A STUDY OF

MUCOSAL RESISTANCE

IN

GASTRIC ULCERATION

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THESIS

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BY

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CHAPTER I

INTRODUCTION

The magnitude of peptic ulceration as a social, economic and community problem has recently been stressed by Blumenthal (1968), who estimated that in 1963 in the United States of America the annual cost in terms of loss in earnings from peptic ulcer was $463 million. Peptic ulceration was the twelfth most common disease responsible for absenteeism from work, and ranked fifteenth in the causes of death for 1965. Furthermore, Blumenthal demonstrated that the prevalence of this disease increased from 14.4 per thousand population per year in 1957-1959 to 19 per thousand per year in 1963-1965. The economic loss from this disease is thus likely to increase.

Peptic ulcer disease was first described by Cruveilhier in 1829 and in 1970, despite manifold theories about the aetiology of this disease, its cause remains unknown. It is not surprising therefore, with therapy directed not at the cause but at manifestations of the disease, that overall the treatment of peptic ulceration is unsatisfactory. Indeed, there is no definitive proof that medical treatment in the long term has a favourable influence on the natural history of the disease, and a high incidence of post-operative problems such as malabsorption, various post-prandial syndromes and recurrent ulceration frustrates the surgical treatment of peptic ulceration. It is apparent that the pathogenesis of the disease must be elucidated if any major advance in the medical treatment of peptic ulceration is to be made.

Chronic peptic ulceration results from an imbalance between aggressive and defensive factors acting on the gastrointestinal mucosa, and at the present time many differences in occurrence and behaviour indicate that gastric and duodenal ulcers should be considered independent diseases (Figure I). Duodenal ulceration is associated with increased acid and pepsin secretion, or increase in aggression. The lack of any demonstrable increase in aggressive factors in primary chronic gastric ulceration has led to the assumption that the ulcer results from diminished mucosal resistance.

Mucosal resistance is provided by two major components - mucus produced by and covering the epithelial cells in the stomach, and the integrity of the epithelial cell layer or mucosa itself. There is no proof that mucus production is altered in patients with primary chronic gastric ulcers, and diminished mucosal resistance resulting from impaired mucus production is unlikely to be an important cause of ulcer formation. However a high incidence of extensive inflammation in the gastric mucosa of patients with chronic gastric ulceration has been widely reported, and this suggests that an abnormality of the mucosa may be implicated in the mechanism of gastric ulcer formation. Histological abnormalities do not per se imply functional abnormality, and investigation of the functional integrity of the gastric mucosa in this disease is therefore necessary.

To date the investigation of the functional capacity of the gastric mucosa in patients with chronic gastric ulceration has been hindered by a lack of suitable techniques. Considerable knowledge of cellular metabolism has been gained over the past two decades by the introduction of radioisotopic techniques into medical research, and indeed the recognition of the rapid proliferation rate of gastrointestinal tissue is in large part attributable to such techniques. Unfortunately many radio-isotopes are potentially hazardous and their application to the study of human subjects is consequently limited by ethical and moral principles. To date therefore, radioisotopic techniques have been largely confined to the investigation of cellular metabolism in animals. Such knowledge from an animal model may not necessarily be validly extrapolated to man (McDonald, Trier and Everett, 1964). Techniques not involving the administration of radioactive substances must be applied to the study of biochemical function of gastrointestinal mucosa if knowledge of the functional integrity or resistance of the gastric mucosa in patients with chronic gastric ulcer disease is to be advanced.

The purpose of the study described in this thesis was to investigate mucosal resistance at a cellular level, particularly in relation to chronic gastric ulceration. The fact that gastrointestinal tissue was known to have a high rate of cellular proliferation suggested that study of nucleic acid

metabolism with its relationship to cell division and protein synthesis might be a rewarding area for investigation. A possible relationship between the activity of some of the enzymes involved in the synthesis of the purine ribonucleotide precursors of nucleic acid, and the rate of nucleic acid metabolism had been previously reported (Murray and Nicholls, 1968). It was considered that investigation of these enzymes in the gastric mucosa of patients with chronic gastric ulceration might reveal a functional abnormality if one were present.

The selection of an animal model for assessment of the purine nucleotide biosynthetic enzymes was difficult because of the low incidence of chronic gastric ulceration in laboratory animals. However, acute gastric erosions or ulceration have been consistently produced by stress restraint, and in this group Imondi, Balis and Lipkin (1968) have described abnormalities of nucleic acid metabolism. Although it was recognised that the acute gastric mucosal lesions in animals were not directly comparable to chronic gastric ulceration in man, a similar model was selected to assess whether the level of activity of selected purine nucleotide biosynthetic enzymes reflected the rate of nucleic acid synthesis.

CHAPTER II

LITERATURE REVIEW

1. *PEPTIC ULCER - THE DIFFERENCES BETWEEN GASTRIC AND DUODENAL ULCER*

Peptic ulcer is a generic term used to describe mucosal lesions occurring in the region of the acid-pepsin secreting mucosa of the gastrointestinal tract, and as such includes gastric and duodenal ulceration. In 1947, Hollander was unable to support the contention that gastric and duodenal ulcer were different diseases. However, a number of differences between these two anatomically distinct ulcers now suggests that duodenal and gastric ulceration are two independent diseases (Wilson, 1962; Shay and Sun, 1964; Frankel and Kark, 1965).

Vesely, Kubickova, Dvorakova and Zvolankova (1968) from a study of 1160 patients in Prague, concluded that peptic ulcers may be divided into duodenal, primary gastric, and combined types, and studied the differing characteristics of each group. Johnson (1957) further subdivided gastric ulcers into three types:

(a) prepyloric, essentially the same as duodenal ulcer;
(b) gastric ulcer associated with pyloric obstruction due to duodenal ulceration; in this group the duodenal ulcer always appears before the gastric lesion (Johnson, 1957; Vesely et alii, 1968); and
(c) primary gastric ulcer associated with a deficiency of mucus and acid secretion, and lying above the angulus of the stomach.

It is probable that a pyloric ulcer may be either duodenal or gastric in type, for Anderson and Grossman (1965) demonstrated that the line of transition of the gastric to duodenal mucous membrane may be at any point in the pyloric canal.

Vesely and co-workers (1968) in support of their classification of peptic ulceration demonstrated a number of differences between the groups. Duodenal lesions appeared at an earlier age than gastric. The average age at time of appearance of duodenal lesions was 33.4 years, while gastric ulcers appeared at an average age of 44.58 years. This finding supported an earlier study (Doll, Jones and Buckatzsch, 1951) that demonstrated duodenal ulcers appeared on average ten years before gastric ulcers. These workers also found that the gastric response to histamine stimulation differed. Hyperacidity prevailed in duodenal ulcer patients, while hypoacidity was common in the combined ulcer and primary ulcer group. The anatomic localization of the ulcer in the gastric ulcer group was important, ulcers below the angulus of the stomach being associated with hyperacidity, while with ulcers above the angulus a low acid response to histamine stimulation was more common.

The importance of differentiating between duodenal type and gastric type ulceration is illustrated by an analysis of blood group and blood group substance secretion in peptic ulcer patients. The first report of an association between blood

group status and gastrointestinal disease was that of Aird and Bentall (1953) who found an association between blood group A and gastric carcinoma. A number of studies have subsequently demonstrated an association between blood group O and duodenal ulcer (Aird, Bentall, Mehigan and Roberts, 1954; Clark, Evans McConnell and Sheppard, 1959; Doll, Swynnerton and Newell, 1960; Doll, Drane and Newell, 1961). Vesely et alii (1968) as well as finding an increased frequency of blood group O in their duodenal and combined ulcer groups, also demonstrated an increased frequency of non-secretors of soluble blood group substances in those patients with duodenal ulcer. The association between blood group O and duodenal ulceration has found general acceptance, but the association between blood group and gastric ulcer is less clear. Aird et alii (1954) found an association between blood group O and gastric ulcer; however Billington (1956) and Clarke, Cowan, Edwards, Howel-Evans, McConnell, Woodrow and Sheppard (1955) did not support this observation. More recently an association between primary gastric ulcer and blood group A has been described (Johnson, Love, Rogers and Wyatt, 1964). These workers have shown that patients with a gastric ulcer above the angulus have a higher than expected incidence of blood group A, while patients with more distal pyloric ulceration have a higher incidence of group O.

Lehrs (1930) demonstrated that a proportion of humans secrete blood group antigens in their saliva and gastric juice. In a

wide population survey in London, Grubb and Morgan (1949) found that the proportion of such secretors approached 80 per cent. The non-secretion of the blood group antigens is more common in duodenal ulcer patients than in the non-ulcerated population (Clarke et alii, 1959; Vesely et alii, 1968). The secretor status of an individual is an hereditary characteristic independent of blood group inheritance (Caldwell and Pigman, 1965). No significant association between secretor status and gastric ulceration has been demonstrated (Vesely et alii, 1968).

The differences in blood group and secretor status of patients with peptic ulcers favour the concept that gastric ulcer and duodenal ulcer are different diseases. As blood group and secretory characteristics are inherited, the possibility that heredity is a significant factor in peptic ulcer genesis is evident. Doll and Buch (1950) found that the incidence of peptic ulceration in the siblings of ulcer patients was more than double that found in a control population. The observation that relatives of patients with gastric ulceration tend to develop gastric ulcers, while duodenal ulcer relatives develop duodenal lesions, led Doll and Kellock (1951) to postulate that independent hereditary factors may operate in the pathogenesis of gastric and duodenal ulcer. Weiner (1967) however was sceptical about the reported relationship between blood group O and duodenal ulcer, and maintained that the relationship was an

artefact based on faulty statistical methods caused by pooling biased data without proper consideration of the obvious heterogeneity of the series used. However his scepticism is shared by few workers in this field.

A recent observation of the relationship between blood group and secretor status and gastric acid response to histamine stimulation (Jori, Mazzacca, Balestrieri, Donnorso, Gallo and Mausi, 1969) reinforces the possible difference in the pathogenesis of gastric and duodenal ulcer. Jori and co-workers (1969) found that acid secretory response to histamine was greater in non-ulcerated patients of blood group O than secretors of group O, and found no difference in response between secretors and non-secretors with blood groups A, B or AB. This observation is of particular interest when considered with the finding of Fodor, Vestea, Urcan, Popescu, Sulica, Iencica, Goia and Ilea (1968) that non-ulcerated members of duodenal ulcer families showed a significantly greater acid response to histamine stimulation when compared to a control non-ulcerated group.

The augmented histamine stimulation test (Kay, 1953) does appear to satisfactorily differentiate between gastric and duodenal lesions (Table I). Duodenal ulcer is usually associated with a greater than normal response to histamine stimulation (Lambert and Martin, 1966; Brewer and Kirsner, 1967; Fodor et alii, 1968), while primary gastric ulcer is usually associated

TABLE 1

Maximal acid response (mEq. HCl per hour) in 33 patients with gastric ulceration. The patients have been subdivided into groups according to the classification of Johnson (1957). Data from Marks and Shay (1959).

PREPYLORIC (10)	25·1
COMBINED (10)	24·1
PRIMARY (13)	10·2
TOTAL (33)	18·9

with a lower than normal response (Vesely et alii, 1968). However, Grossman, Kirsner and Gillespie (1963) disputed the discriminatory value of the test, and emphasized that the range of acid secretion was so great that on the basis of these values alone more than half the patients with duodenal ulcer could not be distinguished from the normal population. In fact Grossman subsequently determined (Wormsley and Grossman, 1965) that only 17 per cent of patients with duodenal ulcers secreted more acid than normal after histalog stimulation. One problem that cannot be avoided is the inclusion in the control group of subjects with a duodenal ulcer diathesis who are hyper-secretors but have not ulcerated up to the time of testing. Any such control group is potentially, at least, weighted towards hypersecretion.

Davenport (1965) maintained that patients with primary gastric ulcers in fact secrete a normal amount of gastric acid, and the hyposecretion revealed by conventional gastric analysis was due to a diffusion of hydrogen ions back into the mucosa. He did not however dispute that gastric ulcer is associated with hypoacidity as assessed by the histamine stimulation test.

Cox (1952) found that the average number of parietal cells per stomach was increased in patients with duodenal ulcer when compared to non-ulcerated controls (Table 2). He estimated that duodenal ulcer patients had an average of 1.8 billion (10^9) parietal cells per stomach, and control patients 1.0 billion cells.

TABLE 2

Parietal cell content (Cox 1952) and response to maximal histamine stimulation (Marks and Shay 1959) in normal subjects and patients with gastric or duodenal ulcer. (Figures in parentheses indicate number of patients in each group).

	PARIETAL CELLS PER STOMACH (COX 1952)	MAXIMAL ACID OUTPUT (mEq. HCl per hour) (MARKS and SHAY 1959)
CONTROL (MALES AND FEMALES)	$0{\cdot}98 \times 10^9$ (107)	20·5 (46)
GASTRIC ULCER (MALES AND FEMALES)	$0{\cdot}8 \times 10^9$ (13)	18·9 (33)
DUODENAL ULCER (MALES AND FEMALES)	$1{\cdot}8 \times 10^9$ (15)	38·1 (63)

Doberneck and Engle (1966) assessed parietal cell numbers in gastric biopsy specimens from patients with active duodenal ulceration, and found increased numbers in these compared with control subjects. On this basis they suggested a relationship between acid production and duodenal ulcer. The Zollinger-Ellison syndrome (Zollinger and Ellison, 1955) provides a clinical example of the role of excess acid, while the role that acid plays in producing the duodenal lesion has been investigated in animals.

The ulcer producing propensity of gastric juice was initially demonstrated by Mathews and Dragstedt in 1932, by diverting gastric juice from Pavlov or Heidenhain pouches into the small intestine of a number of dogs. Chronic ulcers developed in the area maximally exposed to gastric juice. Fogelman, Grossman and Ivy (1949) demonstrated that duodenal ulcers could be produced in dogs by the continuous intragastric infusion of acid and pepsin. They felt that there may be a critical level of acid concentration necessary to produce ulceration. Monkeys studied in a chronic stress situation showed an increase in gastric acid secretion and developed duodenal ulcers, some of which perforated (Polish, Brady, Mason, Thach and Niemack, 1962). Kontureck and Dubiel (1969) stimulated gastric acid production in cats by administering pentagastrin intravenously and produced duodenal ulceration within 36 hours. Diversion of the alkaline biliary and pancreatic juice facilitated ulcer formation in these animals.

Arai and Necheles (1965) produced an experimental model which possibly explains the observed frequency of ulcer formation in the first part of the duodenum. They showed that the digestive action of perfusates of acid and pepsin in rats was potentiated by alternating the perfusion with alkaline tryptic solutions. In fact Rhodes and Prestwick (1966), by inserting tubes designed to record intraluminal pH, demonstrated that wide fluctuations (between pH 2 and pH 6) of pH do occur in the first part of the duodenum in humans in an in vivo situation.

The occurrence of duodenal ulceration particularly in the anxious, tense "executive" subject is well recognized in the clinical situation. Indeed Grundy, Donaldson, Pinderhughes and Barrabee (1967) have found that the personality of the subject may affect gastric acid secretion. In a study of forty male patients with duodenal ulceration they found that anxiety was the predominant personality feature in eight patients with a low basal but high peak acid output, while depression was an obvious emotional feature in eight patients with a low basal and low peak acid output. Accumulating evidence of the many differences in behaviour, occurrence and probable aetiology between gastric and duodenal ulceration suggests that they should be considered as separate diseases, and studied as such.

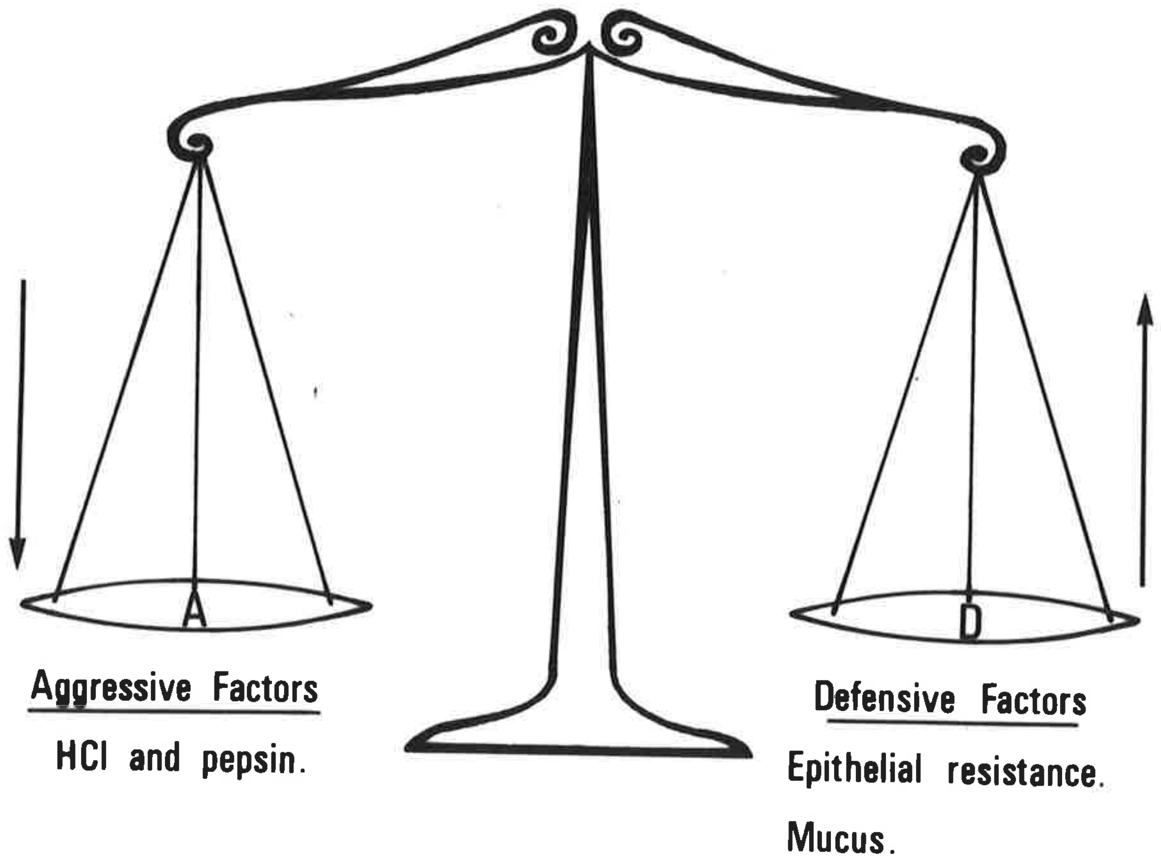
2. *THE PATHOGENESIS OF GASTRIC ULCER*

The current concept of the pathogenesis of peptic ulceration is that the mucosal lesion arises from an imbalance between aggressive and defensive factors (Shay and Sun, 1963). While duodenal ulceration is closely associated with increased secretion of hydrochloric acid and pepsin, or increased aggression, there is no evidence relating gastric ulcer with increase in aggressive factors. In fact the indices of aggression appear reduced in gastric ulcer patients, and if ulceration does arise from an imbalance of these factors, gastric ulcer must result from a reduction in the defensive mechanism.

Mucosal resistance is synonymous with defensive factor. Hollander (1954) postulated that mucosal resistance is provided by a two-component system consisting of a layer of mucus secreted by the mucous cells, and the epithelial cell layer lining the gastrointestinal lumen. Any review of the pathogenesis of gastric ulcer must include a discussion of these two components. Another observation which any theory of gastric ulcer genesis must explain is the apparent association of gastric ulcer with the ageing process. The disease of gastric ulceration has a maximum incidence of onset in the age group 40-55, and appears on average ten years after duodenal ulcer (Doll and co-workers, 1951; Vesely et alii, 1968). Mulsow (1941) observed that 10.5 per cent of peptic ulcers occurred in the group of patients aged 60 years

FIGURE I

PATHOGENESIS OF PEPTIC ULCER.



Ulceration may result when balance is altered in the direction indicated by the arrows.

and over at the time of diagnosis, while Klein and Bradley (1957) reported that in the elderly age range there was a higher incidence of first appearing gastric ulcers than of duodenal lesions. Peptic ulceration is not uncommon in children, but the ulcers present are predominantly duodenal and gastric lesions are very rare (Briguglio and Casaglia, 1967; Tudor, 1967; Coddington, 1968). Both Briguglio and Casaglia, and Coddington noted a positive family history of ulcer disease in half of the children in their series with duodenal ulcers.

An alteration in either the quantity or quality of mucus production leading to increased susceptibility to mucosal lesions is a logical and attractive explanation of gastric ulcer genesis. However no variation from normal mucus secretion has as yet been demonstrated in gastric ulcer patients (Tulin, Gutman and Almy, 1947; Piper, Griffith and Fenton, 1965; Robert, Henninger and Munroe, 1967). Indeed the protective as opposed to lubricant capacity of gastric mucus is open to question. Taking pyloric secretions from guinea pigs and concentrating these, Heatley (1959) obtained a mucosubstance resembling the "visible mucus" which normally lines the gastric mucosa. He demonstrated that in vitro this mucosubstance was an ineffective barrier to acid diffusion and had negligible buffering power per se. Furthermore he demonstrated that mucus in physiological concentrations had no pepsin inhibiting properties. Cooke (1967) after reviewing the

available literature on the mucous barrier concluded that mucus played very little if any part in providing resistance to ulceration, and argued that if mucus were an effective barrier to acid diffusion it would be difficult for acid secretion from cell through mucus to the lumen to occur. Furthermore he emphasized, that because of species variation in susceptibility to ulcer formation, studies of gastric secretion in animals may not necessarily reflect the situation in man.

In the experimental situation there is a correlation between ulcer formation and mucus production. Robert, Bayer and Nezamis (1963) found a correlation between the occurrence of mucosal erosions and reduction in gastric mucus content in fasting rats. Ludwig and Lipkin (1969) demonstrated a reduction in P.A.S. positive mucosubstances in the gastric mucosa of guinea pigs developing erosions induced by restraint. Impaired mucus production may also in part be responsible for the mucosal damage produced by anti-inflammatory drugs (Menguy and Desbaillet, 1967). Menguy and Masters (1965) found that aspirin reduced mucus formation in rats and dogs and postulated that this may result in impaired resistance to proteolysis. Lambert, Martin, Andre, Descos and Vouillon (1968) suggested that the glycoprotein molecules in gastric mucus may inhibit proteolysis by the formation of a resistant complex with the protein of the mucosal cells. However it must be emphasized that the alterations in mucosubstance

secretion in acute experimental ulceration cannot necessarily be extrapolated to chronic ulceration in humans.

James and Pickering (1949) observed that bile staining of the gastric aspirate was more common in patients with gastric ulceration than in ulcer-free subjects. du Plessis (1965) demonstrated an increased concentration of bile acid conjugates in the fasting gastric juice collected from a number of gastric ulcer patients. He was impressed by the high incidence (81 per cent) of severe chronic gastritis in his series of 75 patients with gastric ulceration, and proposed that duodenal reflux producing a chronic gastritis was an aetiological factor in chronic gastric ulcer. However, in by no means all patients with primary gastric ulcer can duodenal reflux be demonstrated. While du Plessis (1965) felt that the gastritis resulting from the reflux of duodenal contents, by lowering mucosal resistance, was the primary factor in the production of gastric ulceration, Capper (1967) suggested that duodenal reflux produced gastric ulceration by tryptic digestion of the gastric mucosa. Gurd (1969) described regurgitation of duodenal contents in shocked animals developing acute gastric ulcers, and also postulated a causal relationship.

The almost invariable association of chronic gastritis with gastric ulcer is at least evidence of some mucosal abnormality in this disease. Magnus (1952) found gastritis in all of 284

partial gastrectomy specimens removed for chronic gastric ulceration. The gastritis was diffuse in 26.5 per cent of cases. Heinkel, Elster and Henning (1956) found evidence of gastritis in fundic biopsy specimens from 70 per cent of gastric ulcer patients, but in only 12 per cent of duodenal ulcer patients. Marks and Shay (1959) maintained that gastritis invariably accompanied chronic gastric ulceration, and also observed that areas of severe atrophic gastritis were particularly prone to ulcerate. This latter observation was confirmed by du Plessis (1965).

Mackay and Hislop (1966) confirmed the association of gastritis and gastric ulcer. In their biopsy series they noted dystrophic changes in the parietal and chief cells, glandular atrophy and a heavy infiltration of lymphocytes and plasma cells in many cases. These changes they interpreted as being consistent with an immunological response. Their findings suggested that ulceration occurred in the active inflammatory type of chronic gastritis rather than in the relatively acellular type associated with pernicious anaemia.

Ritchie and Delaney (1968) produced an experimental model which confirmed that the presence of atrophic gastritis in a localized segment of gastric mucosa renders it highly susceptible to acid-peptic digestion. These workers manufactured a small (3 cm x 3 cm) area of mucosa resembling human gastric atrophy by

surgical exteriorization in dogs, and on reimplantation stimulated acid production with parenteral histamine. Eight of ten operated dogs developed penetrating ulceration in the abnormal mucosa, while only one sham operated control developed a gastric (prepyloric) ulcer.

The gastritis associated with chronic gastric ulceration in humans persists after the ulcer has healed (Palmer, 1951; Mackay and Hislop, 1966; Salupere, 1969) suggesting that the gastritis may well be a primary change and not secondary to the ulcer. In fact, Salupere (1969) maintained that the persistence of gastric inflammation explained the high relapse rate and chronic nature of gastric ulcer.

Vilardell (1963) observed that the drug cincophen could produce chronic gastric ulceration in dogs and felt that the evolution of these ulcers illustrated a possible pathogenetic mechanism of gastric ulcer formation in man. The mucosa initially exhibited a diffuse haemorrhagic erosive gastritis, followed by multiple acute ulcer formation; as the erosions and gastritis resolved, a chronic ulcer remained. The association of gastritis of varying extent and degree with gastric ulcer is well proven, and it seems very likely that it may have a role in gastric ulcer genesis.

