J., Kelly R.W., Russell R.I.
The effects of diets containing fish oil, evening primrose oil

and olive oil on prostaglandin synthesis and histology of rat stomach.

European Journal of Gastroenterology and Hepatology 1990; 2:52-56.

5. Taha, A.S., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I. The interaction between Helicobacter pylori culture filtrate and indomethacin: effects on the integrity of the human gastric antral mucosa and its prostaglandin E_2 production in vitro.

Aliment. Pharmacol. Therap. (In press.)

6. Taha, A.S., Fraser, W.D., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
Inhibition of gastric cyclic AMP production by Helicobacter pylori protein. Possible involvement of mucosal prostaglandin E_2.

Gut (submitted).

Abstracts.

1. Taha A.S., Boothman P., McLaughlin S., McKinlay A., Upadhyay R., Kelly R.W., Russell R.I.
The effect of Campylobacter pylori (C.P.) on gastric prostaglandins in patients with gastric ulcers and non-ulcer dyspepsia.

Gut 1989; 30: A732.

2. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W.,

Sturrock R.D., Russell, R.I.
The effect of repeated therapeutic doses of naproxen and etodolac on gastric and duodenal mucosal prostaglandins (PGs) in rheumatoid arthritis.
Gut 1989; 30:A751.

3. Taha A.S., Tulloch I., Stewart J.P., Kelly R.W., Russell R.I.
The effect of fish oil, primrose oil and olive oil diets on gastric mucosal prostaglandin E_2 synthesis in rats.
Gastroenterology 1989; 96: A500.

4. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell, R.I.
The effects of repeated therapeutic doses of naproxen and etodolac on gastric and duodenal prostaglandins in rheumatoid arthritis (RA).
Gastroenterology 1989; 96: A499.

5. Taha, A.S., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
The effect of Helicobacter pylori (HP) "toxin" on human gastric mucosa and its interaction with indomethacin.
Gastroenterology 1990; 98: A133.

6. Taha, A.S., Fraser, W.D., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
Inhibition of gastric mucosal cyclic AMP by Helicobacter pylori protein.
Scottish Medical Journal (In press).

Letters.

1. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell R.I.
Prevention of NSAID-induced gastric ulcer with prostaglandin analogues.
Lancet 1989; 1: 52.

2. Upadhyay R., Taha A.S., Sturrock R.D., Russell R.I.
Misoprostol and ulcer prophylaxis.
Lancet 1989; 1: 212-13.

3. Taha A.S., Boothman P., Holland P., McKinlay A.W., Upadhyay R., Kelly R.W., Lee F.D., Russell R.I.
Duodenal ulcer and Campylobacter pylori.
Lancet 1989; 1: 333-34.

4. Taha, A.S., McKinlay, A.W., Upadhyay, R., Kelly, R.W., Russell, R.I.
Prostaglandins and Campylobacter pylori.
Lancet 1989; ii: 800-801.

APPENDIX 2.

Presentations to Learned Societies.

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Presentations to Learned Societies..

1. Taha A.S., Kelly R.W., Anderson J., Holland P., McLaughlin S., Russell R.I.
   Microstructural and functional behaviour of fresh versus frozen gastro-duodenal biopsies.
   The Caledonian Society of Gastroenterology
   Falkirk, June 1988.

2. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell R.I.
   The effect on gastric and duodenal mucosal prostaglandin E_2 of repeated intake of therapeutic doses of naproxen and etodolac in rheumatoid arthritis.
   The Caledonian Society of Gastroenterology
   Glasgow, November 1988.

3. Taha A.S., Boothman P., Holland P., McKinlay A., Upadhyay R., Kelly R.W., Lee F., Russell R.I.
   The effect of Campylobacter pylori on gastric prostaglandins in gastric ulcers and non-ulcer dyspepsia.
   The Caledonian Society of Gastroenterology
   Edinburgh, February 1989.

4. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell R.I.
The effects of repeated therapeutic doses of naproxen and etodolac on gastric and duodenal mucosal prostaglandins (PGs) in rheumatoid arthritis (RA).
The British Society of Gastroenterology.
Bradford, April 1989.

5. Taha A.S., Boothman P., Holland P., McKinlay A., Upadhyay R., Kelly R.W., Lee F., Russell R.I.
The effect of Campylobacter pylori (C.P.) on gastric prostaglandins in patients with gastric ulcers and non-ulcer dyspepsia.
The British Society of Gastroenterology.
Bradford, April 1989.

6. Taha A.S., McLaughlin S., Holland P.J., Kelly R.W., Sturrock R.D., Russell R.I.
The effects of repeated therapeutic doses of naproxen and etodolac on gastric and duodenal prostaglandins in rheumatoid arthritis (RA).
The American Gastroenterology Association,
Washington D.C., May 1989.

7. Taha, A.S., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
The effect of Helicobacter pylori (HP) "toxin" on human gastric mucosa and its interaction with indomethacin.
The Caledonian Society of Gastroenterology,
Glasogw, 1990.

8. Taha, A.S., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
The effect of Helicobacter pylori toxin on human gastric mucosa and its interaction with indomethacin.
The American Gastroenterology Association.
San Antonio, Texas, 1990.

9. Taha, A.S., Fraser, W.D., Kelly, R.W., Gemmell, C.G., Lee, F.D., Russell, R.I.
Inhibition of gastric mucosal cyclic AMP by Helicobacter pylori protein.
The Scottish Society for Experimental Medicine.
Edinburgh, 1990.