The classification of gastric ulceration into the three

categories of prepyloric, combined, and primary gastric ulcer (Johnson, 1957) has aetiological significance. The appearance of a gastric ulcer secondary to duodenal ulceration (Vesely et alii, 1968) raises the possibility that duodenal obstruction and gastric stasis may produce gastric lesions. The demonstration by Shay, Komarov, Fels, Marance, Gruenstein and Siplet in 1945 that pyloric ligation in rats was a reliable method of inducing acute gastric ulceration, the occurrence of gastric ulcer following vagotomy with inadequate drainage (Woodward, 1958) and the production of gastric ulcers in rabbits by vagotomy without drainage (Linares, de la Rosa, Woodward and Dragstedt, 1964) support this possibility. The mechanism whereby gastric stasis produces ulceration is unknown, but increased gastrin release stimulating acid production is a likely possibility (Dragstedt, Oberhelman, Evans and Rigler, 1954). Menguy (1965) reported that vagal stimulation decreased mucus secretion, but vagotomy alone does not seem to increase the incidence of gastric ulceration.

Doll, Jones and Pygott (1958) observed that gastric ulceration was more common in smokers than non-smokers, and more marked in cigarette than pipe smokers. However they failed to demonstrate a relative excess of ulceration among heavy compared to moderate smokers. Three large scale studies (Hammond and Horn, 1958; Dorn, 1959; Doll and Hill, 1964) demonstrated that mortality from peptic ulcer was higher in smokers than non-smokers.

However Doll (1964) felt that smoking was not a principal cause of gastric ulcer and concluded that smoking and gastric ulcer were indirectly related to the same cause.

Doniach and Roitt (1964) demonstrated the presence of circulating antibodies to gastric parietal cells in 22 per cent of patients with gastric ulcer, but found no parietal cell antibody in patients with duodenal ulceration. Wangel (1970) has found parietal cell antibodies in 25 per cent of patients with gastric ulceration. Fisher, Mackay, Taylor and Ungar (1967) maintained that these gastric antibodies are related to the presence of gastritis, but Kravetz, van Noorden and Spiro (1967) suggested that parietal cell antibodies develop independently, and unrelated to the gastritis. It is unlikely that the parietal cell antibodies are directly related to gastric ulcer genesis, but their presence is evidence of an antigen-antibody reaction in the stomach of some chronic gastric ulcer patients. Grinshpun (1966) found a high incidence of antibody to a gastroduodenal mucosal antigen in patients with peptic ulceration. Recently Hausamen, Halcrow and Taylor (1969) provided evidence of a possible relationship between antibody presence and lowered mucosal resistance. They demonstrated that the presence of tissue specific antibody reduces DNA synthesis in that tissue. Reduction of DNA synthesis would result in reduced cellular turnover in the stomach and lower mucosal resistance by affecting the

second of Hollander's (1954) components, the epithelial cell layer.

Dragstedt and co-workers (1954) proposed that gastric ulceration was due to antral hyperfunction which released the hormone gastrin, thus stimulating gastric secretion. More recently Dragstedt has re-emphasized this hypothesis (Dragstedt, Woodward, Linares and de la Rosa, 1964). However the available evidence is very much against this possibility. Emas and Fyrö (1964) estimated antral gastrin activity in patients with either duodenal or gastric ulceration and found that the gastrin secretion was higher in the duodenal ulcer group. Celestin (1967) demonstrated that the gastrin response to broth feeding was higher in a group of patients with active duodenal ulceration than in a group with gastric or healed duodenal ulcers. The most forceful argument against the role of gastrin hypersecretion in the pathogenesis of primary gastric ulcer is the absence of demonstrable acid hypersecretion in patients with this disease (Menguy, 1964b). Gastrin stimulates gastric acid secretion (Konturek and Dubiel, 1969) yet patients with gastric ulcer have normal or low secretion of acid (Vesely et alii, 1968). Although Davenport (1965) maintained that the measured acid output in patients with gastric ulceration was not a true reflection of synthesis, Wlodek and Leach (1966) demonstrated that gastrin increased the capacity of the stomach to contain an instilled load of hydrochloric acid. Measurement of acid secretion in the

presence of excess gastrin stimulation therefore should be a more accurate reflection of acid production than in the non-stimulated individual. Thus there is no evidence at the present time relating the occurrence of primary gastric ulceration with excess gastrin release. However, this mechanism may be the explanation of the secondary type of gastric ulcer occurring in association with duodenal ulceration.

3. *DRUGS AND PEPTIC ULCERATION*

Treatment with a number of drugs, particularly anti-inflammatory agents, may be complicated by the ulcerogenic potential of the drug. The incidence and mechanism of ulcer production with each individual drug are not known with certainty at the present time.

Indomethacin and phenylbutazone both produce gastric ulcers in humans and animals. Taylor, Huskisson, Whitehouse, Dart and Trapnell (1968) demonstrated gastric ulceration in nine out of ten patients treated with indomethecin for varied periods of time. Djahanguiri (1969) showed that indomethecin induced gastric ulcers in rats, and that the incidence increased with increasing drug dosage. The ulcer presumably arises from a systemic action of the drug, for parenteral administration produced an ulceration incidence of 100 per cent. Phenylbutazone also produces gastric ulceration by a systemic action. Watt and Wilson (1959) demonstrated gastric ulcers in guinea pigs within two hours of intraperitoneal administration of phenylbutazone.

Sodium salicylate (aspirin) produces gastrointestinal bleeding by inducing an haemorrhagic gastritis (Croft and Wood, 1967; Skyring and Bhanthumnavin, 1967). Although the facility with which sodium salicylate produces an acute gastric lesion is well proven, the relationship between salicylates and chronic gastric ulcer is less clear. Shay and Sun (1963) maintained that there was no conclusive evidence that salicylate was a cause of peptic ulceration. However, Gillies and Skyring (1969) in an epidemiological study of gastric ulceration in Sydney, noted a striking relationship between chronic gastric ulcer and salicylate ingestion. They concluded that salicylates and gastric ulceration were causally related.

Anderson (1965) produced acute gastric erosions in guinea pigs by administering aspirin orally for ten days. He found that coincident administration of atropine reduced the severity of gastric lesions in these animals, and suggested that acid secretion was important in the pathogenesis of aspirin-induced lesions. Stephens, Milton and Loewenthal (1966) were not able to demonstrate that increase in acid secretion played any part in the genesis of the gastritis. St. John and McDermott (1969) found that aspirin increased gastric blood loss in patients with pernicious anaemia or confirmed gastric achlorhydria; evidence against the importance of acid in aspirin-induced lesions. On the other hand, Davenport (1967) found that mucosal damage with

salicylates occurred only in the presence of acid. He suggested that salicylates impaired the gastric mucosal barrier to acid, and that the consequent retrograde flow of acid damaged the mucosa. Salicylate-induced mucosal damage can be prevented by administering the drug with an alkaline solution (Thorsen, Western, Tonaka and Morrissey, 1967). This suggests that the unionized acetylsalicylic acid rather than its sodium salt is the gastric irritant, and the presence of acid in the stomach does favour mucosal damage from salicylates.

Aspirin appears to reduce mucus secretion by the gastric mucosa. Menguy and Masters (1965) demonstrated that parenteral aspirin reduced the amount of P.A.S. staining mucosubstance in the gastric mucosa, and reduced the hexosamine and fucose content of secreted mucus in rats. Mucus production was also impaired by aspirin administration in dogs. Hakkinen, Johansson and Pantio (1968), working with dogs, demonstrated that the secretion and synthesis of a gastric sulphoglycoprotein derived from the superficial mucus cells was reduced after feeding with aspirin. However, some workers have been unable to demonstrate altered mucus production with salicylate administration (Reed, Balint and Powers, 1968).

Originally it was believed that gastritis associated with the use of salicylates was the result of a local intragastric irritative effect of the drug. However, it has now been

conclusively shown that salicylates induce gastritis when given parenterally or in such a way that direct contact with the gastric mucosa is avoided (Grossman, Matsumoto and Lichter, 1961; Menguy, 1966; Max and Menguy, 1970). Croft (1963) measured the DNA content of gastric washings as an index of cell shedding and demonstrated that local irrigation of the gastric mucosa with an aspirin solution increased the number of cells shed into the gastric juice. A similar effect from systemic aspirin has been demonstrated by Max and Menguy (1968). In a subsequent study of the effect of aspirin on the gastric mucosa, Max and Menguy (1970) found that aspirin did not alter cell proliferation. They suggested that aspirin affected the mucosa in such a way that shedding of surface cells was increased without a concomitant increase in the rate of cell replacement.

Thus there is no doubt that aspirin produces an acute gastritis with erosions and bleeding in both man and animals. This appears to be an effect of circulating salicylate, though salicylates may in addition have a local irritative effect. The initiating factor may be a reduction in mucosubstance production leading to increased cell shedding and a more vulnerable mucosa. However, the place of aspirin in the pathogenesis of chronic gastric ulcer remains unresolved.

The role of corticosteroids in the pathogenesis of gastric ulceration is debatable. Following the introduction of cortico-

steroids into clinical medicine a definite impression evolved that steroids exacerbate pre-existing ulcers and possibly produce gastric ulceration 'de novo' (Forbes, 1952). However, this phenomenon has not been scientifically documented and remains a clinical impression. Cooke (1967), after reviewing the available literature on steroid ulceration, concluded that there was no indisputable evidence that steroids produce ulceration in man, and deplored the lack of scientific control underlying the reported observations. Menguy (1967) maintained that Cooke was unduly critical, and felt that there was reasonable evidence that large doses of corticosteroids produced gastric ulceration. Kammerer, Freiberger and Rivelis (1958), in a prospective study, demonstrated an increased incidence of gastric ulceration in a group of patients with rheumatoid arthritis treated by steroids. These workers documented a 31 per cent incidence of ulceration in patients treated with steroids, while only 9 per cent of patients with rheumatoid arthritis not treated with steroids had demonstrable ulcers.

Corticosteroids do produce gastric lesions in the laboratory animal (Robert and Nezamis, 1963; Robert and Nezamis, 1964; Lev, Siegal and Glass, 1970). Melon, Haot and Betz (1967) produced chronic gastric ulcers in rabbits by irradiation, and showed that while the incidence of ulceration was not increased by cortisone, the rate of formation of the ulcers was accelerated.

The mechanism of ulcer formation with steroids is not clear. Although there is no evidence relating gastric ulceration to increased acid secretion, the effect of corticosteroids on gastric acid production has been extensively studied. The influence of the adrenal gland on gastric secretion was noted by Tuerkischer and Wertheimer (1945) when they demonstrated that gastric acid secretion was reduced by adrenalectomy. This observation has subsequently been reinforced by Jones and Harkins (1958), and Bralow, Komarov and Shay (1964). Reports of the effect of exogenous steroids on gastric acid secretion are contradictory. McGee, Blackburn, Lance and Scott (1958) found that massive and prolonged cortisone therapy increased gastric secretion in dogs. Gray (1958) maintained that chronic stimulation with A.C.T.H. increased acid and pepsin secretion in dogs while short term stimulation had no effect. Plainos, Nikitopulu and Vukydis (1962) showed that cortisone increased gastric secretion in dogs, but that the increase was slight. Other workers (Brodie, Marshall and Moreno, 1962; Beck, Fletcher, McKenna and Griff, 1960) have been unable to demonstrate any change in acid secretion with steroid therapy. Weinshelbaum, Fry and Ferguson (1965) demonstrated that cortisone administered to dogs in conjunction with histamine resulted in an increased incidence of duodenal and antral ulceration without a significant increase in acid secretion, and Wiederanders (1965) confirmed the finding that cortisone did not augment histamine stimulated

gastric secretion. Van Nostrand and Nicoloff (1968), however, found that Metopirone, which inhibits endogenous steroid production, reduced the incidence of histamine induced ulceration from 80 per cent to 9 per cent in the dog. They suggested that this reduction was related to the observed decrease in acid secretion.

Because of the conflicting evidence with respect to the induction of gastric acid hypersecretion by corticosteroids, Menguy and Masters (1963) rejected increased acid production as an important aetiological factor in ulcer formation with steroids. Accordingly they examined the effect of corticosteroids on mucus production and found an appreciable decrease in mucus secretion and alteration of mucus content in dogs given cortisone. They suggested that a reduced rate of mucus secretion was responsible for steroid ulceration. Previous observations by Denko (1958) and Kowalewski and Strutz (1959) had indicated that steroids inhibit the synthesis and sulphation of glycoproteins in the stomach. Desbaillets and Menguy (1967) also confirmed that steroid administration produced an alteration in composition of mucus and a reduction in dogs. However, in a recent study by Lev et alii (1970) in dogs, mucosal damage induced by steroids was preceded by increased rather than depressed secretion. This latter report casts some doubt on the effect of steroids on mucus production, and indeed altered mucus production alone is probably

not a sufficient explanation of gastric ulcer genesis (Cooke, 1967).

Steroids apparently affect the second component of Hollander's two component mucosal barrier, the epithelial cell layer. The inhibitory effect of steroids on cell proliferation was demonstrated initially by Hoffman, Hines, Lapan and Post (1955). These workers observed that cortisone reduced the mitotic activity of regenerating rat liver, and further demonstrated that steroids depress nucleic acid, especially RNA, metabolism in this tissue. With particular reference to gastric epithelial cells, Myhre (1960) observed a decreased mitotic rate and inhibition of regrowth of gastric mucosa following corticosteroid treatment in rats. Rasanen (1963) found that a single injection of cortisone inhibited mitosis in the gastric but not the intestinal mucosa of rats. Crean (1967) also demonstrated that large doses of adrenocortical hormones inhibited the growth of gastric mucosa.

The depression of mitosis in the gastric mucosa that occurs with corticosteroids may be the result of depression of nucleic acid metabolism. Cline and Rosenbaum (1968) have demonstrated that steroids inhibit the incorporation of tritiated thymidine and tritiated uridine into leucocytes from patients with acute leukaemia. This presumptive evidence of the ability of steroids to depress DNA and RNA metabolism confirmed the findings of

Makman, Nakagawa and White (1967). These workers found that cortisol inhibited DNA, RNA and protein metabolism in thymocytes. More recently Max and Menguy (1970), working with dogs, have confirmed that corticosteroids inhibit DNA metabolism in the gastric mucosa.

Steroids apparently produce a depression of nucleic acid metabolism, and may thereby inhibit cell proliferation. Teir (1963) suggested that variation in cell turnover may be the cause of decrease in local resistance in the stomach. This may indeed be the mechanism of steroid induced ulceration, for it is unlikely that alterations in acid production or mucus secretion are responsible for the appearance of the gastric lesions seen with steroid therapy.

4. *THE TREATMENT OF GASTRIC ULCERATION*

While the pathogenesis of gastric ulceration remains unknown the treatment of the disease must be empirical. For this reason Doll (1964) stated that "gastric ulcer is one of the few conditions which provide an opportunity to practise 19th century medicine in the second half of the 20th century". However, despite this gloomy sentiment a number of factors have been shown to influence the healing rate of a gastric ulcer.

In 1950 Doll and a number of colleagues initiated a series of trials to assess what factors, if any, influenced the clinical

course of gastric ulceration. In their initial trial (Doll and Pygott, 1952) they compared the healing rate of gastric ulceration in a series of hospital inpatients matched with patients treated on an ambulant outpatient basis. They found that the inpatient group fared significantly better than did the outpatients, and considered that rest in bed in a hospital environment increased the ulcer healing rate. There was no demonstrable benefit from sedation nor ascorbic acid therapy.

Cheney (1952) reported that an extract of cabbage juice, supposedly containing "vitamin U", healed gastric ulcers. At the same time a proprietary preparation called Robaden was marketed. Robaden was an extract of gastrointestinal tissue that had a protective effect against experimentally induced ulcers in rats. The manufacturers claimed possible efficacy for this preparation in gastric ulcer therapy. Doll and Pygott (1954) assessed the effect of Robaden and cabbage juice on gastric ulcer healing. They refuted Cheney's claim by demonstrating that cabbage juice extract did not alter the healing rate of the ulcer in twenty-four patients, and they found that a group of patients given Robaden fared worse than their control group. Two other controlled trials of Robaden therapy (Stolte, 1950; Evans, 1954) demonstrated that Robaden had no effect on gastric ulcer healing, and this preparation was soon discarded.

Doll, Price, Pygott and Sanderson (1956) next analysed the effect of continuous milk drip combined with intensive antacid therapy on gastric ulcer healing. They found that their regime had no beneficial effect, and Doll, Friedlander and Pygott (1956) then assessed the effect of dietary manipulation on gastric ulcer. No evidence was obtained to suggest that alterations in diet from normal to bland, or from high fat to low fat content had any effect on the healing rate of gastric ulcer. The effect of smoking on gastric ulcer genesis has been discussed previously. Illingworth (1953) stated that "clinical opinion generally forms the view that it (smoking) militates against healing of the ulcer." Doll, Jones and Pygott (1958) demonstrated that the healing rate of gastric ulceration is increased in smokers if they stop smoking.

At the present time there is no evidence that long-term anticholinergic therapy influences the course of gastric ulceration, though Sun (1964) observed that long-term anti-cholinergic therapy did prevent recurrence of duodenal ulcer. This observation was in contrast to an earlier study in which no such benefit was demonstrated (Bralow, Spellberg, Kroll and Necheles, 1950). The studies of Doll and his colleagues in particular demonstrated that of the many therapeutic factors studied, rest in bed and cessation of smoking were the only factors contributing to the healing of a gastric ulcer.

An interesting development in gastric ulcer therapy has been the acceptance that liquorice and related compounds accelerate the healing of the ulcer. Although Revers (1948) observed that powdered *succus liquiritiae* was beneficial in patients with gastric ulcer, little attention was paid to this form of treatment until 1962 when the beneficial effect of carbenoxolone sodium was confirmed by a controlled clinical trial (Doll, Hill, Hutton and Underwood, 1962). Carbenoxolone sodium is the disodium salt of 3-0-(β carboxypropionyl) 11-oxo-18 β-olean-12-en-30-oic acid, which is synthesized from glycyrrhetinic acid, one of the many constituents of the liquorice root. A number of clinical trials have subsequently confirmed that carbenoxolone sodium does increase the healing rate of gastric ulcers (Doll, Hill and Hutton, 1965; Horwich and Galloway, 1965; Bank, Marks, Palmer, Groll and Van Eldik, 1967).

Unfortunately the administration of carbenoxolone in standard dosage leads to a high incidence of side effects due to water retention and electrolyte imbalance (Doll and co-workers, 1962; Turpie and Thomson,, 1965). The fluid retention and hypokalaemia produced by carbenoxolone suggested an aldosterone effect. Doll, Langman and Shawdon (1968) found that both the side effects and the beneficial healing effect of carbenoxolone were blocked by an aldosterone antagonist (spironolactone), while a diuretic with a different mechanism of action (chlorothiazide) abolished the

fluid retaining but not the healing potential of this drug. This suggested that the healing effect of carbenoxolone may be related to an aldosterone-like action.

The incidence and severity of side effects with carbenoxolone led Langman (1968) to emphasize the need for a variant of carbenoxolone sodium devoid of side effects. Turpie, Runcie and Thomson (1969) described the use of a deglycyrrhinized liquorice preparation (Caved-S) in the treatment of gastric ulcer. The initial results with this preparation are encouraging. Turpie et alii (1969) found a significant increase in healing rate of gastric ulcers in patients given this deglycyrrhizanated liquorice preparation, while there was no evidence of fluid retention or hypokalaemia in these patients. Russell and Dickie (1968) in a smaller trial noted similar results with Caved-S. Its mechanism of action is unknown.

Another approach to overcoming the problem of side effects associated with carbenoxolone therapy was that of Cocking and MacCaig (1969). They used a low dose regime in ambulant outpatients and found significant healing benefit with the drug and a low incidence of side effects. Doll et alii (1968) demonstrated a lower incidence of side effects with a low dose regime, but also noted that less satisfactory healing resulted from the lowered dose. The value of carbenoxolone sodium is that it increases the healing rate of gastric ulceration in the

ambulant patient and the economic cost of treatment is lowered as hospitalization of the patient becomes unnecessary in many cases. Indeed Middleton, Cooke, Stephen and Skyring (1965) found that the healing effect of carbenoxolone sodium and bed rest in hospital was similar and non-additive, and confirmed that the value of the liquorice preparations was in the outpatient treatment of patients with gastric ulceration.

It is not at present resolved that carbenoxolone sodium is useful in the treatment of duodenal ulceration and published evidence suggests that any beneficial effect on ulcer healing is small. Craig, Hunt, Kimmerling and Parke (1967) found that a timed release capsule of carbenoxolone which disintegrates within the duodenum (Duogastrone) produced symptomatic relief in 13 of 15 patients with duodenal ulcers, but they did not assess healing of the ulcers. Montgomery, Lawrence, Manton, Mendl and Rowe (1968) did not demonstrate a significant increase in healing of duodenal ulceration with Duogastrone. The findings of Cliff and Milton-Thompson (1970) were similar to those of Montgomery et alii (1968). Amure (1970) on the other hand, in a small trial observed that Duogastrone did appear to accelerate the healing of duodenal ulcers in ambulant patients.

The mechanism whereby carbenoxolone increases the healing rate of gastric ulcers is unknown. The anti-inflammatory activity of glycyrrhetinic acid derivatives was first described

by Finney and Somers (1958). Khan and Sullivan (1968), employing a cotton pellet implantation technique for assessing anti-inflammatory activity, demonstrated that parenteral carbenoxolone had one-third the anti-inflammatory activity of hydrocortisone. The drug was ineffective in rats when given orally. Adrenalectomy markedly reduced this anti-inflammatory activity, and Khan and Sullivan (1968) suggested that carbenoxolone was, partially at least, dependent on endogenous corticosteroid production for its anti-inflammatory effect. Atherden (1958) demonstrated that steroid inactivation by the liver is depressed by glycyrrhizic acid derivatives, and this is a possible explanation of the steroid-like effect of these preparations. Carbenoxolone sodium appears to produce both gluco-corticoid and mineralo-corticoid activity when administered to both animals and humans.

An early study of the metabolism of glycyrrhetinic acid compounds suggested that the drug was not absorbed from the gastrointestinal tract but was excreted unchanged in the faeces (Carlat, Margraf, Weathers and Weichselbaum, 1959). Subsequently Parke, Pollock and Williams (1963) demonstrated that the drug was absorbed orally, and excreted almost entirely through the biliary system in conjugated form. Parke (1968) showed that the stomach was the main absorptive site for carbenoxolone, and found that absorption was greatest when the pH of the gastric contents was below pH 2. With this information it is difficult to rationalize

the use of duodenal releasing capsules in the therapy of duodenal ulcer. There is some evidence that carbenoxolone sodium may inhibit gastric acid secretion (Bank et alii, 1967; Cocking and MacCaig, 1969). Although the degree of inhibition is slight, this may be an additional factor in gastric ulcer healing. Furthermore this action may be the explanation of the symptomatic relief provided by the drug in duodenal ulcer patients (Craig et alii, 1967).

Whatever their mechanism of action, the introduction of liquorice root preparations to clinical medicine represents a major advance in gastric ulcer therapy. However, the problem of recurrence of ulceration after treatment persists. Flood and Hennig (1950) reported gastric ulcer recurrence in nearly all patients followed over a five to six year period. Swynnerton and Tanner (1953) in a pre-carbenoxolone study, reported a 76 per cent recurrence rate. Bank et alii (1967) observed a 50 per cent recurrence rate within eighteen months of completion of a course of carbenoxolone, while Horwich and Galloway (1965) found a 44 per cent recurrence rate within twelve months in their treated group. The effect of long term treatment with carbenoxolone sodium on the gastric ulcer recurrence rate has not been assessed.

5. *NUCLEIC ACID METABOLISM AND CELL DIVISION IN THE GASTROINTESTINAL TRACT*

The mucosa of the gastrointestinal tract has a very high metabolic rate and a rapid cell turnover (Messier and Leblond, 1960; Lipkin, Sherlock and Bell, 1963; Lipkin and Deschner, 1968). The rate of cell turnover in tissue such as gastro-intestinal mucosa can at present be assessed by two main techniques - firstly, by the rather inaccurate method of mitotic figure counting in histological specimens, and secondly, by assessing the incorporation of radioactively labelled nucleic acid precursor substances. Lipkin et alii (1963) utilizing an autoradiographic technique demonstrated that epithelial cells in the gastrointestinal tract are produced at a mean rate of one cell per hundred cells per hour. The epithelial cells are produced in a proliferation zone in a gastric gland or intestinal crypt, and while maturing migrate to the mucosal surface from which they are sloughed into the intestinal lumen. McDonald, Trier and Everett (1964) demonstrated that migration from the gastric pits to the surface took between four and six days in humans. Messier and Leblond (1960) had previously observed that in rats and mice migration time from the bottom of the gland to the mucosal surface was between twenty-four and forty-eight hours. It appears that studies of the migration rate in animals cannot be applied unreservedly to man (McDonald et alii, 1964).

Indeed variation exists between different areas of the intestine, and Lipkin and Deschner (1968) have demonstrated in the newborn hamster that the cell proliferation rate was more rapid in the small intestine than elsewhere in the gastrointestinal tract, and was more rapid in stomach than colon.

Five epithelial cell types are present in the gastric mucosa. Chief cells produce pepsinogen and parietal cells produce hydrochloric acid, while the function of the third type of cell, the argentaffine cells, is unknown. Two other cell types are present, mucus secreting cells and mucous neck cells believed to secrete the mucoprotein intrinsic factor. Of these cell types the latter two only divide with significant frequency (Stevens and Leblond, 1953; Baker, 1964). Mitotic division and cell proliferation in surface mucus secreting and mucous neck cells follow a circadian periodicity (Clark and Baker, 1962; Clark and Baker, 1963; Alov, 1963; Pilgrim, Erb and Maurer, 1963). Clark and Baker (1963) could not determine the mechanism of such periodicity but noted that the rhythm persisted after both adrenalectomy and hypophysectomy. Pilgrim et alii (1963) found that mitotic periodicity was not present throughout the gastrointestinal tract, for while they demonstrated periodicity in the stomach of mice, they did not observe periodicity in the small intestine.

Apart from such a circadian rhythm several factors are known to influence the rate of cell division within the gastric mucosa. Administration of adrenocortical hormones depresses cell growth and mitosis in the stomach (Myhre, 1960; Rasanen, 1963; Crean, 1967). Emotional stress also has a marked effect. Rasanen (1963), using a mitotic counting technique, demonstrated that acute and chronic stress decreased the rate of cell division in the gastric mucosa. The effect of stress in reducing cell proliferation has been confirmed by other workers using radio-isotope incorporation techniques (Kim, Kerr and Lipkin, 1967; Lahtiharju and Rytomaa, 1967; Imondi, Balis and Lipkin, 1968). Rasanen and Lahtiharju (1967) found that heparin administration reduced cell division in the stomach and skin of mice, and postulated that endogenous heparin release may play a part in the acute gastric ulceration induced by stress. Brown, Levine and Lipkin (1963) found that prolonged starvation reduced intestinal cell renewal in animals by 50 per cent.

The most accurate and easily applicable methods for studying gastrointestinal epithelial proliferation are those employing radioactively labelled nucleic acid precursor substances, the most commonly used radionuclide being tritium. Unfortunately the carcinogenic (Lisco, Baserga and Kisieleski, 1961) and mutagenic (Bateman and Chandley, 1962) potential of the radio-nuclides precludes their unrestricted use in man. The use of

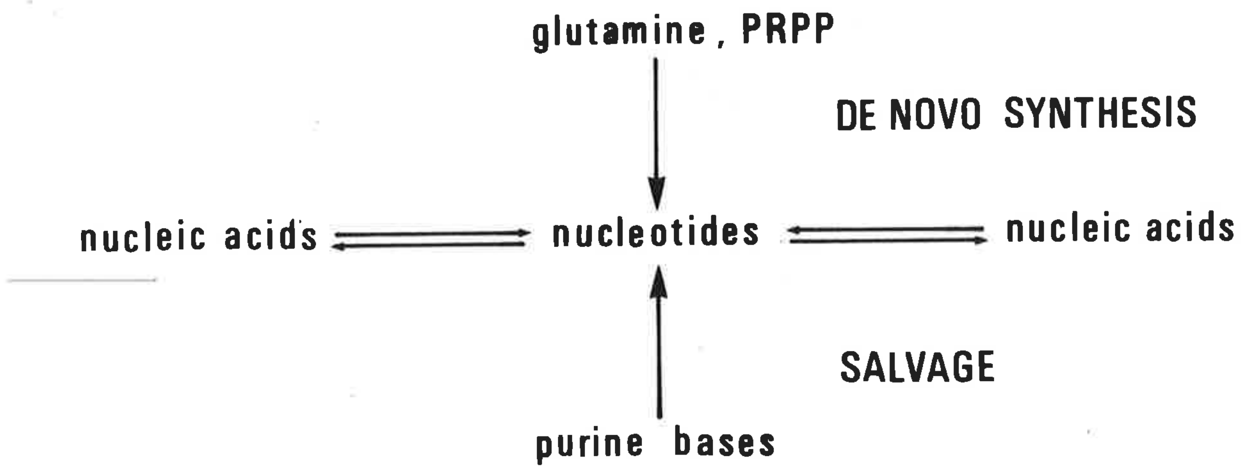
Tritium has been limited in humans to the assessment of cell proliferation rates in patients with undoubted terminal disease (McDonald et alii, 1964).

6. *PURINE NUCLEOTIDE BIOSYNTHESIS*

The maintenance of normal nucleic acid metabolism is of fundamental importance to the normal function and viability of each individual cell. The availability of precursor substances essential for normal nucleic acid synthesis is presumably tailored to meet the particular requirement of each cell. The supply of these prerequisites, in particular the nucleoside triphosphates, is controlled by a sensitive enzymatic system whereby feedback inhibition of rate controlling enzymes by the immediate nucleic acid precursors ensures that a minimum of energy is expended in maintaining an adequate supply of these precursors. As observed by Murray, Elliott and Atkinson (1969) the ultimate control of cell growth, function and replication is probably not achieved by regulation of nucleotide biosynthesis, for this mechanism would be too unselective and inefficient. Nevertheless, altered nucleotide synthesis may well be reflected in altered cell growth and function.

The purine nucleotide biosynthetic pathway is illustrated in figure 2. Kornberg (1957) emphasized the existence of two pathways leading to the formation of purine nucleotides. Nucleotides may be formed by a 'de novo' pathway from simple

FIGURE II



Representation of purine nucleotide biosynthesis.

metabolites such as sugars and amino acids, or alternatively by a 'salvage' pathway in which preformed purine bases or nucleosides are converted directly to their nucleotides. Selective chemotherapy with inhibitors of purine nucleotide formation is possible because of the varying dependence of different tissues on these two pathways of nucleotide biosynthesis (Murray et alii, 1969).

The enzymes involved in the salvage pathway of nucleotide biosynthesis are nucleoside kinases, catalysing the reaction:

nucleoside + A.T.P. $\rightleftharpoons$ nucleoside S phosphate + A.D.P.

and purine phosphoribosyltransferases, controlling the reaction:

purine base + *P.R.P.P. $\rightleftharpoons$ nucleotide + PP_i.

The importance of the purine phosphoribosyltransferases in clinical disease was demonstrated in 1967 by Seegmiller, Rosenbloom and Kelley. They demonstrated a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase in a group of patients with a syndrome of mental retardation, choreo-athetosis, self-mutilation and hyperuricaemia, originally described as a clinical entity by Lesch and Nyhan in 1964. Seegmiller et alii (1967) suggested a causal relationship between deficiency of this enzyme and the disease process.

In most animal tissues the purine ring was assumed to arise

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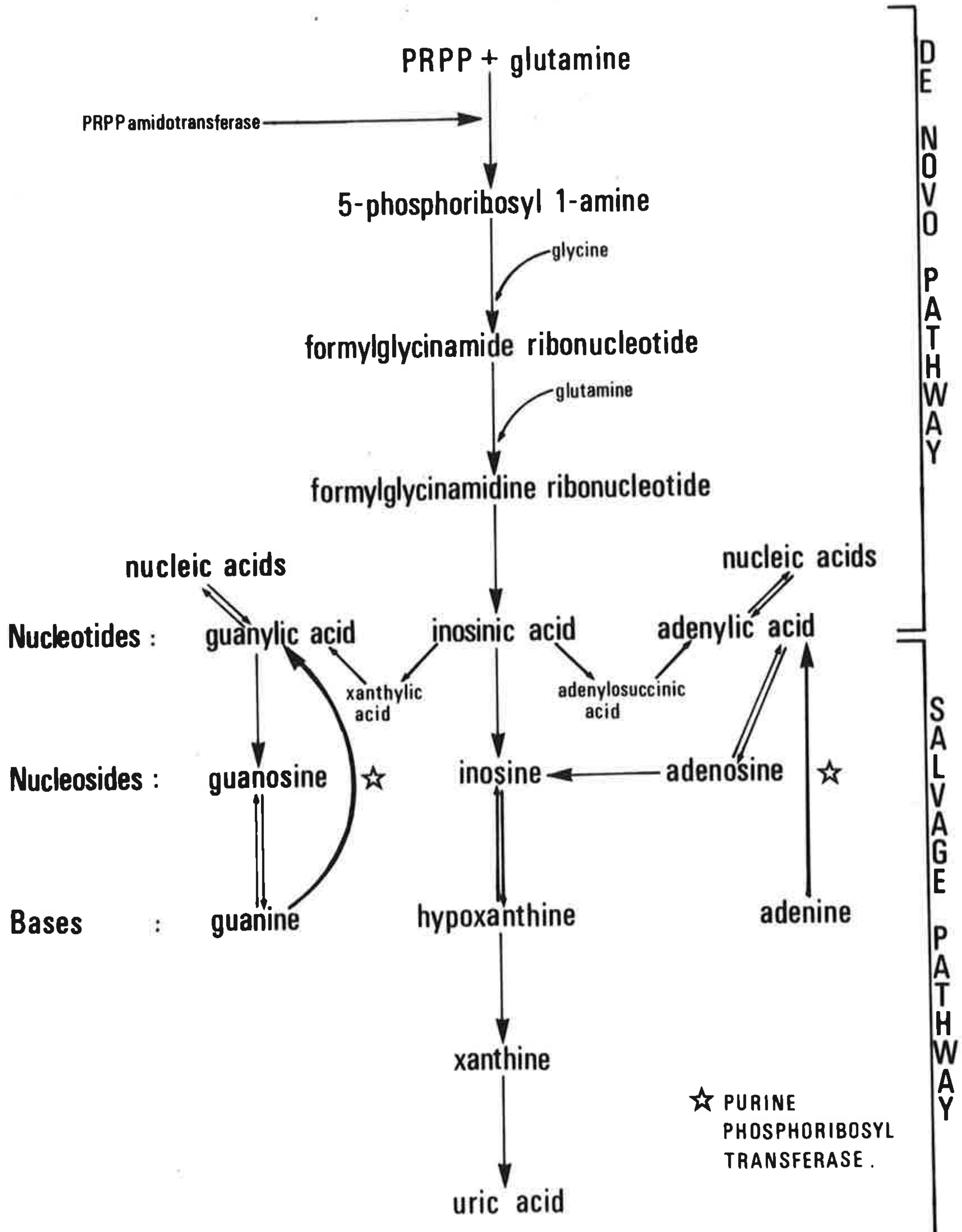
*Phosphoribosyl pyrophosphate

FIGURE III



by new biosynthesis from non-cyclic precursors (Buchanan and Hartman, 1959). However, Smellie, Thompson, Goutier and Davidson (1956) had demonstrated that bone marrow cells and Ehrlich ascites-tumour cells could utilize purines derived from other tissues and possibly from dietary sources. This was confirmed by Henderson and Le Page (1959) and Kimball and Le Page (1963). Lajtha and Vane (1958) suggested that although bone marrow cells had a limited capacity for 'de novo' synthesis of purines, they depended to a great extent on the liver for a supply of preformed purines. This suggestion was in agreement with that of Smellie et alii (1956) that there may be two types of tissue - one type like liver which could synthesize sufficient purine ring for its requirement, and other like bone marrow which was dependent on a supply of purine formed in other tissues.

If preformed purines are utilized by some tissues the purine phosphoribosyltransferases may play an important part in the nucleotide metabolism of these tissues. However, although there are many studies indicating the utilization of preformed purines in the formation of tissue nucleic acids, the most persuasive evidence of the physiological importance of purine salvage is indirectly derived. For example, Lajtha (1959) stressed the importance of purine ring salvage in the human, and estimated that 80 per cent of the daily purine requirement is met by purine reutilization. More recently Murray (1971), arguing from data derived from studies of uric acid production in patients with the

Lesch-Nyhan syndrome, estimated that up to 40 mg per kg body weight per day of purine is normally reutilized through the purine salvage pathway. Indeed, as suggested by Murray (1971), the gross over-production of uric acid in the Lesch-Nyhan syndrome is the most cogent evidence of a significant physiological salvage of the purine ring, and furthermore studies of xanthine and hypoxanthine body pools and daily turnover in patients with xanthinuria indicate that a large proportion of the hypoxanthine pool is reutilized by the body. Fontenelle and Henderson (1969) further emphasized the importance of the purine salvage mechanism when they demonstrated that the first enzyme of the 'de novo' pathway, phosphoribosylpyrophosphate amidotransferase, was not present in mouse or human red blood cells. The absence of this enzyme is an indication of inactivity of the 'de novo' pathway in this tissue.

Hori and Henderson (1966) suggested that the purine phospho-ribosyltransferases have a predominantly anabolic role catalysing the irreversible reaction:

$$\text{purine} + \text{P.R.P.P.} \rightarrow \text{nucleotide} + PP_i.$$

These enzymes have weak, if any, phosphorolytic activity. Two purine phosphoribosyltransferases have been identified - one utilizing adenine as a substrate (adenine phosphoribosyltransferase E.C.2.4.2.7) to form A.M.P., the other predominantly utilizing hypoxanthine or guanine as substrate (hypoxanthine-guanine phospho-ribosyltransferase E.C. 2.4.2.8) to form I.M.P. or G.M.P. Kelley,

Rosenbloom, Henderson and Seegmiller (1967) showed that a single enzyme was involved in the reaction with the two purine bases hypoxanthine and guanine by demonstrating a similar rate of heat inactivation with both substrates. Furthermore activity for both hypoxanthine and guanine conversion is absent in patients with the Lesch-Nyhan syndrome (Seegmiller, Rosenbloom and Kelley, 1967).

There is considerable evidence that the enzymes involved in purine nucleotide biosynthesis and interconversion are controlled by feedback or end product inhibition (Mager and Magasanik, 1960; Wyngaarden and Greenland, 1963; Nierlich and Magasanik, 1965). Murray (1966a) demonstrated that this was the case with the purine phosphoribosyltransferases. He found that adenine phosphoribosyltransferase was inhibited by its immediate product A.M.P., and to a lesser extent by A.D.P., A.T.P. and G.M.P., while hypoxanthine phosphoribosyltransferase was inhibited by I.M.P. and G.M.P. Each of the immediate products of the reactions catalysed by these enzymes inhibit the respective enzyme by competing with phosphoribosylpyrophosphate (P.R.P.P.). However the degree of inhibition is not great and Murray (1966a) found that nucleotide synthesis proceeded in the presence of low purine concentrations provided that the concentration of P.R.P.P. was relatively high. Murray and Wong (1967) further suggested that control of the activity of the purine phosphoribosyltransferases in vivo depended on the relative local concentrations of nucleotides and P.R.P.P.

There is accumulating evidence that the purine phosphoribosyltransferases have an important role in the metabolism of rapidly growing tissue (Atkinson and Murray, 1965; Murray, 1966b; Nicholls and Murray, 1968). Murray (1966b) found that the activity of both enzymes was increased in rapidly growing ascites tumour cells and in regenerating rat liver, and furthermore demonstrated that tissues with the most rapid rates of cell proliferation (rat and mouse spleen, rat bone marrow) contained the highest phosphoribosyltransferase activity. Nicholls and Murray (1968) provided evidence of a relationship between adenine phosphoribosyltransferase activity and RNA biosynthesis. Key in 1964 suggested that plant hormones exert their control of growth through the regulation of RNA synthesis. Nicholls and Murray demonstrated that coincident with stimulating growth, the plant hormone kinetin produced a two-fold increase in adenine phosphoribosyltransferase activity in stimulated tissue. This indicates that measurement of phosphoribosyltransferase activity may be an index of nucleic acid, particularly RNA, synthetic activity.

Davidson and Winter (1964) demonstrated elevated activity of the purine phosphoribosyltransferases in the metabolically active leukaemic cells from patients with leukaemia. However, Troncale, Hertz and Lipkin (1971) did not demonstrate elevated activity of these enzymes in the metabolically active tissue from colonic neoplasms. The association between purine salvage activity and nucleic acid synthesis is therefore far from established.

Imondi, Balis and Lipkin (1969) demonstrated greater activity of the purine phosphoribosyltransferases in the differentiated cells at the top of the intestinal villi than in the undifferentiated crypt zone cells. Their data argues against the importance of these enzymes in nucleic acid synthesis, which occurs predominantly in the crypt zone (Räsänen, 1963; Shorter and Creamer, 1962), and by analogy with the development of disaccharidase activity in the intestinal cell suggest an absorptive function for these enzymes. Indeed, Imondi et alii (1969) suggest that the distribution of purine phosphoribosyltransferase activity in the intestinal mucosa indicates that a function of these enzymes may be the salvage of intraluminal purines.

The purine phosphoribosyltransferases have been extensively studied in relation to gout (Kelley et alii, 1967; Seegmiller, Rosenbloom and Kelley, 1967; Rosenbloom, Kelley, Miller, Henderson and Seegmiller,1967; Balis,1968) and neoplasia (Atkinson and Murray, 1965; Murray, 1966a; Murray and Wong, 1967). Krenitsky (1969), to elucidate their relative importance in the purine metabolism of tissue, studied these enzymes in various tissues from the rhesus

monkey. However he did not extend his studies to gastrointestinal epithelium. In fact, in view of the high metabolic activity of gastrointestinal mucosa, surprisingly little study has been made of purine biosynthesis in this tissue. Rosenbloom and co-workers (1967) in the course of a study of the Lesch Nyhan syndrome referred to purine phosphoribosyltransferase activity in this tissue. They also found that these enzymes were stable with storage at $- 20^{\circ}C$. Balis (1968) studied purine phosphoribosyltransferase activity in the stomach, duodenum, jejunum, ileum and colon from mice, and found that there was greater activity of the hypoxanthine than adenine enzyme in all areas. This was particularly marked in the duodenum where hypoxanthine converting activity was sixty times that of adenine. Imondi et alii (1969), in extending this observation, noted greater hypoxanthine than adenine activity in rat jejunum. On the other hand Berlin and Hawkins (1968), in a preparation of hamster small intestine, demonstrated a much higher level of adenine phosphoribosyltransferase than of the hypoxanthine converting enzyme. The limited amount of published work on these enzymes in gastrointestinal tissue does not allow any definite conclusion at present concerning their relative concentration.

The synthesis of purine nucleotides by the 'de novo' pathway and the series of enzymically-controlled reactions has been elucidated (Buchanan and Hartman, 1959). The 'de novo' purine

biosynthetic pathway provides an example of rate controlled product formation by end product or feedback inhibition of an early enzymatically controlled step. In this case phosphoribosylpyrophosphate amidotransferase (E.C.2.4.2.14) is inhibited by monoribonucleotides, the end products of 'de novo' synthesis (Wyngaarden and Ashton, 1959; Caskey, Ashton and Wyngaarden, 1964; Reem and Friend, 1969). Assessment of the activity of this enzyme is an index of the rate of formation of nucleotides by the 'de novo' pathway. However the study of phosphoribosylpyrophosphate amidotransferase is difficult because of the instability of the enzyme and its relatively low activity in most mammalian tissues (Reem and Friend, 1969).

Phosphoribosylpyrophosphate amidotransferase is present in liver (Caskey et alii, 1964), Ehrlich ascites-tumour cells (Tay, Lilley, Murray and Atkinson, 1969) and spleen (Reem and Friend, 1969) but Fontenelle and Henderson (1969) were unable to demonstrate activity of this enzyme in mature erythrocytes from mice or man. These findings provide further support for the concept of Smellie et alii (1956) that some tissues have not the capacity to synthesize the purine ring and are dependent on the conservation of preformed purines for provision of purine nucleotides. While activity of the salvage pathway has been demonstrated in gastrointestinal tissue (Balis, 1968; Imondi et alii, 1969), no information about the 'de novo' pathway in this tissue is available.

As observed by Imondi et alii (1969), it is possible that the purine salvage pathway is of particular importance in gastrointestinal tissue in the salvage of intraluminal purines. A number of studies indicate that dietary purines are absorbed and made available for nucleic acid synthesis (Brown, Roll, Plentl and Cavaleiri, 1948; Balis, Marrian and Brown, 1951). However, the problem with such studies is that high, possibly non-physiological, doses of purine were administered and the data derived may not reflect the normal physiological situations; the evidence for significant utilization of dietary purine is therefore largely presumptive. Nevertheless, studies involving the administration of radio-labelled pyrimidines (Shorter and Creamer, 1962; Brown, Levine and Lipkin, 1963; Cutright, 1965; Lawson, 1969) demonstrate the appearance of freshly labelled cells some days after dose administration. It is probable, therefore, that reutilization of nucleic acid precursors does occur in gastrointestinal mucosa.

Until recently it was assumed that in the absence of dietary purine there was no body source of adenine available. However, Zimmerman, Gersten and Miech (1970) have now demonstrated the presence of adenosine phosphorylase activity in rat liver, spleen

and blood, thus negating the former concept. The apparent inactivity of the de novo pathway of purine nucleotide synthesis in bone marrow (Lajtha and Vane, 1958) has drawn attention to the role of the liver as the body factory for purine synthesis. The work of Pritchard, Chavez-Peon and Berlin (1970) indicates that purines are released from the liver to become available for nucleotide synthesis in non hepatic tissues, and it is probable that normally, let alone under the artificial conditions of purine deprivations, the liver is an important source of purine for such tissue as bone marrow and gastrointestinal mucosa. The demonstration of adenosine phosphorylase activity (Zimmerman et alii, 1970) indicates that the liver may be a source of adenine for other body tissues.

In developing the concept of the physiological importance of the purine salvage enzymes in metabolically active tissue, the apparently paradoxical finding of high levels of activity of these enzymes in brain, especially basal ganglion tissue, warrants further comment. There is no ready explanation of this finding, but it is of interest that the neurological manifestations of the Lesch-Nyhan syndrome are indicative of a basal ganglion disorder, and that the purine salvage enzymes are normally at greatest activity in this region of the brain. It is possible that the high activity of hypoxanthine-guanine phosphoribosyltransferase

in the brain is not related to metabolic activity but an acquired characteristic to prevent the accumulation of hypoxanthine or a toxic compound derived from this substance.

7. *EXPERIMENTAL ULCERATION*

Acute gastric ulceration can be produced with relative ease in animals but an experimental model producing chronic gastric ulceration is more difficult to establish. Shay et alii (1945) described a simple, reliable method of producing acute gastric ulceration in animals. They found that ligation of the pylorus induced a high incidence of rumenal ulcers in rats, and that the incidence increased with time post-ligation. Shay, Gruenstein,

Siplet and Komarov (1948) then demonstrated that feeding these animals a protein-rich diet prior to pyloric ligation reduced the incidence of ulceration. In 1956 a non-surgical technique for induction of gastric ulceration was described by Rossi, Bonfils, Liefooghe and Lambling. These workers found that physical restraint resulted in gastric lesions in rats, and Sawrey and Weisz (1956) coincidentally produced gastric ulcers in rats by invoking environmental stress. Similar acute gastric lesions are produced by stress situations in man (Cushing, 1932; Davis, Wetzel and Davis, 1955; Sevitt, 1967). These lesions are not however strictly comparable to the chronic gastric ulcers in man.

On the other hand the work of French, Porter, Cavanaugh and Longmire (1954) with monkeys suggested that a pathogenetic relationship existed between acute and chronic lesions, and Vilardell (1963) observed a transition from acute to chronic ulcers in animals treated with the drug cincophen. Study of the mechanisms involved in the production of acute gastric ulceration in animals may therefore add to the understanding of the genesis of primary chronic gastric ulcer in man. Furthermore, experimental gastric lesions induced by pyloric ligation are closely comparable to the chronic gastric ulcers seen in patients with pyloric obstruction consequent upon duodenal ulceration (the 'combined' type of gastric ulcer). Both the acute lesions produced experimentally and the chronic lesions observed in the clinical

situation may be induced by acid hypersecretion resulting from increased release of the hormone gastrin from the antral mucosa (Dragstedt et alii, 1964). The mechanism of ulcer production through the application of stress by restraint is not fully understood, and a better understanding of this mechanism hopefully may add to the understanding of the pathogenesis of primary chronic gastric ulcer.

Restraining procedures induce mucosal lesions in mice and rats (Brodie and Hanson, 1960), guinea pigs (Ludwig and Lipkin, 1969), pigs, (Labie, 1969) and dogs (Edlich, Urdaneta and Hansen, 1969) but are apparently ineffective in rabbits and monkeys (Brodie and Hanson, 1960). A number of factors influence the incidence of mucosal lesions in susceptible animals. The duration of restraint was found to be important by Brodie and Hanson (1960) who reported that the incidence and severity of ulceration increased with increasing duration of restraint. They further demonstrated that repeated periods of restraint produced more gastric lesions than a single period of restraint. These observations were not confirmed by Guth and Mendick (1964) who found that rats appeared to adapt to the repeated insult and that the incidence of ulceration was lower with repeated periods of restraint. However they studied a small number of animals, and the period of restraint was shorter (four hours) than that selected by Brodie and Hanson (eighteen hours). These latter

workers also observed that a period of starvation prior to immobilization increased the incidence of ulceration, and prolonged the time taken for the animals to recover from restraint. The enhancing effect of pre-stress starvation was confirmed by Buchel, Gallaire and Levy (1963), and Hanson (1963) but Robert, Phillips and Nezamis (1966) found to the contrary that fasting prior to restraint actually reduced the incidence of ulceration in rats. Such conflicting reports confirm the confusion in understanding of factors influencing the production of acute gastric lesions by the restraining method.

Wilson and Whitaker (1969) found an inverse relationship between body weight and susceptibility to ulceration in rats. The relationship of age to ulcer susceptibility is not clear.

The demonstration by Truelove (1960) that stilboestrol increased the likelihood of healing of a chronic gastric ulcer led to studies of the effect of pregnancy upon experimental gastric lesions. Luther, Heistad and Sparker (1969) investigated the effect of pregnancy on restraint induction of ulcers. They found no alteration in ulceration incidence in early pregnancy in mice but demonstrated that the severity of ulcer formation was increased in late pregnancy in these animals.

A number of drugs appear to have a protective effect in experimentally induced gastric ulceration. Among these are

anticholinergics (Brodie, Marshall and Moreno, 1962; Robert and Nezamis, 1964), acetazolamide (Djahanguiri, 1968) and carbenoxolone sodium (Lipkin and Ludwig, 1968). Evidence of the effect of corticosteroids is conflicting. Some workers (Bonfils, Liefooghie, Rossi and Lambling, 1957; Flandre and Damon, 1964) have been unable to demonstrate that corticosteroids influence the course of experimental ulceration. Robert and co-workers (1966) however, found that corticosteroids protected against the appearance of stress induced gastric erosions. The findings of Brodie and Hanson (1960) that reduction of endogenous steroid production following adrenalectomy increased the incidence of restraint ulceration also suggests that steroids may have a protective effect in this situation. Groza, Buzoianu, Constantuiescu and Ionescu (1968) observed that another adrenocortical hormone, the mineralocorticoid aldosterone, afforded protection from cincophen induced gastric ulceration in dogs. The effect of aldosterone on the course of experimentally induced ulceration requires further study, particularly as Groza and his colleagues suggested that aldosterone may not have the same protective action in man. However, the efficacy of carbenoxolone sodium on chronic gastric ulcer healing in man, together with the aldosterone-like effects of this drug, suggests that aldosterone per se may provide protection against experimental ulcer formation and promote ulcer healing in man.

The effect of restraint induced stress on gastric function has

been studied by a number of workers, and the understanding of the pathogenesis of gastric ulceration resulting from restraint is increasing. Bonfils, Liefooghe, Rossi and Lambling (1959) observed an increase in total acid output with restraint in rats, and Ritchie, Breen, Grigf and Wangensteen (1966) likewise suggested that the observed increased parietal cell secretion of acid may be one factor in the pathogenesis of these lesions. Certainly the protection against the formation of stress induced lesions provided by vagotomy and anticholinergics (Brodie et alii, 1962) suggests that acidity may be instrumental in initiating the mucosal defect. The finding of increased acid production with restraint has not been universal. In 1960 Menguy, with restrained rats also subjected to pyloric ligation, found that ulceration occurred despite a decrease in free acid output. Singh, Sharma and Kar (1967) and Ludwig and Lipkin (1969) demonstrated no alteration in gastric acid output with restraint. Robert and co-workers (1963), inducing ulceration by starvation and not immobilization, reported decreased acid production in rats developing gastric lesions. Alteration in acid production is probably not the primary cause of ulceration induced by restraint.

In the same study, Robert et alii (1963) observed that the gastric mucosal content of mucosubstances was increased by fasting. The effect of restraint on gastric mucosal content of acid polysaccharides was studied by Hakkinen, Hartiala and Lang

(1966). They found a marked reduction in amino sugar content of the acid polysaccharides in the gastric mucosa of rats with stress-induced lesions. Kim, Kerr and Lipkin (1967) noted a reduction in P.A.S. positive staining mucosubstance in the gastric mucosa of restrained mice. Lambert, Andre and Martin (1969), by measuring the incorporation of radiosulphate into the gastric mucosa of rats subjected to restraint, demonstrated a significant reduction in mucosubstance production in the ulcerated animals when compared to non-stressed control animals and restrained animals without demonstrable mucosal lesions. In a subsequent study to determine whether the alterations in mucosubstances were a cause or effect of ulceration Terho and Hartiala (1970) found that restraint did not alter the turnover rate of sulphomucopolysaccharides. Measurement of radiosulphide incorporation into the gastric mucosa indicated that inhibition of sulphation did not begin until the appearance of ulceration, and in animals not developing lesions during the restraint period sulphation was increased. These workers, while not offering any conclusive proof, postulated that the alterations in gastric mucosubstance production were a result rather than a cause of ulceration.

Ludwig and Lipkin (1969) demonstrated that restraint stress reduced the amount of P.A.S. positive staining substance in the gastric mucosa of guinea pigs. Furthermore they found that prefeeding the animals carbenoxolone sodium protected the animals

from stress-induced erosions and increased the amount of P.A.S. positive substance in the gastric mucosa. Dean (1968) found that carbenoxolone sodium qualitatively altered gastric mucus in the rat, the amount of mucus adhering to the mucosa being greater in carbenoxolone-fed animals. Mucosubstance production does appear to be altered by restraining procedures. Whether this is causally related to the ulcers produced by these methods is not resolved, although by analogy with chronic gastric ulcer genesis in man (Cooke, 1967) it is unlikely to be a primary cause of the mucosal lesion.

In 1966 Guth and Hall, while investigating gastric mucosal changes induced by restraint, observed degranulation and decrease in the number of mast cells, and engorgement of small vessels below the surface epithelium. Guth and Kozbur (1969), by neutralizing the gastric contents with an alkaline solution prior to a 30 minute period of restraint, demonstrated that these changes were not due to local acid irritation and suggested that they may result from vagal stimulation or increased adrenocortical hormone secretion. Indeed, Goldman and Rosoff (1968) reported evidence of vagal overactivity in restrained animals and noted mucosal vascular abnormalities prior to ulceration. An interesting relationship between mast cell degranulation and stress induced ulceration is apparent from the observation that heparin depresses mitotic activity in the rat stomach (Rasanen, 1963). Rasanen and

Lahtiharju (1967) further demonstrated that tritiated thymidine incorporation was reduced in the stomach and skin of heparinized mice, indicative of reduced DNA synthesis in these animals. Mast cells are known to contain heparin, and release of heparin from the mucosal mast cells, by reducing cellular proliferation in the gastric epithelium, may lead to the development of mucosal lesions.

The least conflicting area of study of the pathogenesis of acute gastric ulceration induced by stress appears to be the effect of restraint on cellular proliferation in the gastric epithelium. Brown et alii (1963) found that severe starvation reduced the rate of cell renewal in the intestinal epithelium of mice to 50 per cent of the normal rate, and that the rate of migration of the epithelial cell from crypt to villus tip was also reduced. This observation may be an explanation of the effect of pre-stress starvation on the susceptibility to stress-induced gastric lesions. By comparing mitotic figure counts in the glandular stomach of stressed and unstressed rats, Rasanen (1963) demonstrated that non-specific stress reduced cellular proliferation in the stomach. He observed a similar change following a single injection of cortisone or adrenocorticotrophic hormone. In 1966 Lipkin, Kerr and Kim demonstrated that DNA synthesis was reduced prior to the development of mucosal lesions, and suggested that this indicated the primary importance of a

failure of cell replication in the mechanism of ulceration induced by stress. Kim and his colleagues (1967) correlated the reduction in mitotic activity in the gastric mucosa of restrained mice with the incorporation of tritiated thymidine and found that both indices of cellular proliferation were comparably reduced. However they found no difference in tritiated L-leucine incorporation between the groups and concluded that protein synthesis was not altered by stress. Lahtiharju and Rytöma (1967) similarly demonstrated that immobilization reduced the rate of DNA synthesis in the fore and glandular stomach of mice. They also found that tritiated thymidine incorporation was reduced in the skin of the restrained animals, probably indicating that stress reduces cell proliferation through a common, systemic mechanism.

Imondi et alii (1968) and Ludwig and Lipkin (1969) also using restrained animals measured the incorporation of tritiated uridine as an index of RNA metabolism and quantitatively assessed the RNA and DNA content of the mucosa. Imondi and his colleagues demonstrated that fasting for periods up to 40 hours had little if any effect on DNA or RNA metabolism in the gastrointestinal tract. However, they found that restraint produced alteration of both DNA and RNA metabolism, but with some regional differences. As assessed by tritiated thymidine incorporation DNA synthesis was reduced throughout the gastrointestinal tract while the mucosal content of DNA was reduced by restraint to a lesser degree. By

contrast, RNA metabolism was affected only in the stomach of restrained mice. There was decreased synthesis of RNA in the stomach, indicated by decreased uptake of tritiated uridine and the level of mucosal RNA was also reduced. Immobilization however produced no fall in mucosal content of RNA in either the small intestine or the colon. The appearance of mucosal lesions in these animals was coincidental with the reduced uridine incorporation, and they suggested that depression of RNA metabolism, particularly the messenger RNA, may be responsible for the development of stress induced gastric erosions.

Ludwig and Lipkin (1969) studied nucleic acid metabolism in the gastric mucosa of guinea pigs. They found a similar reduction in DNA synthesis in the gastric mucosa of restrained animals, and observed a reduction in mucosal content of RNA in the restrained animals. An indication of impaired RNA synthesis was the observation that the amount of RNA in the mucosa progressively decreased with time. However, unlike Imondi et alii (1968), Ludwig and Lipkin observed an increased incorporation of tritiated uridine into the gastric mucosa. One possible explanation of this difference is the period of restraint selected by these two groups. Ludwig and Lipkin restrained their animals for 4, 18 and 24 hours and observed increased tritiated uridine incorporation at 18 and 24 hours. On the other hand Imondi and co-workers selected 16 and 40 hours as restraint periods; they

found a slightly increased incorporation of uridine at 16 hours and a reduced incorporation at 40 hours. This suggests that reduction of uridine incorporation into RNA may occur late in the sequence of restraint-induced events. Furthermore, as Ludwig and Lipkin observed, ribosomal RNA is not labelled to any extent by a one hour pulse of uridine (Graham and Rake, 1963) yet this constitutes the majority of the RNA within a cell. This may further explain the disparity between gastric mucosal RNA content and measured uridine incorporation in restrained animals. However it is apparent that restraint-induced stress reduces nucleic acid metabolism and cell proliferation in the gastro-intestinal tract. In particular, the demonstration of the altered RNA metabolism in the stomach but not the intestine of restrained animals suggests a causal relationship between RNA metabolism and stress-induced gastric lesions.

The mechanism by which stress produces alteration in nucleic acid metabolism is not clear. The demonstration that cortico-steroids reduce the mitotic rate in the stomach (Myhre, 1960; Rasanen, 1963) suggests that they may be responsible for the restraint ulcer phenomenon. However there is no evidence that concurrent administration of steroids increases the incidence of mucosal lesions (Bonfils et alii, 1957; Flandre and Damon, 1964; Robert and co-workers, 1966).

The normal circadian periodicity of mitosis and cell division

(Clark and Baker, 1962) suggested that disturbance of a central cortical-hypophyseal mechanism might be related to the changes observed in stressed animals. However, as the circadian rhythm persists after hypophysectomy and adrenalectomy, a centrally controlled mechanism is unlikely (Clark and Baker, 1963). Foltz (1964) however, maintained that gastric ulceration was related to a disturbance of the cerebral cortex-hypothalamus-pituitary inter-relationship, and suggested that ultimately peptic ulceration in humans would be treated by brain surgery!

The reduction in granulation and number of mucosal mast cells that occurs with A.C.T.H. and cortisone therapy (Rasanen, 1960) and with restraint (Guth and Kozbur, 1969) suggest a possible pathogenetic relationship between mast cells and stress erosions, and the demonstration that heparin affects cell division (Rasanen, 1963) supports this possibility. Whatever the mechanism, it is likely that reduction in nucleic acid metabolism leading to impaired cell division and replacement is the primary factor in the pathogenesis of stress induced gastric ulceration.

CHAPTER III

PURINE NUCLEOTIDE BIOSYNTHESIS IN GASTROINTESTINAL TISSUE

INTRODUCTION

Gastrointestinal epithelium is a rapidly proliferating tissue (Messier and Leblond, 1960) and the probable physiological importance of purine nucleotide biosynthetic enzymes in such tissue has been discussed in the previous chapter. However, few studies of these enzymes in gastrointestinal mucosa have been reported, nor have enzyme levels been correlated with the rate of nucleic acid synthesis in this tissue. Furthermore, the relative importance of the alternative pathways of purine nucleotide synthesis - de novo or salvage - remains unresolved. The purpose of this initial investigation was:

1. To confirm the presence of the purine phosphoribosyltransferases in gastrointestinal tissue, and to examine the distribution of these enzymes within the gastrointestinal tract.

2. To determine the relative importance of the alternative pathways of purine nucleotide synthesis. This was considered necessary because of the suggestion by Smellie et alii (1956) that there were two types of tissue. These workers postulated that there was one type capable of synthesizing the purine ring from non-cyclic precursors, and another type dependent on a

supply of preformed purine for conversion into tissue nucleic acids. Assessment of the activity of the de novo pathway, not previously studied in gastrointestinal mucosa, is necessary before any conclusion as to the relative importance of the two pathways can be drawn.

3. To extend the animal work to the human situation by establishing reliable methods of assaying these enzymes in the small specimens of tissue provided by standard intestinal biopsy tubes.

MATERIALS AND METHODS

A. *REAGENTS*

1. *Radionuclides*

(a) 100 microcuries of adenine 8-C^{14}, specific activity 51.1 millicuries per millimole (Amersham), was dissolved in 83.3 ml of glass distilled water to result in a concentration of 23.5 micromolar adenine (1.2 µCi/ml).

(b) 100 microcuries of hypoxanthine 8-C^{14}, specific activity 60.1 mCi per mM (Amersham) was dissolved in 10 ml of glass distilled water to result in a concentration of 55.4 µM hypoxanthine (3.33 µCi per ml).

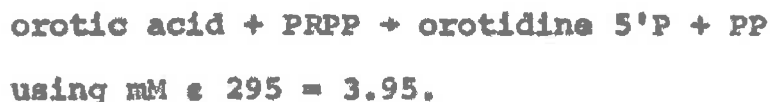
(c) 50 microcuries of L glutamine C^{14}, specific activity 36.3 mCi per mM (Amersham), was dissolved in 5 ml of glass distilled water to result in a concentration of 276 μM L glutamine (10 μCi per ml). Aliquots of the above radionuclide solutions were stored at - 15°C.

2. *Phosphoribosylpyrophosphate (PRPP)*

100 mg of the magnesium salt of PRPP (Sigma Chemical Company) was converted to the sodium form because of the greater stability of the sodium salt with storage (Atkinson and Murray, 1969). The following procedure was adopted to achieve the ionic exchange, and conducted in an environmental temperature of 4°C.

100 mg of magnesium PRPP was dissolved in 2.0 ml of glass distilled water at 2°C with 0.8 grams of Chelex-100 (sodium form, Bio-Rad Laboratories) and allowed to stand for 10 min. This was then eluted through a 0.5 cm x 3.4 cm column of Chelex-100 previously adjusted to pH 7 with glass distilled water at 2°C. The PRPP remaining on the column was further eluted with 1.6 ml of water, and the eluate adjusted to pH 7 by the addition of an appropriate volume of 0.1 N hydrochloric acid. The PRPP thus obtained was stored at - 15°C.

The concentration of this PRPP was then determined by the method of Kornberg, Lieberman and Simms (1955). This is an enzymatic assay based on the observed decrease in optical density at 295 mμ per the following reaction:



orotic acid + PRPP → orotidine 5'P + PP

using mM ε 295 = 3.95.

The enzyme catalysing this reaction, oritydilic pyrophosphorylase, was prepared from brewers yeast (South Australian Brewing Company) by the method of Murray, Wong and Friedrichs (1969).

A final concentration of 15 mM PRPP was achieved by dilution, and aliquots of 0.3 ml were stored at - 15°C.

All other reagents employed in the enzyme assays were of Analytical grade and are detailed in Appendix A.

B. COLLECTION OF BIOLOGICAL SAMPLES

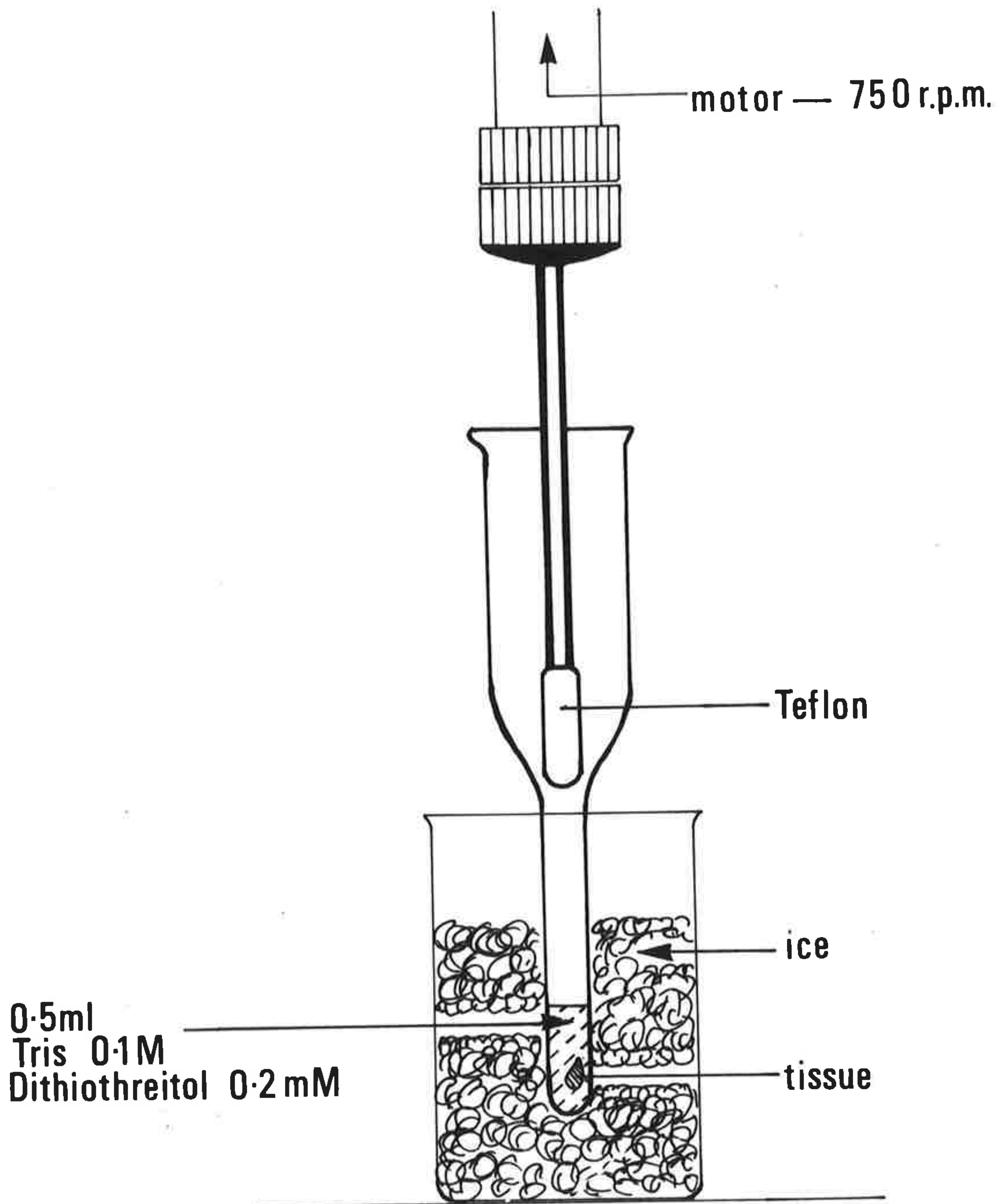
1. Guinea pigs: All animals were from a line selectively inbred for more than 20 years at the Institute of Medical and Veterinary Science, Adelaide. The animals were matched for age, sex and weight at the Institute and then allowed two days in which to adapt to the new environment of the animal room in the Department of Medicine, Royal Adelaide Hospital. Here they

were kept in separate metabolic cages (30 cm x 60 cm) and allowed free access to food (M & V rabbit pellets, William Charlick Limited) and water.

The guinea pigs were then fasted for 12 hours and killed by a blow on the head. Tissues obtained were placed in 0.9% sodium chloride on ice. Full thickness specimens of mucosa of equal size and approximating 30 mg wet weight were cut with a No. 3 cork borer from the proximal stomach (fundus), distal stomach (antrum), duodenum, jejunum and ileum. Each mucosal specimen was homogenized in 0.5 ml of 2 mM dithiothretol, 0.1 M Tris chloride buffered at pH 8.0 in a Potter-Elvehjam homogenizer with teflon pestle. The homogenizer was supported in a beaker of crushed ice, and blending was carried out for 10 to 15 seconds at 750 r.p.m. (Figure IV). The homogenate was then submitted to centrifugation at 8000 g for 10 min at 4°C. The supernatant was maintained at 2°C until assayed for enzyme activity, invariably within 30 minutes of specimen collection.

2. Humans. (a) Gastric specimens: Fundic and antral biopsy specimens were obtained from healthy volunteers using a Watson intestinal biopsy capsule (Crosby type, Crosby and Kugler, 1957). The subjects fasted overnight and next morning swallowed the capsule without prior pharyngeal anaesthesia. In each case the biopsy capsule was localized to the appropriate area (greater curve fundus, or pyloric canal) under radiological control and

FIGURE IV



TISSUE HOMOGENIZATION

the specimen was cut immediately after localization.

(b) Duodenal and jejunal specimens: Duodenal and jejunal tissue was obtained from healthy volunteer subjects using the Watson intestinal biopsy capsule. The same procedure as for gastric biopsy was employed with radiological localization of the capsule to the second part of the duodenum or the jejunum just distal to the ligament of Trietz. Metoclopramide (Beecham) was administered intravenously in each case to facilitate passage of the capsule through the pylorus.

(c) Ileal and colonic tissue: Ileal and proximal colonic tissue was obtained from three patients undergoing partial or total colectomy (Appendix C). Immediately after removal, surgical specimens were placed on ice and full thickness mucosal specimens were cut with a No. 3 cork borer. These tissues were then placed in 0.9% sodium chloride at $2^{\circ}C$.

3. Preparation of human samples: All tissues obtained from a human source were processed as described previously for guinea pig tissue.

C. LABORATORY APPARATUS AND EQUIPMENT

All apparatus and equipment used in the assay procedures described in this chapter are detailed in Appendix B.

METHODS

1. *DETERMINATION OF PROTEIN CONCENTRATIONS OF THE HOMOGENATES*

The protein concentration in tissue homogenates was initially determined by the method of Lowry, Rosebrough, Farr and Randall (1951) utilizing the Folin-Ciocalteu reagent. The standard protein used was bovine serum albumin, grade II, in a crystalline form (Sigma). This method, however, provided some inconsistent results in this laboratory and was discarded in favour of the method described by Bramhall, Noack, Wu and Loewenberg (1969) utilizing naphthalene blue-black as the colorimetric reagent. The specific enzyme activities reported in this thesis are in terms of the protein concentration as determined by this latter method.

2. *ASSAY OF PHOSPHORIBOSYLPYROPHOSPHATE AMIDOTRANSFERASE ACTIVITY (E.C.2.4.2.14)*

The method employed was that described by Tay, Lilley, Murray and Atkinson (1969) in which the rate of L-glutamate C^{14} formation from L-glutamine C^{14} is the measure of activity of this enzyme.

Because of the expected low levels of activity in gastro-intestinal tissue, the reaction mixture was incubated for 60 min at 30°C. The final volume of the reaction mixture was 0.1 ml and contained:

25 mM Tris-chloride (pH 8.0

5.5 mM $MgCl_2$

0.5 mM dithiothretol

0.75 mM PRPP

1.0 mM L-glutamine

0.0276 mM L-glutamine C^{14} (0.1 µCi).

The amino acids (glutamine and glutamate) were separated by chromatography in ethanol, n butyl alcohol, formic acid and water (12:4:1:3) for 16 hours, and radioactivity in the glutamate zone assessed by liquid scintillation counting.

8. PURINE PHOSPHORIBOSYLTRANSFERASE ASSAYS

(a) Adenine phosphoribosyltransferase (E.C.2.4.2.7). The method used was that described by Murray, Wong and Friedrichs (1969), in which the rate of formation of C^{14} AMP from adenine 8-C^{14} is the measure of the activity of this enzyme. The final volume of the reaction mixture was 0.1 ml and contained:

80 mM Tris-chloride pH 7.8

8.mM $MgCl_2$

4.7 µM adenine 8-C^{14} (0.024 µCi)

0.75 mM PRPP

0.01 ml of tissue homogenate was added and incubation carried out for 3 minutes at 30°C.

(b) Hypoxanthine phosphoribosyltransferase (E.C.2.4.2.8). The method used was essentially similar to the method used for the determination of adenine phosphoribosyltransferase with 11.1 μM hypoxanthine 8-C^{14} (0.67 μCi) instead of adenine 8-C^{14} and with internal markers of hypoxanthine and AMP for the chromatographic separation procedure.

RESULTS

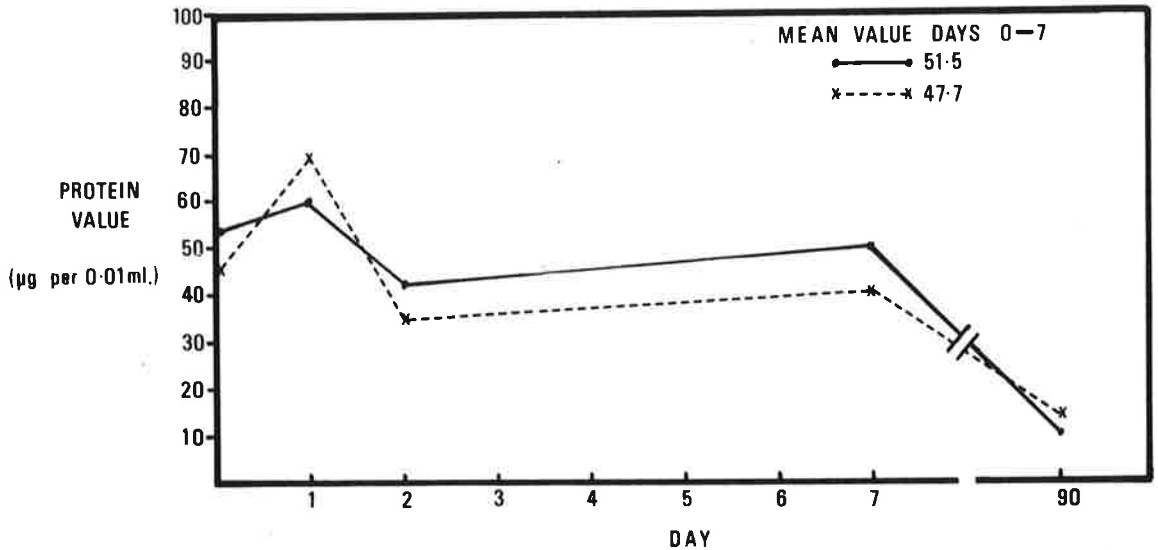
1. *PROTEIN ASSAY*

In the early stages of this study the protein concentration of tissue homogenates was determined by the Lowry method some weeks after preparation of the specimen. The homogenates were stored at - 15°C in the interim. A chance observation that protein assays performed on stored tissue samples yielded considerably lower values than assay of freshly prepared tissue led to further analysis of this phenomenon.

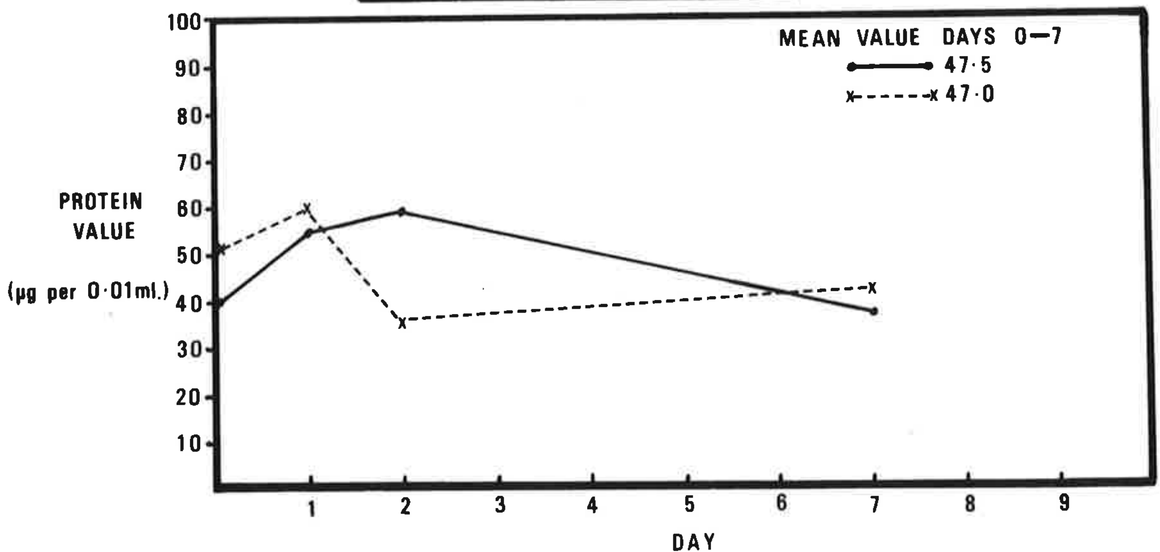
A freshly homogenized sample of gastric tissue was assayed in duplicate on the day of preparation and after 1, 2 and 7 days storage at - 15°C. The duplicate assays were performed by two individuals working independently. Freshly prepared reagents and separate protein standards were used. The results are detailed in figure 5. The mean values obtained in independent duplicate assays over this period were comparable. However, there was considerable variation of determined protein value within this time period, and no consistent pattern was apparent. Indeed

FIGURE V

Reproducibility of protein values on tissue sample as determined by the Lowry - Folin method.



Duplicate samples (•——•, x- - - - -x) on tissue homogenate determined at intervals after tissue preparation. (Worker A).



Duplicate samples (•——•, x- - - - -x) on tissue homogenate determined at intervals after tissue preparation. (Worker B).

TABLE 3. REPRODUCIBILITY OF PROTEIN ASSAY BY METHOD OF BRAMHALL ET ALII (ONE SAMPLE OF GUINEA PIG STOMACH)

NO. OF ASSAYS	35
RANGE	38 - 44 µg per 0.01 ml
MEAN	41.7 µg per 0.01 ml
S.D.	1.31
COEFFICIENT OF VARIATION	3.1%

TABLE 4. COMPARISON OF LOWRY AND BRAMHALL METHODS

(Values in μg per 0.01 ml homogenate)

SPECIMEN	LOWRY	BRAMHALL
1	45	34
2	55	37
3	48	32
4	54	32
5	48	32
6	51	32
7	40	32
8	70	41
9	36	23
10	42	32
11	42	31
12	40	24
13	34	21
14	38	31
15	45	33
16	37	30
17	38	30
18	50	32
19	37	28
20	38	35
21	29	32

Mean difference between Lowry protein and Bramhall protein + 12.6 μg per 0.10 ml.

variation of 20% from the mean recorded value was not uncommon.

The impression that the protein concentration of the homogenates was reduced by prolonged storage at - 15°C was confirmed by the gross reduction in protein concentration in samples stored for 3 months. The protein concentration of samples stored for 6 months, (not represented in graph) was consistently less than 10 μg per 0.01 ml of homogenate compared with a baseline value of approximately 50 μg per 0.01 ml of homogenate.

The consistency of the colorimetric determination of protein concentration by the method of Bramhall et alii (1969) was then assessed. The results of these determinations are illustrated in Table 3, and are comparable to those described by Bramhall and colleagues. These workers found that the coefficient of variation within a test ranged from 2 to 5%.

The protein concentration of 21 different specimens of guinea pig gastrointestinal mucosa was determined coincidentally by the Lowry and the Bramhall methods (Table 4). The Lowry method consistently revealed a higher value than did the Bramhall method, and a mean difference of + 12.6 μg per 0.01 ml was recorded. This consistent elevation is in agreement with the finding of Bramhall et alii (1969) that the presence of buffering and reducing agents such as those used in preparation of the homogenate elevated the protein value as assessed by the Folin-Lowry technique.

TABLE 5. RADIOACTIVE COUNTS (IN C.P.M. CORRECTED FOR QUENCHING INEFFICIENCY) FOR ASSAYS OF PRPP AMIDOTRANSFERASE IN GUINEA PIG STOMACH, INTESTINE AND LIVER

STOMACH	1	2	3	4	5	6	7	8	9	10
$^*B_1$	540	289	371	295	432	326	724	417	702	330
B_2	564	245	382	304	354	297	680	348	858	320
T_1	482	426	320	412	540	357	534	462	596	316
T_2	556	450	375	376	576	287	570	415	746	350
INTESTINE	1	2	3	4	5	6	7	8	9	10
B_1	319	257	202	271	225	419	394	270	300	294
B_2	382	266	223	263	282	387	360	311	315	306
T_1	525	270	286	265	278	312	627	242	354	410
T_2	558	300	273	314	296	450	593	272	372	
LIVER	1	2	3	4	5					
B_1	1048	770	1710	1435	998					
B_2	1120	742	1585	1360	874					
T_1	6574	5524	7004	5550	4323					
T_2	6934	6027	6990	5874	4560					

$^*B_1$, B_2 — Control assays (in duplicate) with no added PRPP

T_1, T_2 — Test assays (in duplicate) with added PRPP

2. *PHOSPHORIBOSYLPYROPHOSPHATE AMIDOTRANSFERASE ACTIVITY*

The results of the assay of this enzyme, to the stage of assessment of radioactive glutamate formation, are illustrated in Table 5. The values are expressed in counts per minute and have been corrected for scintillation fluid quenching.

Ten specimens of guinea pig gastric mucosa and ten specimens of guinea pig jejunum were studied. The difference between control and test assays (a reflection of net P.R.P.P.-dependent glutamate formation) was variable, and in no sample was the mean difference between test and control assays greater than 200 counts per minute. In some specimens the mean control value was greater than the test value. Protein concentration of the mucosal homogenates was 30 to 50 µg per 0.01 ml.

Specimens of liver from five guinea pigs were assayed for phosphoribosylpyrophosphate amidotransferase activity. The values, in counts per minute, are illustrated in Table 5. In each case there was very much greater glutamate-associated radioactivity in test assays than in the controls. The mean specific activity of this enzyme in guinea pig liver was 35 nanomoles glutamate formed per mg protein per hour (range 26-41). The average protein concentration of liver homogenates was 82 µg per 0.01 ml.

Five specimens of human gastric mucosa and two specimens of

TABLE 6. ACTIVITY OF PHOSPHORIBOSYLPYROPHOSPHATE AMIDO-TRANSFERASE IN GUINEA PIG AND HUMAN TISSUE

Species	Tissue	Activity
Guinea pig (5 animals)		
	fundus antrum duodenum jejunum ileum	No activity detected (counts per minute glutamate C^{14} - 100 to + 200)
	liver	35.0 nanomoles glutamate formed per mg protein per hour (counts glutamate C^{14} average 5400)
Human (5 persons)		
	fundus antrum	No activity detected (counts per minute L-glutamate C^{14} - 100 to + 200)

jejunal tissue were assayed for phosphoribosylpyrophosphate amidotransferase activity; the values obtained were comparable to those for guinea pig mucosa and are not represented. Human liver was not assayed.

3. *PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY*

With each tissue extract the rate of nucleotide formation in the absence of P.R.P.P. (control assay) was always less than 7 per cent of the rate in the presence of this compound. The rates, or enzyme activities, reported in this thesis have been corrected for this control activity which is presumably due to the presence of some P.R.P.P. in the tissues homogenized.

All enzyme activities are expressed as nanomoles of nucleotide formed per milligram of homogenate protein per hour (nM/mg/hour).

(a) Guinea pigs. Gastric mucosal specimens obtained from ten animals were studied and the mean values obtained together with the range and standard deviation of values are illustrated in Table 7. In nine animals purine phosphoribosyltransferase activity was also assayed in specimens obtained from proximal and distal small intestine (Table 7).

Four duodenal specimens were obtained and studied. The determined mean value of hypoxanthine phosphoribosyltransferase

TABLE 7. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GUINEA PIG GASTROINTESTINAL MUCOSA

(in nanomoles nucleotide formed per mg of homogenate protein per hour)

Site	Substrate	Statistic	Value
Fundus (10 animals)			
	Hypoxanthine	Mean	38.6
		S.D.	3.6
		Range	33.5 - 47.4
	Adenine	Mean	28.8
		S.D.	6.0
		Range	21.5 - 41.2
Antrum (10 animals)			
	Hypoxanthine	Mean	35.2
		S.D.	5.6
		Range	24.3 - 45.7
	Adenine	Mean	32.6
		S.D.	4.6
		Range	24.8 - 41.6
Jejunum (9 animals)			
	Hypoxanthine	Mean	19.8
		S.D.	2.1
		Range	17.2 - 23.7
	Adenine	Mean	67.3
		S.D.	9.2
		Range	57.2 - 87.4
Ileum (9 animals)			
	Hypoxanthine	Mean	13.6
		S.D.	3.0
		Range	9.8 - 18.7
	Adenine	Mean	45.9
		S.D.	8.6
		Range	36.7 - 65.3

activity in this tissue was 20.0 (range 16.6 to 22.5) nM/mg/hour, while the value for the adenine converting enzyme was 35.2 (range 28.9 to 42.1) nM/mg/hour.

(b) Humans. The Watson intestinal biopsy capsule delivers a specimen ranging from 8 to 20 mg wet weight. The hypoxanthine and adenine phosphoribosyltransferase activities in human fundic, antral, duodenal, jejunal, ileal and colonic tissue are illustrated in Table 8.

COMMENT

1. *PROTEIN CONCENTRATIONS*

The consistency of the method used to determine the protein concentration of the tissue homogenates is critical to the accurate expression of enzyme activity. The determined protein value is a denominator in the calculation of the enzyme specific activity, and any inconsistency in protein values will be reflected in the final expression of enzyme activity. It therefore follows that the more reproducible the method of protein determination, the more reproducible are the enzyme values.

The ideal expression of enzyme activity is in terms of the actual enzyme protein content of the sample assayed. However, with impure enzyme preparations this ideal cannot be attained, and enzyme activities are therefore commonly expressed in terms of the

TABLE 7. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GUINEA PIG GASTROINTESTINAL MUCOSA

(in nanomoles nucleotide formed per mg of homogenate protein per hour)

Tissue	Substrate	Statistic	Value
Fundus (10 animals)			
	Hypoxanthine	Mean	38.6
		S.D.	3.6
		Range	33.5 - 47.4
	Adenine	Mean	28.8
		S.D.	6.0
		Range	21.5 - 41.2
Antrum (10 animals)			
	Hypoxanthine	Mean	35.2
		S.D.	5.6
		Range	24.3 - 45.7
	Adenine	Mean	32.6
		S.D.	4.6
		Range	24.8 - 41.6
Jejunum (9 animals)			
	Hypoxanthine	Mean	19.8
		S.D.	2.1
		Range	17.2 - 23.7
	Adenine	Mean	67.3
		S.D.	9.2
		Range	57.2 - 87.4
Ileum (9 animals)			
	Hypoxanthine	Mean	13.6
		S.D.	3.0
		Range	9.8 - 18.7
	Adenine	Mean	45.9
		S.D.	8.6
		Range	36.7 - 65.3

total protein concentration of the enzyme preparation (Dixon and Webb, 1964). While the method described by Lowry et alii (1951) is a simple and sensitive method, it suffers from interference by many compounds. As Bonitati, Elliott and Miles (1969) observed, the chief shortcoming of this method is poor specificity. The determination of protein concentration of specimens containing mixed proteins is further complicated by the protein standard required for the calculation of the homogenate protein concentration. In most cases a relatively pure protein, such as bovine serum albumin, is utilized, and this increases the lack of specificity of the method. Ideally, a protein composite resembling the mixed proteins in mucosal homogenates should be the protein standard. Furthermore, a number of substances almost certainly present in mucosal homogenates, such as purines, sugars and free amino acids, interfere in this assay (Lowry et alii, 1951; Diamant, von Redlich and Glick, 1967; Bonitati et alii, 1969). In addition the buffering and reducing agents used in the process of tissue homogenization provide interference with the Lowry reaction (Robson, Goll and Temple, 1968; Bramhall et alii, 1969).

In this study the finding that the Lowry method provided a higher apparent protein value than that determined by the Bramhall method was anticipated. Bramhall et alii (1969) in describing their method, observed that the presence of buffering and reducing

agents elevated the Lowry value, but did not alter the protein value determined by their technique. However, the lack of reproducibility of protein values determined by the Lowry method in this laboratory was unexpected and disturbing. This lack of reproducibility cannot be adequately explained by the presence of interfering substances as their concentration should remain reasonably constant despite the lack of acid precipitation. Lowry et alii in 1951 stressed that the Folin-Ciocalteu reagent should be rapidly added to and mixed with the other reagents, and variation in methodology here may explain some of the inconsistency However, in this initial study standardisation of technique from test to test was maintained. One must conclude therefore, that in this laboratory the Lowry method of protein determination does not provide consistent results.

Progressive decrease of protein content of tissue homogenates was recorded during storage at - 15°C. It was originally supposed that the tyrosine and tryptophan residues in a protein provided the colour reaction in the Lowry method, but Bailey (1967) recently pointed out that any peptide bond will yield some colour, and the amino acid sequence in a peptide chain is important to the colour yield of any particular protein. For this reason enzymatic digestion of protein at - 15°C with consequent loss of peptides appears the likely explanation of the reduction in determined protein concentration that occurs with storage.

In this study therefore, all enzyme activities are expressed in terms of the protein value as determined by the method of Bramhall et alii (1969) on the day of tissue preparation. The lower, but more reproducible values recorded with this method means that the enzyme activities reported herein are strictly comparable only to values achieved with this method of protein assay.

2. *PHOSPHORIBOSYLPYROPHOSPHATE AMIDOTRANSFERASE ACTIVITY*

This enzyme is difficult to assay with accuracy because of its structural instability (Rowe and Wyngaarden, 1968; Tay et alii, 1969) and relatively low concentrations in mammalian tissue (Reem and Friend, 1969). Tay and his colleagues emphasized that as the regulatory properties of this enzyme are readily modified on storage, studies of its activity must be carried out on freshly prepared tissue. This point was strictly observed in this study.

The demonstration of radioactivity associated with the glutamate zone in control or PRPP-independent assays indicates that conversion of glutamine to glutamate does occur even in the absence of P.R.P.P. This conversion is not apparent with purified phosphoribosylpyrophosphate amidotransferase preparations and is due to the presence of L-glutamine amidohydrolase (E.C.3.5.1.2.) in the tissue homogenate (Atkinson and Murray, 1969) This latter enzyme is highly active in liver and the finding of

greater P.R.P.P.-independent glutamate formation in liver than intestinal tissue is compatible with this explanation of the high control values.

The rate of P.R.P.P.-dependent glutamate formation, that is the difference between test and control assays per unit time, is an index of phosphoribosylpyrophosphate amidotransferase activity, and the presence of some apparent P.R.P.P. dependent glutamate formation in some mucosal samples suggests that this enzyme may be present in gastrointestinal tissue. Possibly the method of enzyme distraction or the assay conditions were not optimal for the assessment of the activity of this enzyme. However, guinea pig liver processed and assayed in the same way demonstrated moderate levels of this enzyme. Presumably, therefore, if the de novo pathway of purine nucleotide biosynthesis was active and of major importance in gastrointestinal mucosa, greater activity of the rate controlling enzyme should be apparent. One may conclude that using the method described by Tay et alii (1969) for measuring phosphoribosylpyrophosphate amidotransferase activity, there is negligible demonstrable activity of this enzyme in gastro-intestinal epithelium.

Since a supply of purine nucleotide is fundamental to continuing nucleic acid and nucleotide coenzyme synthesis, the corollary of this finding is that gastrointestinal tissue is relatively dependent on purine salvage mechanisms for provision of

purine nucleotide. Gastrointestinal tissue is particularly favoured with respect to supply of exogenous purine, and it is conceivable that dietary purines may be utilized during the transport from intestinal lumen to blood stream. In the absence of dietary purine, one could predict continuing salvage of purines derived from such sources as sloughed intestinal cells and bacterial nucleic acids liberated by luminal nucleases.

Furthermore, even in the absence of dietary purine, the low activity of the de novo pathway is still compatible with the generally accepted concept of the liver being the major source of purine synthesized de novo. The recent work of Pritchard, Chavez-Peon and Berlin (1970) indicated that purines are released from the liver to become available for nucleotide synthesis in non-hepatic tissues, and it is probable that even under normal physio-logical conditions, let alone the artificial situation of dietary purine deprivations, the liver is an important source of purine for gastrointestinal tissue. This concept is supported by the demonstration of significant activity of both hypoxanthine and adenine phosphoribosyltransferase in the jejunal mucosa of rats 12 weeks after a jejunal by-pass operation to eliminate luminal nutrition (Dowling, Elias and Mackinnon, unpublished observations).

Presumably, therefore, gastrointestinal mucosa is relatively dependent on the salvage mechanism for the adequate provision of purine nucleotides, and it is conceivable that measurement of the

activity of the purine salvage pathway enzymes may provide an index of the rate of purine nucleotide synthesis in such tissue. Although purine nucleotides are utilized both for nucleic acid and nucleotide coenzyme synthesis, it is possible that information of the rate of nucleic acid synthesis may be extrapolated.

3. *PURINE PHOSPHORIBOSYLTRANSFERASES*

These enzymes were present in all areas of the gastro-intestinal tract studied, thus confirming previous reports (Rosenbloom et alii, 1967; Balis, 1968; Imondi et alii, 1969) of their presence in gastrointestinal mucosa. In contrast to the findings of Balis (1968) and Imondi et alii (1969), in which studies enzyme activity was expressed in terms of mucosal DNA content, the present study demonstrated greater overall activity of the adenine than the hypoxanthine converting enzyme. Here activity is expressed in terms of mucosal homogenate protein content, but it is unlikely that this difference in expression is the explanation of the apparent difference between these studies. Furthermore the finding of greater levels of adenine converting enzyme is in agreement with the study of Berlin and Hawkins (1968) who also found greater adenine than hypoxanthine converting activity. It appears therefore, that no definite significance can be attached to the regional variation in ratios of these enzymes. In particular this study found no support for the observation of Balis (1968) that the duodenum was markedly different from other areas in the gastrointestinal tract with respect to the ratio of these enzymes.

CHAPTER IV

STUDIES ON THE EFFECT OF STRESS RESTRAINT ON PURINE NUCLEOTIDE METABOLISM IN GUINEA PIG GASTROINTESTINAL MUCOSA

INTRODUCTION

Acute gastric ulceration may be produced in animals by a number of techniques. Those most widely reported are pyloric ligation (Shay et alii, 1945) and the technique of inducing psychic stress by immobilization (Rossi et alii, 1956). While the mechanism of acute ulceration induced by immobilization or restraint has been extensively investigated (Brodie and Hanson, 1960; Lahtiharju and Rytomaa, 1967; Kim, Kerr and Lipkin, 1967; Ludwig and Lipkin, 1969) the ultimate cause of the mucosal lesion has not yet been determined.

Assessing the rate of DNA synthesis by tritiated thymidine incorporation with autoradiography, Lahtiharju and Rytomaa (1967) demonstrated that non-specific stress reduced the rate of DNA synthesis in the stomach and skin of mice. Imondi and co-workers (1968) found that the rate of DNA synthesis was reduced throughout the gastrointestinal tract in restrained mice. However, reduction in RNA synthesis was confined to the gastric mucosa. Ludwig and Lipkin confirmed that both DNA and RNA metabolism in the gastric mucosa were affected by restraining the animal, and commented " .. in the guinea pig under stress, loss of RNA and decreased DNA synthesis and cell proliferation develop in the gastric mucosa. These events are accompanied by loss of cells from the mucosa and the development of progressively increasing numbers of gastric erosions."

From such experiments it is firmly established that restraint procedures producing acute gastric mucosal injury in animals give rise to impaired nucleic acid metabolism in the gastrointestinal mucosa. It was considered that the model of restraint induced gastric mucosal injury in guinea pigs would be suitable for testing the hypothesis that the level of activity of the purine phosphoribosyltransferases in gastrointestinal mucosa reflected the rate of nucleic acid synthesis in the tissue. Therefore the purpose of the investigations described in this chapter was:

1. To confirm, in a limited pilot study, that nucleic acid metabolism was reduced by restraint procedures.

2. To establish whether there is a relationship between the activity of the purine salvage pathway enzymes and the rate of nucleic acid synthesis in gastrointestinal tissue.

MATERIALS AND METHODS

1. ASSESSMENT OF NUCLEIC ACID METABOLISM IN GASTROINTESTINAL MUCOSA

Four guinea pigs, matched for age, sex and weight, were obtained from the Institute of Medical and Veterinary Science, Adelaide and maintained for two days in the Department of Medicine as previously described. Two of these animals (controls) were

FIGURE VI

GUINEA PIG RESTRAINED IN WIRE MESH ENVELOPE AND SUSPENDED IN METABOLIC CAGE.

fasted for 40 hours and then given 0.5 microcuries per gram body weight of 6-^{3}H-thymidine (Amersham, 22.8 curies per mM) intraperitoneally. One hour later the animals were killed by a blow on the head and gastrointestinal tissue was obtained and processed as described below.

Two animals were completely immobilized for 40 hours in a wire mesh envelope (Figure VI). On removal from the restraint envelope, 6-^{3}H-thymidine was administered and the animals were killed one hour later.

(a) Autoradiography. Full thickness non-ulcerated mucosal specimens from the fundus, antrum, jejunum and ileum were cut and placed in 10 per cent formalin for 24 hours. The tissues were paraffin-embedded and sections were cut at 4 microns and mounted on glass slides. The slides had previously been cleaned in chromic acid, washed in distilled water and dipped into a solution containing 0.5 per cent bacteriological gelatin (Davis) and 1:20,000 merthiolate in distilled water. Section-bearing slides were then passed through xylol, absolute, 90, 70 and 50 per cent alcohol and finally distilled water.

The sections were coated with Ilford K5 nuclear research emulsion (gel form) in a darkroom illuminated by an Ilford safe-light (S902). Twenty grams of emulsion was mixed with 20 ml of distilled water in a glass beaker and melted at 40°C in a water

bath for 15 minutes. The emulsion was stirred gently to avoid local over-heating. A thin film of emulsion was obtained by dipping a thin wire loop, 2.5 cm in diameter into the emulsion, and the film suspended on the loop was then applied to the slides over the tissue sections. The emulsion was then dried in a stream of warm air, and when dry the slides were placed in a plastic slide box (Kartell) which was sealed with black plastic tape and placed in a light-proof container. After two weeks exposure at room temperature the slides were removed from the container in the darkroom and developed.

The slides were immersed in Ilford ID2 developer diluted 1 in 3 with glass distilled water for 5 minutes at 20°C. They were then rinsed with distilled water and placed in Ilford Hypan liquid fixer for 10 minutes. After washing in running water for 15 minutes, they were dried at room temperature. The sections were then stained with haematoxylin and eosin, dehydrated in alcohol and mounted.

Thymidine incorporation was assessed by random counting of the number of labelled nuclei per thousand cell nuclei.

(b) DNA content of the mucosa. Two full thickness mucosal specimens were cut with a No. 3 cork borer from the fundus, antrum, jejunum and ileum. These specimens, which were selected to avoid ulcerated mucosa, were homogenized in 0.5 ml of 0.1 M

Tris chloride pH 8.0 by the method previously described. These were then prepared for nucleic acid determination by the method of Schneider (1957). A 0.5 ml aliquot of the final solution was assayed for DNA content by the diphenylamine procedure (Schneider, 1957) using calf thymus DNA (Sigma) as the standard. The radioactivity contained in a further 0.5 ml aliquot was then measured in a Packard liquid scintillation counter with automatic external standardization.

2. *PURINE PHOSPHORIBOSYLTRANSFERASES*

Thirty-two guinea pigs obtained from the Institute of Medical and Veterinary Science and matched for age, sex and weight were examined.

Eight animals (control series) were fasted for 40 hours, then killed by a blow on the head. The stomach was removed from each animal, opened along the greater curvature and placed in 0.9 per cent sodium chloride at 2°C. The mucosa was inspected under a variable power dissecting microscope (Olympus) and the appearance recorded, noting in particular the extent of ulceration when present. Full thickness mucosal specimens were cut from the fundic and antral mucosa carefully excluding areas of ulceration, and from the jejunum and ileum. These tissues were processed for enzyme assay as previously described. In addition mucosal specimens from each area were placed in 10 per cent formalin and sent to the Institute of Medical and Veterinary Science for sectioning and

staining with haematoxylin and eosin, periodic acid-Schiff and Hales colloidal iron stains.

Eight animals were fasted and completely immobilized in separate cages for 40 hours. Immobilization was achieved by placing the animal in a flywire envelope, and then restricting available space by stapling the wire in close proximity to the animal's body (Figure VI). On removal from the envelope the animals were killed and the tissues were removed, inspected and processed for enzymic assay. Specimens were also sent to the Institute of Medical and Veterinary Science for staining for mucosubstances.

A further eight animals were immobilized for 20 hours and another group of eight for 10 hours. In both instances gastric tissue only was selected for enzyme assay.

RESULTS

1. *TRITIATED THYMIDINE INCORPORATION*

This study was too small for valid statistical analysis, but certain trends were apparent. There was a reduction in tritiated thymidine incorporation throughout the gastrointestinal tract as assessed by the two techniques of autoradiography and liquid scintillation counting (Tables 9 and 10). The reduction was more apparent with the liquid scintillation counting method, where there was greater than 50 per cent reduction in recorded radio-activity in all areas. However, the reduction in tritiated

TABLE 9. D.N.A. CONTENT AND TRITIATED THYMIDINE INCORPORATION OF GASTRIC MUCOSA FROM 40 HOUR RESTRAINED AND UNRESTRAINED (CONTROL) GUINEA PIGS

		μg D.N.A. (per 0.50 ml solution)	Tritiated thymidine incorporation (c.p.m. per 0.50 ml solution)	Autoradiographic assessment of tritium incorporation (Labelled cells per 1000)
UNRESTRAINED				
I	Fundus	310	1506	62
I	Antrum	240	2100	71
I	Jejunum	330	4620	140
I	Ileum	280	4340	97
II	Fundus	325	1820	68
II	Antrum	285	1600	70
II	Jejunum	380	4130	124
II	Ileum	265	3900	94
RESTRAINED				
III	Fundus	250	480	35
III	Antrum	210	840	28
III	Jejunum	270	1420	87
III	Ileum	240	1500	64
IV	Fundus	285	730	32
IV	Antrum	210	750	32
IV	Jejunum	290	1250	84
IV	Ileum	260	1720	53

TABLE 10. MEAN VALUES OF D.N.A. CONTENT, TRITIATED THYMIDINE INCORPORATION. GASTRIC MUCOSA FROM RESTRAINED AND UNRESTRAINED GUINEA PIGS

	μg D.N.A. (per 0.5 ml solution)	Tritium incorporation (c.p.m. per 0.5 ml solution)	Autoradiography (Labelled cells per 1000)	
UNRESTRAINED				
Fundus	317	1663	65	(neck zone)
Antrum	262	1850	70	(neck zone)
Jejunum	355	4375	132	(crypt zone)
Ileum	272	4120	95	(crypt zone)
RESTRAINED				
Fundus	267	605	33	(neck zone)
Antrum	210	795	30	(neck zone)
Jejunum	280	1330	85	(crypt zone)
Ileum	262	1610	58	(crypt zone)

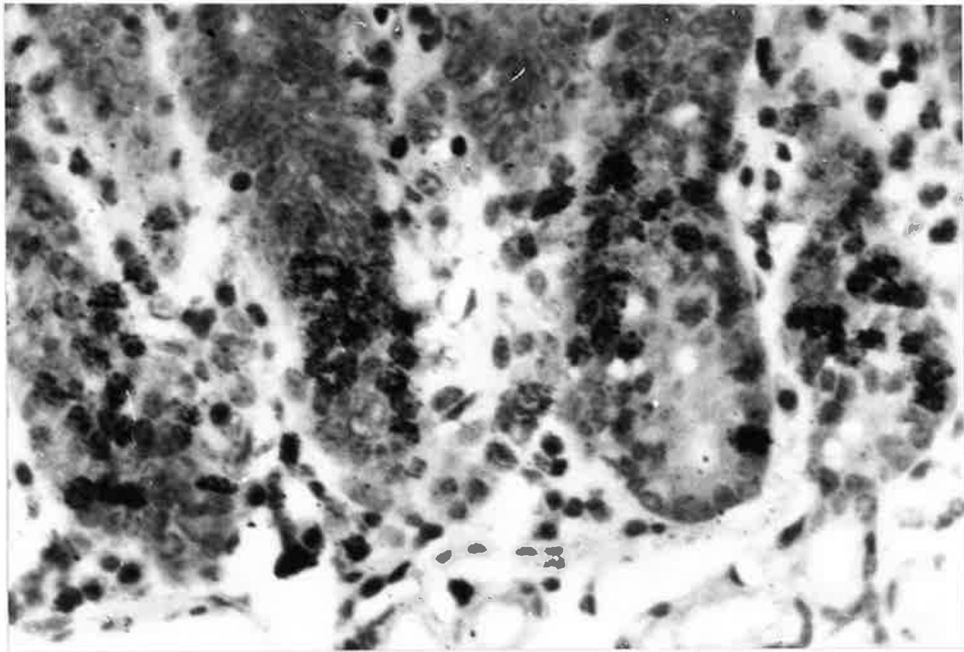
thymidine incorporation was apparent with the autoradiographic method and the autoradiographic appearance is illustrated in Figures VII and VIII. In contrast there was little difference in mucosal DNA content between the retrained and unrestrained animals (Table 10) but a trend towards lower DNA mucosal content was apparent in the restrained group, in that a difference of 20 per cent was recorded for antral mucosa.

2. *PURINE PHOSPHORIBOSYLTRANSFERASES*

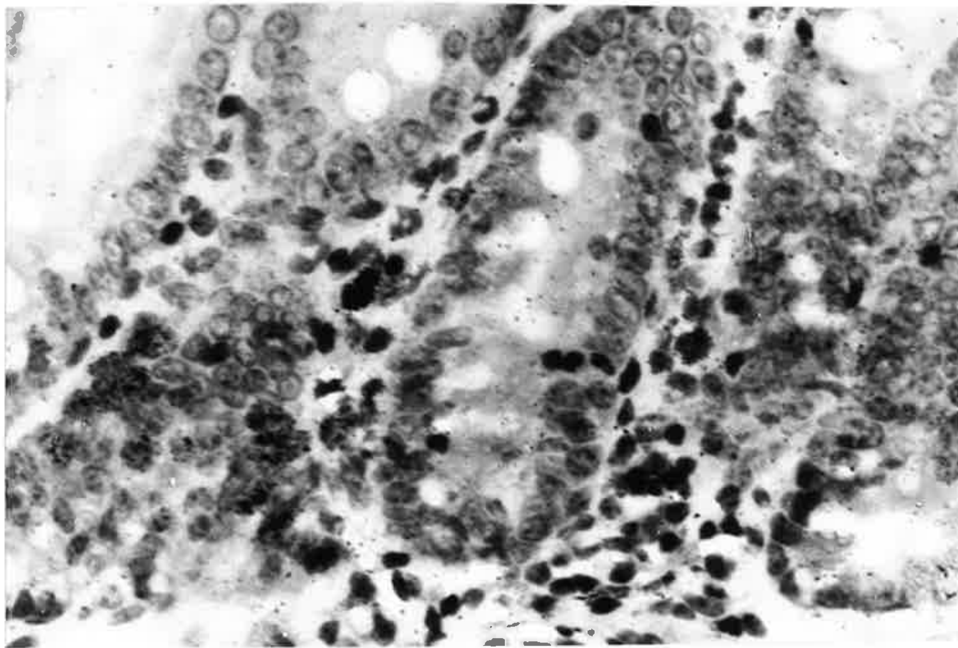
Control animals. The gastrointestinal mucosa of all control animals was non-ulcerated and normal in appearance with a surface covering of abundant mucus. The activity of adenine and hypoxanthine phosphoribosyltransferase recorded in the gastrointestinal mucosa of these animals is illustrated in Tables 11 and 12.

Animals restrained for a 10 hour period. In 3 of 8 guinea pigs there was no evidence of mucosal damage. Two animals had small haemorrhagic fundic erosions (3 lesions in 1 animal, 1 lesion in the other), and 3 animals had antral erosions of minor degree. The enzyme activities are illustrated in Table 11. At 10 hours there was a significant fall in hypoxanthine and adenine phosphoribosyltransferase activity confined to the antral area. In the antral mucosa the mean hypoxanthine phosphoribosyltransferase activity was 24.0 nanomoles of nucleotide formed per mg protein per hour compared to the control value of 32.9 units ($P < 0.002$), while

FIGURE VII: AUTORADIOGRAPHY

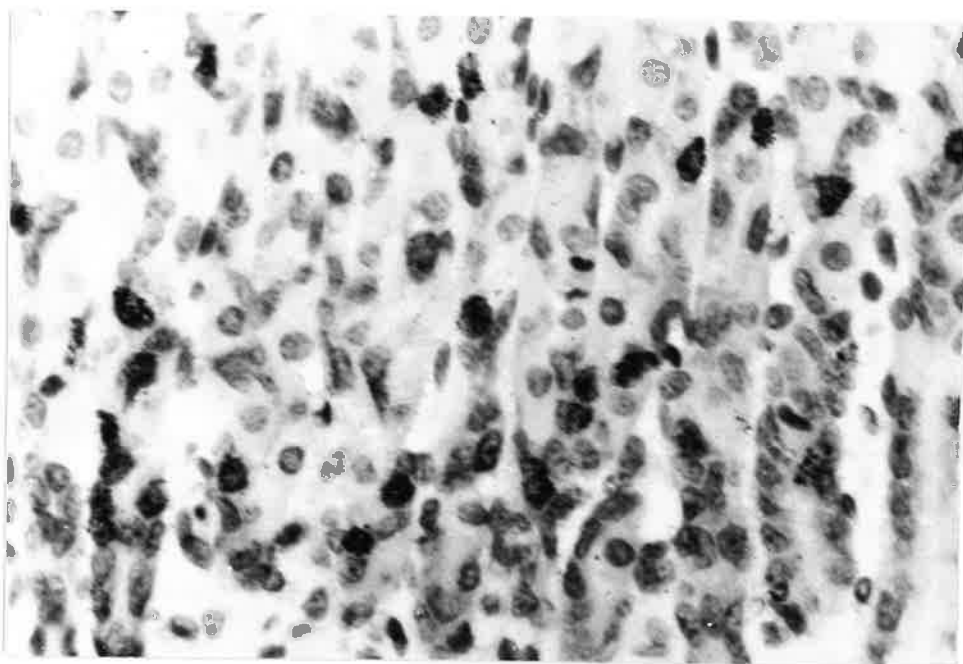


(A) UPTAKE OF TRITIATED THYMIDINE: JEJUNAL MUCOSA OF UNRESTRAINED GUINEA PIG X 500

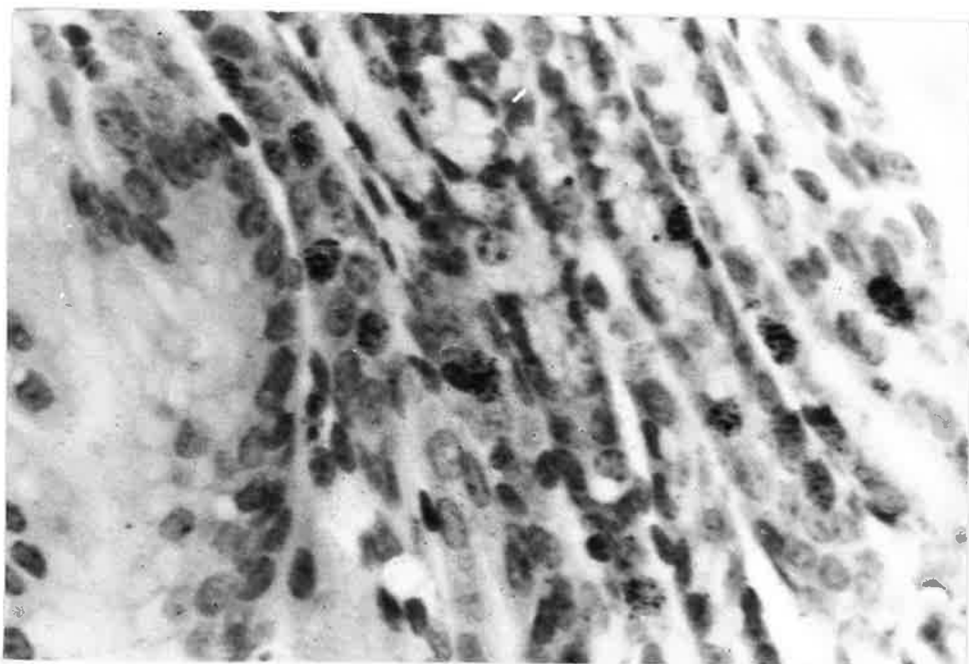


(B) UPTAKE OF TRITIATED THYMIDINE: JEJUNAL MUCOSA OF RESTRAINED GUINEA PIG X 500

FIGURE VIII: AUTORADIOGRAPHY



(A) TRITIATED THYMIDINE UPTAKE: ANTRAL MUCOSA OF UNRESTRAINED GUINEA PIG X 500



(B) TRITIATED THYMIDINE UPTAKE: ANTRAL MUCOSA OF RESTRAINED GUINEA PIG X 500

TABLE 11. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GUINEA PIG GASTRIC MUCOSA AFTER 10, 20 AND 40 HOURS OF RESTRAINT. EIGHT ANIMALS IN EACH GROUP

(in nanomoles of nucleotide formed per mg of protein per hour)

	Control	10 hours	20 hours	40 hours
FUNDUS				
HYPOXANTHINE				
Mean	38.3	36.5	34.5	36.0
S.D.	4.6	5.9	6.3	4.8
Range	33.2-48.3	26.5-44.9	22.5-46.0	30.4-45.2
Deviation (t)	-	0.62	1.26	0.98
Probability (P)	-	> 0.1	> 0.1	> 0.1
ADENINE				
Mean	29.1	29.5	30.1	23.8
S.D.	5.8	4.1	4.3	4.8
Range	21.8-40.0	21.8-34.7	21.6-37.4	20.0-36.4
Deviation (t)	-	0.43	0.4	1.99
Probability (P)	-	> 0.1	> 0.1	> 0.05
ANTRUM				
HYPOXANTHINE				
Mean	32.9	24.0	21.7	16.6
S.D.	5.5	2.6	4.6	6.3
Range	24.8-45.0	20.7-27.6	15.3-29.4	9.0-30.1
Deviation (t)	-	4.0	4.2	5.5
Probability (P)	-	< 0.002	< 0.001	< 0.001
ADENINE				
Mean	32.8	24.2	20.6	14.8
S.D.	5.8	4.2	3.4	2.6
Range	24.2-41.3	18.1-29.5	16.4-27.3	13.1-21.0
Deviation (t)	-	3.22	4.9	8.0
Probability (P)	-	< 0.01	< 0.001	< 0.001

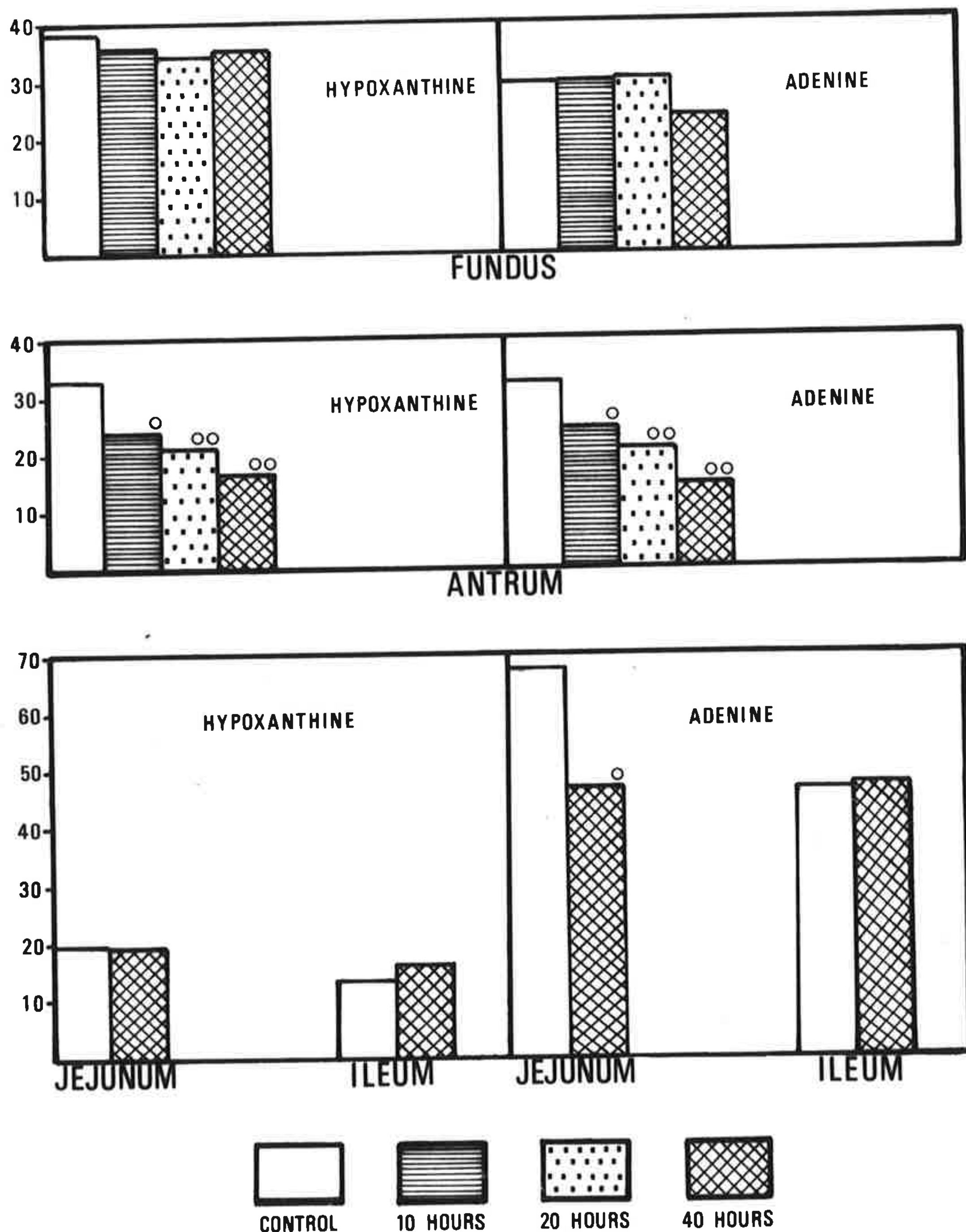
TABLE 12. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GUINEA PIG INTESTINAL MUCOSA; CONTROL AND RESTRAINED (40 HOURS) ANIMALS. EIGHT ANIMALS IN EACH GROUP

(in nanomoles of nucleotide formed per mg of protein per hour)

	Control	40 hours
JEJUNUM		
HYPOXANTHINE		
Mean	19.4	19.3
S.D.	2.0	4.4
Range	17.2 - 23.7	10.0 - 24.2
ADENINE		
Mean	67.5	46.4
S.D.	12.0	13.3
Range	54.1 - 90.7	18.4 - 62.6
Deviation (t)	-	3.35
Probability (P)	-	< 0.01
ILEUM		
HYPOXANTHINE		
Mean	13.4	15.8
S.D.	3.5	5.1
Range	9.1 - 20.7	7.0 - 21.1
ADENINE		
Mean	46.3	47.4
S.D.	9.9	18.6
Range	32.4 - 67.9	17.7 - 85.5

FIGURE XI

Purine phosphoribosyltransferase activity in the fundic, antral and intestinal mucosa of control animals and guinea pigs restrained for 10, 20 and 40 hours.
(In nanomoles nucleotide formed per mg. protein per hour).



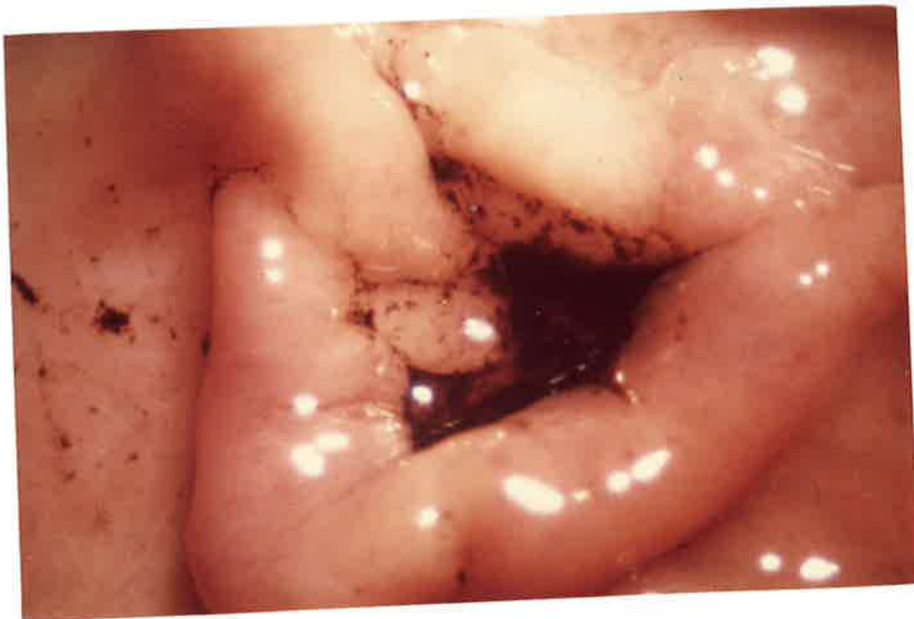
mean adenine phosphoribosyltransferase activity was 24.2 units compared to the control 32.8 units ($P < 0.01$).

20 hour period of restraint. One animal had normal non-ulcerated mucosa, while 7 animals showed numerous antral lesions with evidence of fresh haemorrhage. Each animal had more than 5 circumscribed lesions per stomach. The enzyme activities are illustrated in Table 11 and show a marginally greater depression in the antral area than was recorded after 10 hours of restraint. Mean hypoxanthine phosphoribosyltransferase activity in antral mucosa was 21.7 units compared with control values of 32.9 ($P < 0.001$), mean adenine phosphoribosyltransferase activity being 20.6 units (control 32.8, $P < 0.001$).

40 hour period of restraint. All 8 animals had antral lesions with fresh haemorrhage. Each animal had more than 5 ulcerated areas per stomach, but in every case the lesions were confined to the distal half of the stomach. A superficial antral erosion is illustrated in Figure IX. Surface mucus coating, assessed visually, was reduced in each case.

There was a marked difference in P.A.S. and Hales colloidal iron staining between the mucosa from restrained and unrestrained guinea pigs. There was abundant P.A.S. positive and colloidal iron staining mucosubstance in the gastric mucosa of unrestrained animals with a marked reduction in the 40 hour restrained group. The difference in P.A.S. staining is illustrated in Figure X.

FIGURE IX



SUPERFICIAL ANTRAL EROSION: GUINEA PIG RESTRAINED FOR 40 HOURS.

Table 11 demonstrates the recorded enzyme activities in the gastric mucosa of the 40 hour restraint group, and the activity in the intestinal mucosa is illustrated in Table 12.

The mean activity of hypoxanthine phosphoribosyltransferase in the antral mucosa of these 40 hour restrained guinea pigs was 16.6 units ($P < 0.001$ compared to mean control value), while mean adenine phosphoribosyltransferase activity was 14.8 nanomoles nucleotide formed per mg protein per hour in antral mucosa ($P < 0.001$ when compared with control values) in this group of animals.

The enzyme values in each particular group are seen in Figure XI. There was a highly significant difference in adenine and hypoxanthine phosphoribosyltransferase activity between the control group antrum and the antral mucosa from the 20 and 40 hour restraint groups. The difference at 10 hours was significant There was no difference in the enzyme activities in the fundic or ileal mucosa, but a significant difference in adenine phosphoribosyltransferase activity was demonstrated in the jejunum. There was, however, no corresponding depression of hypoxanthine phosphoribosyltransferase in this area.

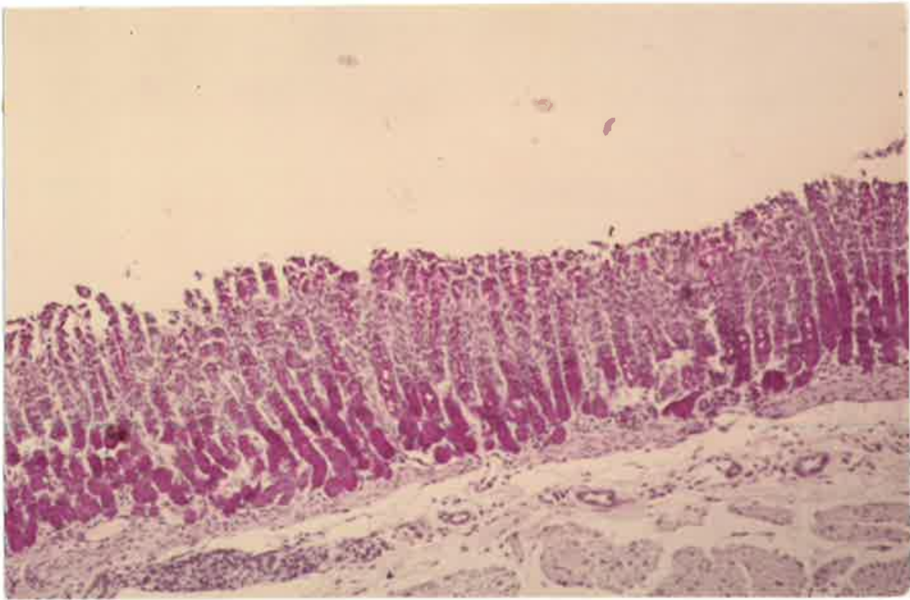
COMMENT

The psychic stress applied through restraint has been shown to be effective in producing acute gastric mucosal ulceration in

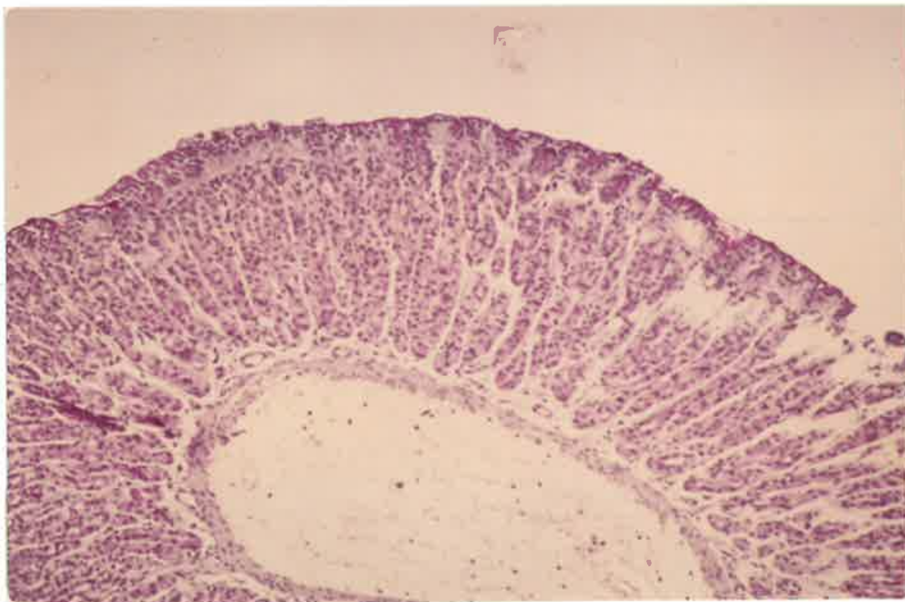
a guinea pig model. In this study it was found that the incidence of ulceration was related to duration of restraint and that the lesions were concentrated in the distal or antral region of the stomach. Although Ludwig and Lipkin (1969) have recorded the severity of the mucosal lesions on a scale of size, no such assessment of individual lesions was carried out in this study; it was considered virtually impossible to assess the size of a pale pink erosion against a predominantly pale pink background with accuracy. Erosions developed at the base of rugal folds, and it was considered that this was an indication that abrasion of the mucosal surfaces played little if any part in the pathogenesis of the lesions.

Restraint does produce alteration in the rate of nucleic acid metabolism. The study of 2 restrained and 2 unrestrained animals reported in this chapter is too small for statistical analysis or to allow definite conclusions to be drawn. Nevertheless the pilot study suggested that DNA synthesis is reduced throughout the gastrointestinal tract of restrained animals with acute gastric ulceration. The effect of non specific stress on the rate of tissue DNA synthesis was studied by Lahtiharju and Rytomaa (1967). They used an autoradiographic technique to demonstrate that DNA synthesis was reduced in the fore and glandular stomach and skin of restrained mice, and commented that ".. non-specific stress interacts with proliferating cells through some common mechanism without any apparent organ dependence per se".

FIGURE X: P.A.S. STAINING



(A) ANTRAL MUCOSA OF NORMAL UNRESTRAINED GUINEA PIG X 100



(B) ANTRAL MUCOSA OF GUINEA PIG RESTRAINED FOR 40 HOURS X 100

Kim, Kerr and Lipkin (1967) studied tritiated thymidine incorporation in restrained mice by autoradiography and confirmed that DNA synthesis is affected by restraint stress. Imondi, Balis and Lipkin (1968) noted a regional difference between DNA and RNA metabolism in restrained mice. They found that the rate of DNA synthesis was reduced throughout the gastrointestinal tract, while RNA synthesis was affected in the stomach alone. Ludwig and Lipkin (1969), working with guinea pigs, confirmed that both DNA and RNA metabolism in the gastric mucosa are depressed by the stress associated with immobilization.

The reduction in stainable mucosubstance in the gastric mucosa of restrained animals confirms previous reports (Robert et alii, 1963; Hakkinen and colleagues, 1966; Ludwig and Lipkin, 1969). However, it is likely that such changes are secondary to some primary causal factor. Indeed, Imondi et alii (1968) and Ludwig and Lipkin (1969) have suggested that the reduction in mucus production may be secondary to impaired RNA metabolism. They observed that the ribosomes are the site of protein synthesis and so any reduction in ribosomal RNA would lead to impaired protein synthesis and so to a reduction in mucoprotein formation. The synthesis of RNA is also necessary for the initiation of DNA synthesis and cell division. Impairment of RNA metabolism could therefore result in reduced cell replacement and decreased mucus production, thereby lowering both factors contributing to mucosal resistance.

The main purpose of this study was to establish the relationship, if any, between the purine phosphoribosyltransferases and the reduction in nucleic acid metabolism resulting from restraint stress. There was a significant reduction of both hypoxanthine and adenine phosphoribosyltransferase activity in the antral mucosa after 10, 20 and 40 hours of restraint. The difference from control values was most marked at 40 hours, at which stage the mucosal ulceration was also most marked. Although occasional lesions were evident in the fundic or proximal part of the stomach, the antrum was the area which appeared to be most affected by stress. The reduction in purine phosphoribosyltransferase activity in this area is, therefore, of possible pathogenetic significance. It should be noted that the present study is not comparable to that of Imondi et alii (1969b) who studied the activity of various enzymes involved in nucleic acid synthesis in the gastric mucosa of restrained animals prior to the onset of impaired DNA synthesis and found no alteration in activity. This implies that enzyme biosynthetic activity does not regulate nucleic acid synthesis. However, the reduced activity of the purine phosphoribosyltransferases in restrained guinea pig gastric mucosa after the initiation of impaired nucleic acid synthesis suggests that the activity of these enzymes reflects the rate of nucleic acid synthesis, and that this is more reduced in antral than fundic mucosa.

The fact that reduction of enzyme activity was not universal

but concentrated to the ulcerated antrum suggests that this reduction is more a reflection of RNA than DNA metabolism, the latter being affected by restraint stress throughout the gastro-intestinal tract (Imondi et alii, 1968). One problem of interpretation here may arise from the observation (McManus and Isselbacher, 1970) that as little as 12 hours of fasting markedly reduces the cell turnover of the intestinal mucosa; in the particular study cited 12 hours of fasting resulted in a 12 per cent reduction in jejunal mucosal mass in rats. Thus there would almost certainly be another factor operating to reduce DNA synthesis in both the control animals (fasted for 40 hours) and the restrained animals fasted for 10, 20 or 40 hours. The implications of this are two-fold. Firstly, the comparison of animals restrained for 10 and 20 hours with a control group fasted for 40 hours is not ideal, for changes induced by starvation may mask those induced by restraint. Secondly, the changes in DNA turnover induced by starvation may mask those theoretically inducible by restraint, and the enzyme assay may not be a sensitive enough technique to differentiate fractional changes. Certainly this may explain why enzyme activity in the intestinal mucosa showed little change from control activity, while radioisotope incorporation studies have demonstrated that restraint reduces DNA synthesis in the intestine (Ludwig and Lipkin, 1969). Thus enzyme activity may only be demonstrably altered where nucleic acid metabolism, both RNA and DNA, is maximally and concurrently depressed.

The localization of the mucosal lesions to the anatomic region of the antrum was particularly marked in this study. Ludwig and Lipkin (1969) did not record such localization in guinea pigs restrained for 24 hours. Furthermore, they did not divide the stomach into proximal and distal areas for assessment of nucleic acid metabolism. Thus there is no evidence that RNA metabolism is affected by restraint to any different extent in these two areas. However, the fact that RNA metabolism is impaired in the stomach alone while DNA metabolism is reduced throughout the gastrointestinal tract, together with the marked reduction in adenine and hypoxanthine phosphoribosyltransferase activity in the ulcerated antral mucosa, suggests that the activity of these enzymes reflects the demand for RNA precursor substances.

A causal relationship between the depression of purine phosphoribosyltransferase activity and the appearance of mucosal lesions is unlikely. The reduced level of purine nucleotide formation in the stressed animals may result from an increase in nucleotide degradation rather than decrease in nucleotide formation. Indeed, the degradative enzyme 5'nucleotidase is present in calf intestinal mucosa (Center and Behal, 1966) and in guinea pig gastric mucosa (Murray, 1970). Increased activity of this enzyme may account for the apparent reduction in purine nucleotide formation reported in this study. However, the present study demonstrates that

availability of purine nucleotides is reduced in a situation in which nucleic acid metabolism is correspondingly depressed. It may be concluded therefore, that although nucleotidase activity was not studied, measurement of the activity of the purine phosphoribosyltransferases provides an index of the rate of nucleic acid synthesis.

CHAPTER V

THE EFFECT OF ASPIRIN AND PREDNISOLONE ON PURINE NUCLEOTIDE METABOLISM IN GUINEA PIG GASTRIC MUCOSA

INTRODUCTION

A number of anti-inflammatory drugs such as aspirin, cortisone and indomethacin have the capacity to produce gastric mucosal injury. The mechanism of this phenomenon is not clear. Mucosal injury does not appear to be associated with excessive production of either acid or pepsin, and the changes in mucus secretion induced by these drugs (Menguy and Masters, 1963; Hakkinen et alii, 1968) seem insufficient to explain the primary mucosal injury.

There is evidence that corticosteroids reduce the rate of cell proliferation in the gastric but not the intestinal mucosa (Rasanen,1963), and it is likely that such a reduction in cell replacement in the gastric mucosa may lead to an increased susceptibility to mucosal trauma leading to ulceration. The effect of aspirin on the rate of mucosal cell turnover has only recently been studied by Max and Menguy (1970), who reported that the rate of mucosal cell turnover in rats was not altered by aspirin administration. In view of the observation that the activity of the purine phosphoribosyltransferases appeared to reflect the rate of nucleic acid synthesis, the effect of aspirin and corticosteroids on these enzymes in the gastric mucosa of guinea pigs was studied.

MATERIALS AND METHODS

A. *ENZYME ASSAYS*

The reagents and methods used in the assay of the purine phosphoribosyltransferases were identical to those described in detail in Chapter III.

B. *ANIMALS*

Sixteen guinea pigs from the line bred by the Institute of Medical and Veterinary Science, Adelaide were matched for age, sex and weight. Their average weight was 530 gm. The animals were maintained in separate metabolic cages in the animal room, Department of Medicine, Royal Adelaide Hospital, for three weeks and allowed free access to food and water during this period. The drinking water was supplemented with either sodium acetylsalicylic acid (Rotary Tableting Corporation Pty. Ltd.) or prednisolone (Intercontinental Pharmaceuticals). The animals drank this fluid freely, and no deviation from normal feeding pattern was seen in this study. It was estimated that each animal drank between 20 and 30 ml of fluid daily.

Eight animals were given prednisolone, 50 mg per litre, in their drinking water. While prednisolone is relatively insoluble in water, the tablets disintegrated readily in water and the particulate matter settled around the mouth of the water bottle ensuring that the animals ingested the drug with their drinking water. It was impossible to accurately assess the amount of

prednisolone each animal received. After three weeks of prednisolone supplementation the animals were fasted for 12 hours and then killed by a blow on the head. Fundic and antral tissue from the stomach of each animal was processed for purine phosphoribosyltransferase assay.

Sodium acetylsalicylic acid, in a concentration of 3 gm per litre in the drinking water, was administered to eight animals. The solubility of this preparation, 1 in 10, is such that approximately 0.3 gm of the aspirin preparation was dissolved per litre. However, as was the case with prednisolone, the tablets disintegrated readily in water and the settling of the fine particulate material around the bottle outlet ensured that the animals ingested additional undissolved aspirin, although it was impossible to accurately assess each animal's daily intake of the drug. The animals were killed after three weeks of aspirin administration, fundic and antral tissue being processed for purine phosphoribosyltransferase assay by the previously described method.

C. STATISTICAL METHODS

Statistical methods employed in this chapter are detailed in Appendix C.

RESULTS

A. PREDNISOLONE-FED ANIMALS

No attempt was made to examine mucus production either

quantitatively by staining of sections for mucosubstances or qualitatively by estimation of carbohydrate residues. However to the naked eye all eight animals treated with prednisolone appeared to have less adherent mucus than the animals fed an unadulterated diet. There were definite antral mucosal defects in 5 animals, and in 3 of these the defects were multiple. Two animals showed evidence of recent gastric haemorrhage.

The results of the purine phosphoribosyltransferase assays on the gastric mucosa from the prednisolone-fed animals are illustrated in Table 13. These values are compared to those obtained from animals in the control group described in Chapter III which were fed a drug-free diet. There was a marked reduction in activity of both enzymes in the fundic and antral mucosa. The mean value for hypoxanthine phosphoribosyltransferase in the fundic mucosa was 20.1 nanomoles nucleotide formed per mg protein per hour (range 13.8 - 24.4), while the mean value for the activity of this enzyme in antral mucosa was 11.2, range 4.9 - 15.6, nanomoles nucleotide formed per mg protein per hour. The difference from control values was highly significant in each case ($P < 0.001$). Adenine phosphoribosyltransferase activity was respectively 18.6 (range 15.3 - 23.9) nanomoles nucleotide formed per mg protein per hour for fundic mucosa and 12.3 (range 5.5 - 17.9) nanomoles nucleotide formed per mg protein per hour for antral mucosa. The difference from control values was highly significant for both areas ($P < 0.001$).

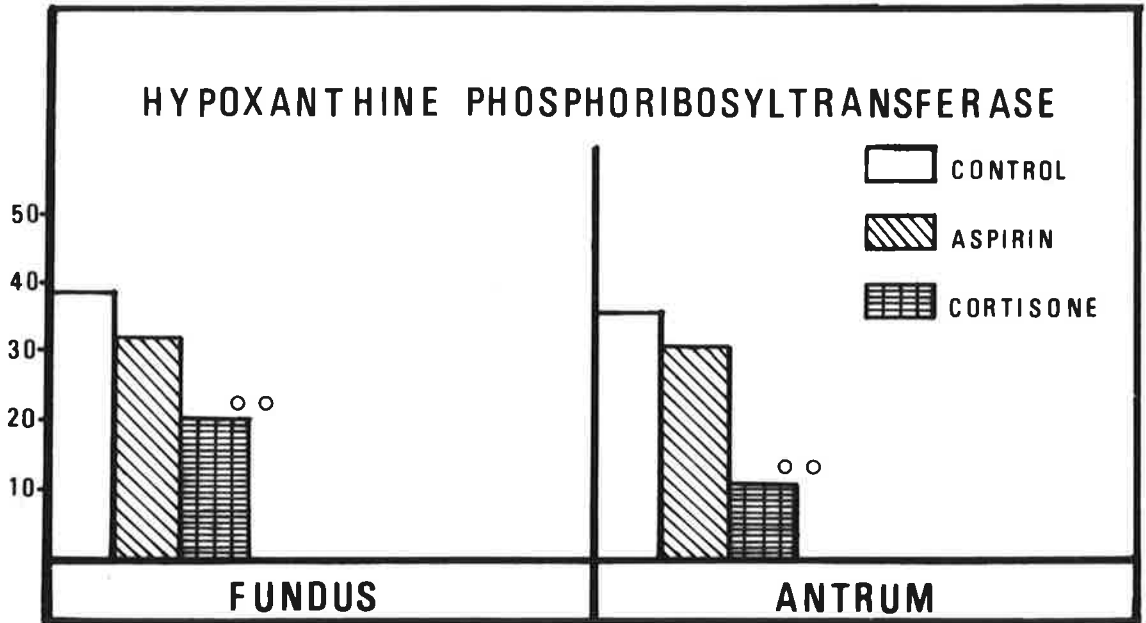
TABLE 13. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN THE GASTRIC MUCOSA OF GUINEA PIGS FED PREDNISOLONE OR ACETYLSALICYLIC ACID (ASPIRIN)

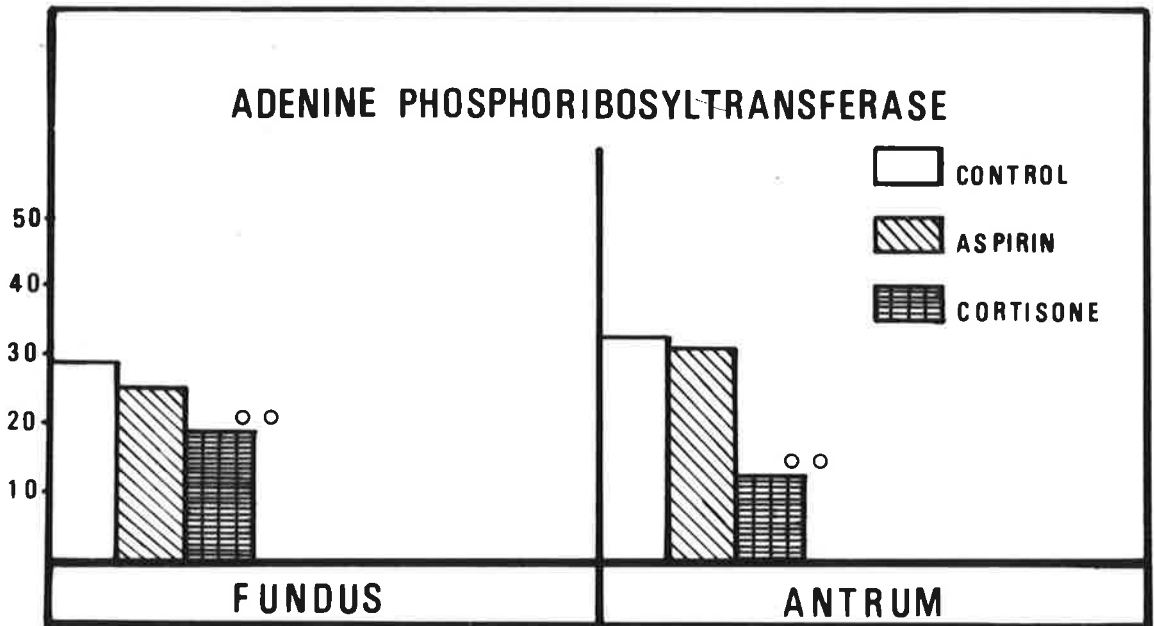
(in nanomoles nucleotide formed per mg protein per hour)

	Control	Prednisolone	Aspirin
FUNDUS			
HYPOXANTHINE			
Mean	38.6	20.1	32.2
S.D.	3.6	3.2	5.3
Range	33.5-47.5	13.8-24.4	25.6-40.1
Deviation (t)	-	11.6	2.92
Probability (P)		< 0.001	.02 > P > 0.01
ADENINE			
Mean	28.8	18.6	25.4
S.D.	6.0	2.7	5.0
Range	21.5-41.2	15.3-23.9	19.7-34.2
Deviation (t)	-	4.8	1.31
Probability (P)	-	< 0.001	> 0.10
ANTRUM			
HYPOXANTHINE			
Mean	35.2	11.2	31.3
S.D.	5.6	3.2	7.3
Range	24.3-45.7	4.9-15.6	22.3-48.2
Deviation (t)	-	11.4	1.25
Probability (P)	-	< 0.001	> 0.10
ADENINE			
Mean	32.6	12.3	31.1
S.D.	4.6	3.6	5.2
Range	24.8-41.6	5.5-17.9	24.8-39.5
Deviation (t)	-	10.6	0.64
Probability (P)	-	< 0.001	> 0.10

FIGURE XII

Purine phosphoribosyltransferase activity in the gastric mucosa of control guinea pigs and animals fed aspirin and prednisolone (In namomoles nucleotide formed per mg. protein per hour).





○○ HIGHLY SIGNIFICANT DIFFERENCE FROM CONTROL VALUE ($P < 0{\cdot}001$).

B. *ASPIRIN-FED ANIMALS*

There were well-defined macroscopic changes in the gastric mucosa of all 8 animals which had been fed aspirin. The mucosa appeared thinner than normal and rugal patterning was reduced. The amount of adherent mucus was macroscopically reduced in each case. However, in contrast to the prednisolone-fed animals where lesions were confined to the antrum, the mucosal abnormality in these animals was in the fundic area. The fundic mucosa appeared reddened and congested in all 8 animals, and in 6 there was evidence of fresh haemorrhage from the fundic mucosa.

The purine phosphoribosyltransferase activity in the gastric mucosa obtained from these animals is illustrated in Table 13. In this instance the mean activity of the hypoxanthine converting enzyme in the fundic mucosa was 32.3, range 25.6 - 40.1, nanomoles nucleotide formed per mg protein per hour. This was significantly different from control values at the 2 per cent level ($0.02 > P > 0.01$). The mean activity of this enzyme in antral mucosa was 31.3, range 22.3 - 48.2, nanomoles nucleotide formed per mg protein per hour, and did not differ significantly from normal ($P > 0.10$). The mean value for the activity of adenine phosphoribosyltransferase in fundic mucosa was 25.4, range 19.7 - 34.2, nanomoles nucleotide formed per mg protein per hour, while for antral mucosa the mean value was 31.1, range 24.8 - 39.5. These values showed no significant variation from control values ($P > 0.10$).

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The difference in effect of prednisolone and aspirin on the activity of the purine phosphoribosyltransferases in fundic and antral mucosa is illustrated in Figure XII.

COMMENT

These studies have shown that both prednisolone and aspirin produce gastric mucosal lesions. The prednisolone induced antral lesions were of particular interest in that some investigators (Dean, 1968) have been unable to induce gastric mucosal defects with corticosteroids. The effect of these drugs differed in two main respects. Firstly, prednisolone produced antral but not fundic lesions while aspirin produced fundic lesions alone. Secondly, prednisolone administration was associated with a marked reduction of both enzymes in both the fundic and antral mucosa, while aspirin was associated with a reduction only of the hypoxanthine converting enzyme in the fundic mucosa. It seems probable therefore, that prednisolone and aspirin produce gastric mucosal damage through different mechanisms.

Shortly after the experiments described above were completed, the independent study of Max and Menguy (1970) was published. These workers studied the effect of cortisone, adrenocorticotrophin aspirin and phenylbutazone on the rate of exfoliation and the rate of renewal of gastric mucosal cells in dogs and rats. They found that aspirin increased the rate of exfoliation of gastric epithelial cells, but did not affect the mitotic frequency as

assessed in gastric mucosal biopsies from dogs. Neither did aspirin affect the rate of uptake of tritiated thymidine by the gastric mucosa in rats. On the other hand, they found that corticosteroid preparations reduced the rate of exfoliation of surface epithelial cells and decreased the apparent rate of mitosis in the gastric mucosa. They concluded "..that one of the mechanisms of gastric mucosal injury by adrenocorticotropin and cortisone may be a reduced rate of renewal of surface epithelial cell Aspirin..on the other hand appears to affect the gastric mucosa in such a way that shedding of surface epithelial cells increases without a concomitant increase in the rate of cell renewal".

By analogy with the findings of Max and Menguy (1970), the depressant effect of corticosteroids on the purine phosphoribosyltransferase enzymes may be interpreted as indicating that corticosteroids do reduce the rate of nucleic acid synthesis in the gastric mucosa. The minimum depressant effect of aspirin on these enzymes is consistent with a minimal effect of this drug on the rate of cell renewal. The results reported in this chapter support the hypothesis that measurement of the activity of purine phosphoribosyltransferases provides an index of purine nucleotide biosynthesis in gastrointestinal mucosa, and furthermore the biosynthesis of nucleic acids in this tissue. Although unproven, the results indicate that nucleic acid rather than nucleotide coenzyme synthesis is reflected in this assay.

CHAPTER VI

A STUDY OF THE EFFECT OF CARBENOXOLONE SODIUM ON PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GASTRIC MUCOSA

INTRODUCTION

The beneficial effect of carbenoxolone sodium on the healing rate of chronic gastric ulcers has been well established in clinical trials (Doll et alii, 1965; Bank and co-workers, 1967; Doll, Langman and Shawdon, 1968). The mechanism by which the drug promotes the healing of the ulcer has not been proven. However the effect of carbenoxolone sodium in increasing the rate of gastric ulcer healing closely parallels its mineralocorticoid effect (Doll et alii, 1968; Cocking and MacCaig, 1969) and may be associated with the glucocorticoid-like anti-inflammatory action of the drug described by Khan and Sullivan (1968).

The assumption that gastric ulceration is the result primarily of a decrease in mucosal resistance leads to the conclusion that carbenoxolone sodium exhibits its effect by augmenting mucosal resistance and thereby facilitating ulcer healing. The present study was designed to examine the effect of this drug on the activity of the purine phosphoribosyltransferases in normal gastric mucosa and so determine whether nucleic acid metabolism was influenced by the drug. It was conceived that carbenoxolone sodium might have two effects; either to stimulate nucleic acid metabolism resulting in increased cell replication, protein synthesis and mucoprotein secretion, or conversely a corticosteroid like effect with reduction in nucleic acid metabolism and enzyme activity.

A protective effect has been ascribed to carbenoxolone sodium in acute experimental gastric ulceration (Dean, 1968; Lipkin and Ludwig, 1968). In view of the possible corticosteroid-like action of carbenoxolone and the previously described ulcerogenic potential of glucocorticoids, it was considered necessary to confirm the possibly paradoxical effect of carbenoxolone in acute experimental ulceration.

MATERIALS AND METHODS

The effect of carbenoxolone sodium on the gastric mucosa was examined in three situations: its effect on gastric mucosal purine phosphoribosyltransferase activity was studied in normal guinea pigs, and similarly in normal subjects, and a protective effect in animals subsequently submitted to restraint stress was sought.

A. *EFFECTS ON NORMAL GUINEA PIGS*

Ten guinea pigs, matched for age, sex and weighing between 350 to 550 g were obtained from the Institute of Medical and Veterinary Science, Adelaide. They were retained in separate metabolic cages in the Department of Medicine, Royal Adelaide Hospital for four weeks, and were fed a standard diet of rabbit pellets and water, supplemented with carbenoxolone sodium. Fifty mg of carbenoxolone sodium was added to each 400 ml water bottle, the bottles were changed daily and it was estimated that each animal ingested a

minimum quantity of 2 mg of carbenoxolone per day. After four weeks of carbenoxolone supplemented feeding the animals were killed by a blow on the head. The stomach was removed from each animal, opened along the greater curvature and placed in 0.9 per cent sodium chloride at 4°C. The gastric mucosa was viewed through a variable power dissecting microscope (Olympus), the presence of mucosal abnormalities was noted and the amount of adherent mucus was assessed visually. Mucosal specimens were cut with a No. 3 cork borer from the fundic and antral regions. Tissue specimens were prepared for enzyme assay and also placed in 10 per cent formalin for forwarding to the Institute of Medical and Veterinary Science for sectioning and P.A.S. staining.

B. ACTION OF CARBENOXOLONE IN GUINEA PIGS SUBSEQUENTLY UNDERGOING RESTRAINT STRESS

A further 8 guinea pigs, corresponding in age, weight and sex to the previously described 40 hour restraint group (Chapter IV) were fed a minimum quantity of 2 mg of carbenoxolone sodium per day for 2 weeks. They were then restrained in a wire mesh envelope for 40 hours, were killed and the gastric mucosa examined under a variable power dissecting microscope for evidence of mucosal lesions.

C. HUMAN STUDIES

Three healthy males in their mid-twenties volunteered for a

course of carbenoxolone sodium with pre- and post-treatment gastric biopsies to assess the effect of the drug on purine phosphoribosyltransferases in uninflamed human gastric mucosa. Fundic biopsies were taken with radiological localization using a Watson intestinal biopsy capsule. The tissue obtained was divided; some tissue being placed in 10 per cent formalin for subsequent sectioning and staining with haematoxylin and eosin, the remaining tissue being processed for enzyme assay. The volunteers then undertook a two week course of carbenoxolone sodium, 300 mg per day in divided doses. A fundic biopsy was obtained on the completion of this course and the tissue was processed for purine phosphoribosyltransferase assay.

RESULTS

A. *GUINEA PIGS TREATED WITH CARBENOXOLONE SODIUM ALONE*

All animals behaved normally while taking carbenoxolone sodium, in particular their daily fluid intake was unaltered by the addition of this drug to their drinking water. The gross appearance of the gastric mucosa differed from normal in that there appeared to be less adherent mucus coating in all animals. However, the variation in mucus production noted macroscopically was not confirmed by histological examination which revealed no difference in the amount of P.A.S.-positive staining mucosubstance in the fundic or antral mucosa of the normal and carbenoxolone-fed animals. Two animals had petechial lesions in the antral

ABLE 14. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN GASTRIC MUCOSA OF NORMAL AND CARBENOXOLONE-FED GUINEA PIGS

	NORMAL	CARBENOXOLONE
UNDUS		
HYPOXANTHINE		
Mean	38.6	28.3
S.D.	3.6	11.4
Range	33.5-47.5	11.4-43.2
Deviation (t)	-	2.71
Probability (P)	-	< 0.02
ADENINE		
Mean	28.8	23.0
S.D.	6.0	10.9
Range	21.5-41.2	9.4-51.7
Deviation (t)	-	1.45
Probability (P)	-	> 0.10
NTRUM		
HYPOXANTHINE		
Mean	35.2	16.5
S.D.	5.6	8.9
Range	24.3-45.7	3.7-34.7
Deviation (t)	-	5.43
Probability (P)	-	< 0.001
ADENINE		
Mean	32.6	15.2
S.D.	4.6	5.6
Range	24.8-41.6	5.8-24.4
Deviation (t)	-	7.25
Probability (P)	-	< 0.001

mucosa, and there was frank ulceration midway along the lesser curvature in one animal.

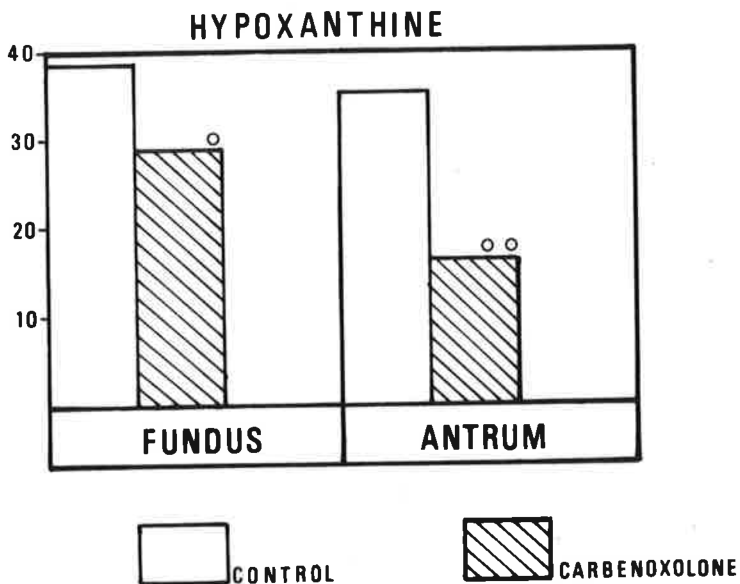
Purine phosphoribosyltransferase activity in the gastric mucosa of the carbenoxolone-fed animals is illustrated in Table 14 and Figure XIII, and is compared to the control values reported in Chapter IV. The mean value for hypoxanthine phosphoribosyltransferase was 38.6 units (standard deviation ± 3.6 units) in the fundic and 35.2 ± 5.6 units in the antral mucosa in the control animals, compared with 28.3 ± 11.4 units and 16.5 ± 8.9 units in the carbenoxolone-fed animals. These differences were both significant ($P < 0.02$ for fundic mucosa and $P < 0.001$ for antral mucosa respectively). Antral adenine phosphoribosyltransferase activity was significantly reduced from a mean value of 32.6 ± 4.6 units in control animals to 15.2 ± 5.6 units in treated animals ($P < 0.001$). However the reduction in adenine phosphoribosyltransferase activity in the fundic mucosa did not approach a significant level.

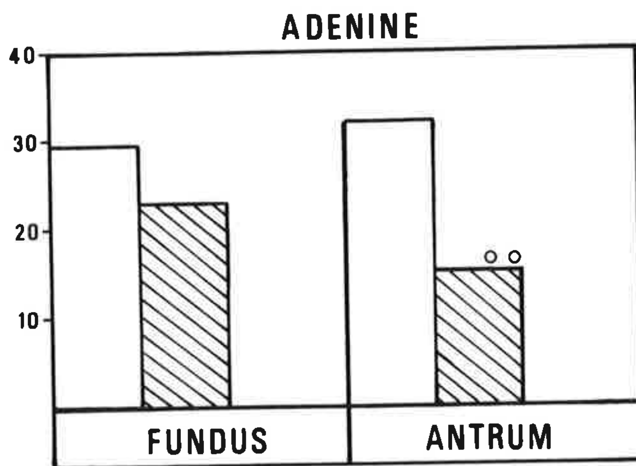
B. *GUINEA PIGS FED CARBENOXOLONE SODIUM AND SUBSEQUENTLY RESTRAINED*

Prefeeding with carbenoxolone sodium had no apparent effect on the incidence of mucosal lesions induced by a 40 hour period of restraint. One animal died during the restraint period (the only death recorded during restraint procedures) and at autopsy had extensive deep antral lesions and a faeculent peritonitis. This was presumed to be due to perforation of a gastric ulcer. All of

FIGURE XIII

The effect of carbenoxolone sodium on purine phosphoribosyltransferases in guinea pig gastric mucosa (values in nanomoles nucleotide formed per mg. protein per hour).





o SIGNIFICANT DIFFERENCE FROM NORMAL (P < 0·05)

o o HIGHLY SIGNIFICANT DIFFERENCE FROM NORMAL (P < 0·001)

ABLE 15. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN THE FUNDIC MUCOSA OF THREE HUMANS BEFORE AND AFTER A TWO WEEK COURSE OF CARBENOXOLONE

(in nanomoles nucleotide formed per mg protein per hour)

		Pre-treatment	Post-treatment
.B. Male Age 23			
	HYPOXANTHINE	24.4	17.7
	ADENINE	44.6	37.7
.L. Male Age 23			
	HYPOXANTHINE	21.5	18.4
	ADENINE	40.2	36.8
.H. Male Age 24			
	HYPOXANTHINE	23.7	11.5
	ADENINE	42.3	17.4
EAN VALUES			
	HYPOXANTHINE	23.2	15.9
	ADENINE	42.4	30.6

the remaining 7 animals had extensive antral mucosal damage with fresh haemorrhage, the mucosal appearance in these animals being indistinguishable from that seen in the non-carbenoxolone fed 40 hour restraint group (Chapter IV).

C. *HUMAN STUDIES*

The activity of the purine phosphoribosyltransferases in the fundic mucosa of the three human subjects before and after a course of carbenoxolone sodium is illustrated in Table 15. The mean value for hypoxanthine phosphoribosyltransferase activity was 23.2 units before treatment, and 15.9 units after carbenoxolone treatment, a reduction in activity of the order of 30 per cent. The corresponding values for adenine phosphoribosyltransferase activity were 42.4 and 30.6 units, a reduction of 30 per cent in activity after treatment. The greatest reduction in activity was seen in subject B.H., where post treatment activity was less than 50 per cent of initial activity.

COMMENT

The administration of carbenoxolone sodium was associated with a reduction in the activity of the purine phosphoribosyltransferases in both unrestrained guinea pig and human gastric mucosa. The reduction in enzyme activity in guinea pig mucosa was comparable to that seen with prednisolone administration (Chapter V) and tentatively suggests that these drugs may be acting through a common mechanism.

One problem associated with studies of this type is that the addition of a drug into the animal's drinking water may alter its feeding pattern and indirectly disturb its metabolism. As far as could be ascertained, the carbenoxolone animals behaved normally in all respects throughout this study, and alterations in feeding pattern with chronic partial starvation is an unlikely explanation of the observed reduction in enzyme activities. Furthermore the three human volunteers altered their feeding pattern only with respect to thrice daily carbenoxolone ingestion, yet a reduction in mucosal enzyme activity was associated with the drug. It is most likely, therefore, that the reduction in purine phosphoribosyltransferase activity is a real effect of the drug carbenoxolone sodium. This finding, in association with the previous studies, suggests that the drug intrinsically reduces the rate of purine nucleotide formation in the gastric mucosa, and is associated with a reduced supply of nucleotide for nucleic acid or nucleotide coenzyme biosynthesis in this situation.

The dosage of carbenoxolone employed in the human studies corresponds to the normal therapeutic dosage of between 150 and 300 mg daily. As previously observed this dose regime

was associated with a reduction in enzyme activity. The dosage in the guinea pigs was more difficult to quantitate, but on the basis of the average weight of the animals being 450 g it was estimated that each animal ingested between 2 - 3 mg daily. This dose corresponds on a body weight basis to that administered to the human volunteers. The results reported in this study suggest that the animal dosage was comparable to the normal human therapeutic dose of this drug, the reduction of activity in the guinea pig being comparable to that in humans.

Pre-treatment with carbenoxolone sodium did not affect the incidence, nor indeed the apparent severity of acute gastric mucosal damage induced by 40 hours of restraint in the guinea pigs. This finding is in marked contrast to the experience of other workers (Lipkin and Ludwig, 1968; Dean, 1968). However, a number of differences between the present study and those previously reported may explain this anomaly. Firstly, the period of restraint imposed in this study was 40 hours, whereas Lipkin and Ludwig immobilized guinea pigs for a 24 hour period. Dean (1968) maintained that carbenoxolone provided protection only if the ulcerogenic stimulus was not too strong, and the near maximal stress of 40 hours immobilization may explain the apparent lack of protection afforded by the drug in the present study. Secondly, the dosage of the drug employed differed,

the dose in the present study being lower than the dose of between 6 to 10 mg/kg/day used in the other studies previously cited. It is conceivable that the lower dose selected in the present study was not sufficient to exert a protective effect, although the effect on enzyme activity was comparable to that observed in humans. Thus this study differs from those previously reported in two respects, either of which could explain the anomalous findings.

The lower dose of carbenoxolone employed in the present study may explain the disparate findings with respect to gastric mucus between this study, in which there appeared to be a decreased amount of mucus present after carbenoxolone administration, and the studies of Dean (1968) and Lipkin and Ludwig (1968).

The data reported in this chapter indicate that at the dosage employed carbenoxolone did not protect animals from the gastric mucosal injury induced by 40 hours of immobilization. However, carbenoxolone administration was associated with reduced activity of the purine phosphoribosyltransferases in the gastric mucosa of both the humans and the guinea pigs studied. This latter finding suggests that

carbenoxolone may intrinsically reduce purine nucleotide formation or is associated with a mucosal situation of reduced demand for purine nucleotides. Therefore carbenoxolone sodium may conceivably affect the rate of nucleic acid synthesis or nucleotide coenzyme synthesis in the gastric mucosa.

CHAPTER VII

PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN THE GASTRIC MUCOSA OF PATIENTS WITH CHRONIC GASTRIC ULCERATION

INTRODUCTION

The activity of the enzymes hypoxanthine and adenine phosphoribosyltransferase in the gastric mucosa of normal humans was presented in Chapter III. The relationship between the activity of these enzymes and the rate of nucleic acid synthesis has been developed in subsequent chapters.

The purpose of the present investigation was to determine the activity of these enzymes in the fundic mucosa of patients with proven chronic gastric ulceration. After base-line enzymic estimations suitable patients were treated with carbenoxolone sodium, and on completion of therapy purine phosphoribosyltransferase activity in the fundic mucosa was determined to assess whether any change in enzyme activity had occurred.

MATERIALS AND METHODS

The patients studied attended the Royal Adelaide Hospital or the Gastroenterology Unit of the Queen Elizabeth Hospital during the period November 1969 - September 1970. Nineteen patients were studied and all had radiological evidence of gastric ulceration. In many instances the diagnosis of gastric ulceration was confirmed and degree of healing assessed by gastroscopy (Olympus GTF-A).

A fundic mucosal biopsy was obtained from each patient using

the Watson intestinal biopsy capsule with radiological localization. In all cases the biopsy site was well distant from the site of ulceration. The mucosal specimen was divided, with some tissue being placed in 10 per cent formalin and sent to the Institute of Medical and Veterinary Science for sectioning and staining with haematoxylin and eosin, and periodic-acid Schiff stains. The majority of the tissue was retained for determination of mucosal enzyme activity by the method described in Chapter III.

Patients in good general health and accessible for regular medical examination were then commenced on a course of carbenoxolone sodium, 200 mg daily on an outpatient basis. Twelve of the original nineteen patients were so treated. After four weeks treatment with this drug, ulcer healing was assessed radiologically and where possible by gastroscopy, and a second fundic mucosal biopsy was obtained. This tissue was processed for purine phosphoribosyltransferase assay, but not for assessment of mucosal histology. Additional therapy in these patients consisted of antacids for relief of pain, but smoking habit was not altered and anticholinergics were not administered.

The haematoxylin and eosin stained sections were examined for evidence of gastritis, and were assessed as being normal or having chronic superficial gastritis or atrophic gastritis according to the classification of Wood and Taft (1958). The P.A.S. stained sections were classified as having normal or reduced P.A.S.-positive

TABLE 16A. PATIENTS TREATED WITH CARBENOXOLONE

Patient No.	Name, age and sex	Hospital record No.		†Enzyme levels pre-treatment	post-treatment	ΔType of ulcer
1	V.G., 37, F	161224	Hypoxanthine	26.1	26.3	Prepyloric
			Adenine	44.9	39.6	
2	V.D., 63, F	155164	Hypoxanthine	11.0	24.5	Primary
			Adenine	39.5	54.2	
3	E.H., 69, F	163989	Hypoxanthine	22.4	29.8	Primary
			Adenine	52.9	77.0	
4	I.M., 47, M	126015	Hypoxanthine	15.5	19.3	Primary
			Adenine	43.7	40.3	
5	J.V., 75, M	*070178	Hypoxanthine	23.2	36.0	Primary
			Adenine	44.7	69.1	
6	A.E., 45, M	132396	Hypoxanthine	22.9	34.1	Primary
			Adenine	46.8	83.4	
7	N.P., 72, F	N.P.P.	Hypoxanthine	18.6	16.5	Prepyloric
			Adenine	35.6	28.9	
8	C.T., 59, M	161231	Hypoxanthine	12.5	24.3	Primary
			Adenine	76.8	58.8	
9	M.M., 43, F	159401	Hypoxanthine	9.1	32.0	Primary
			Adenine	51.9	66.2	
10	H.L., 60, M	169337	Hypoxanthine	15.9	25.4	Primary
			Adenine	39.4	45.2	
11	J.D., 42, M	*109492	Hypoxanthine	14.9	30.4	Prepyloric
			Adenine	23.6	55.2	
12	A.S., 64, F	117940	Hypoxanthine	17.5	19.9	Prepyloric
			Adenine	40.1	36.9	

*Queen Elizabeth Hospital patient (all others Royal Adelaide Hospital)
†Enzyme values expressed in nanomoles nucleotide formed per mg protein per hour
ΔUlcers classified according to Johnson (1957)

TABLE 16B. PATIENTS TREATED WITH CARBENOXOLONE

Patient No.	Name, age and sex	Mucosal histology	Result of therapy	Side effects from carbenoxolone
1	V.G., 37, F	Normal	Complete healing (XR)*	Nil
2	V.D., 63, F	Mild superficial gastritis	Complete healing (XR)	Hypertension Hypokalaemia
3	E.H., 69, F	Mild superficial gastritis	Complete healing (XR)	Hypokalaemia
4	I.M., 47, M	Moderate superficial gastritis	Partial healing (XR & G)*	Weight gain
5	J.V., 75, M	-	Complete healing (XR & G)	-
6	A.E., 45, M	Moderate atrophic gastritis	Complete healing (XR)	Hypertension
7	N.P., 72, F	Moderate superficial gastritis	No healing (XR & G)	Epistaxis
8	C.T., 59, M	Moderate atrophic gastritis	Complete healing (XR & G)	Nil
9	M.M., 43, F	Severe superficial gastritis	Complete healing (XR)	Hypertension
10	H.L., 69, M	Moderate superficial gastritis	Complete healing (XR)	Nil
11	J.D., 42, M	-	Complete healing (XR & G)	Nil
12	A.S., 64, F	-	Partial healing (XR & G)	Nil

*XR Barium meal confirmation
G Confirmed by gastroscopy

TABLE 17. PATIENTS WITH CHRONIC GASTRIC ULCERATION BUT NOT TREATED WITH CARBENOXOLONE

ms values in nanomoles nucleotide formed per mg protein per hour)

age, sex and tal record no.	Enzymes	Type of ulcer	Gastritis
75, F 076245			
Hypoxanthine	19.0	Primary	-
Adenine	41.2		
79, F 005882			
Hypoxanthine	16.7	Primary	Moderate to severe atrophic gastritis
Adenine	27.5		
67, M -			
Hypoxanthine	21.2	Primary	-
Adenine	84.8		
70, F 074215			
Hypoxanthine	19.2	Primary	Moderate atrophic gastritis
Adenine	38.9		
74, F 023637			
Hypoxanthine	24.6	Prepyloric	-
Adenine	61.4		
64, F 148896			
Hypoxanthine	52.9	Combined	-
Adenine	93.0		
42, F 050417			
Hypoxanthine	18.6	Primary	-
Adenine	34.7		

staining mucosubstance. Fundic mucosal biopsies obtained from five normal subjects were used as controls.

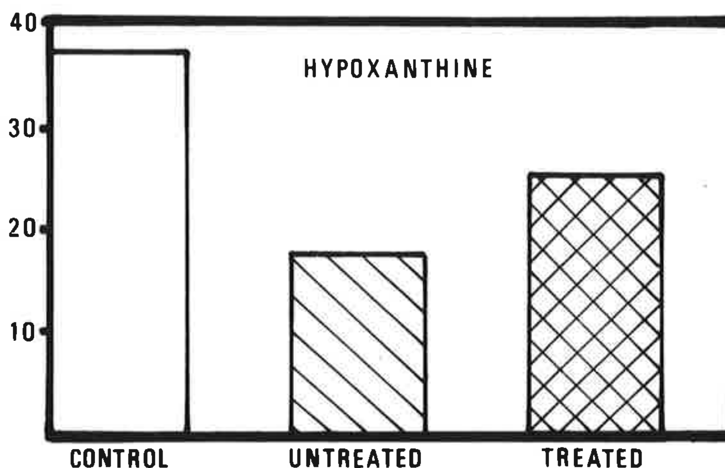
RESULTS

Nineteen patients with gastric ulceration were studied (Tables 16a, 16b and 17). Thirteen of these had a primary type of gastric ulcer, five had prepyloric ulcers and one patient had both gastric and duodenal lesions. The gastric mucosa from the patient with combined ulceration (patient D.Y., Table 17) yielded hypoxanthine and adenine phosphoribosyltransferase activities respectively of 52.9 and 93.0 nanomoles of nucleotide formed per mg protein per hour. In view of the fact that these activities were markedly higher than those from other patients, and that the pathogenesis of the combined type of gastric ulcer probably differs from that of the primary and prepyloric types, these values were excluded from overall statistical analysis.

Twelve patients completed a course of carbenoxolone and attended for post-treatment biopsy. Tables 16a and 16b illustrate the pre- and post-treatment enzyme values, mucosal appearance, and result of therapy in each case. All but one of the patients who exhibited complete healing of the ulcer during the course of carbenoxolone therapy showed an increase in activity of both purine phosphoribosyltransferases. In 3 of the 12 cases a greater than two-fold increase in activity was apparent. There was evidence of gastritis in 10 of the 11 pre-treatment fundic specimens

FIGURE XIV

Effect of carbenoxolone sodium on purine phosphoribosyltransferase activity in fundic mucosa of patients with gastric ulceration. (In namomoles nucleotide formed per mg. protein per hour).



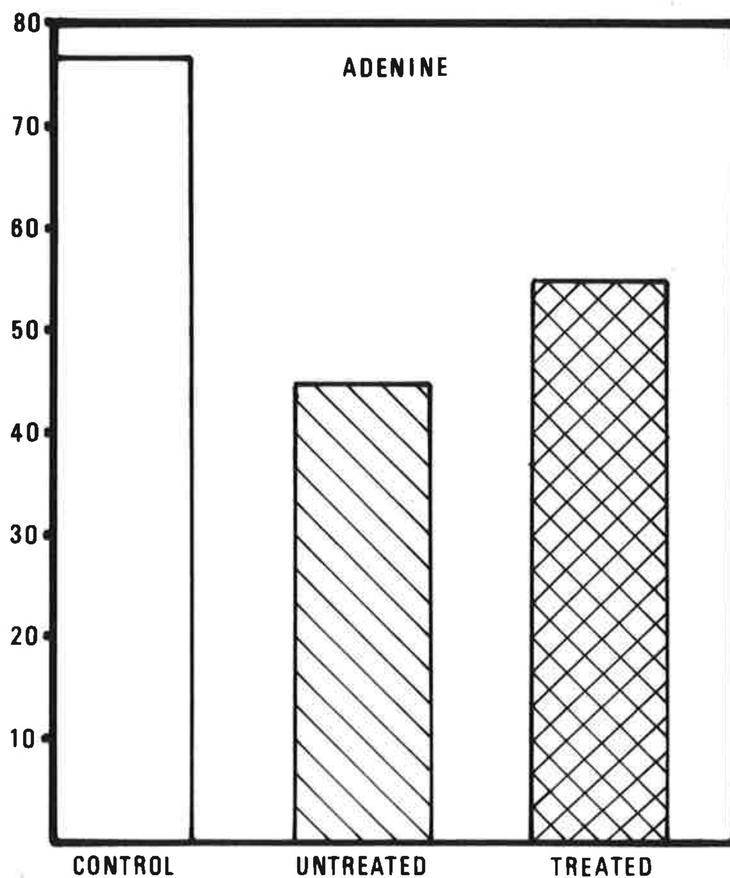


TABLE 18. PURINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY IN FUNDIC MUCOSA OF PATIENTS WITH GASTRIC ULCERATION BEFORE AND AFTER TREATMENT WITH CARBENOXOLONE

(in nanomoles nucleotide formed per mg protein per hour)

	Normal (10)	All gastric ulcer patients (18)	Carbenoxolone treated Pre-treatment (12)	Carbenoxolone treated Post-treatment (12)
HYPOXANTHINE				
Mean	37.4	18.3	17.5	26.5
S.D.	5.9	4.5	5.1	5.9
Range	27.5-47.6	9.1-24.6	9.1-23.2	16.5-36.0
Deviation (t)	-	8.83	8.33	4.31
Probability (P)	-	< 0.001	< 0.001	< 0.001
ADENINE				
Mean	77.0	45.4	44.1	54.6
S.D.	23.3	15.1	12.3	16.4
Range	47.6-124.0	23.6-84.8	23.6-76.8	28.9-83.4
Deviation (t)	-	3.68	3.80	2.42
Probability (P)	-	< 0.002	< 0.002	> 0.02

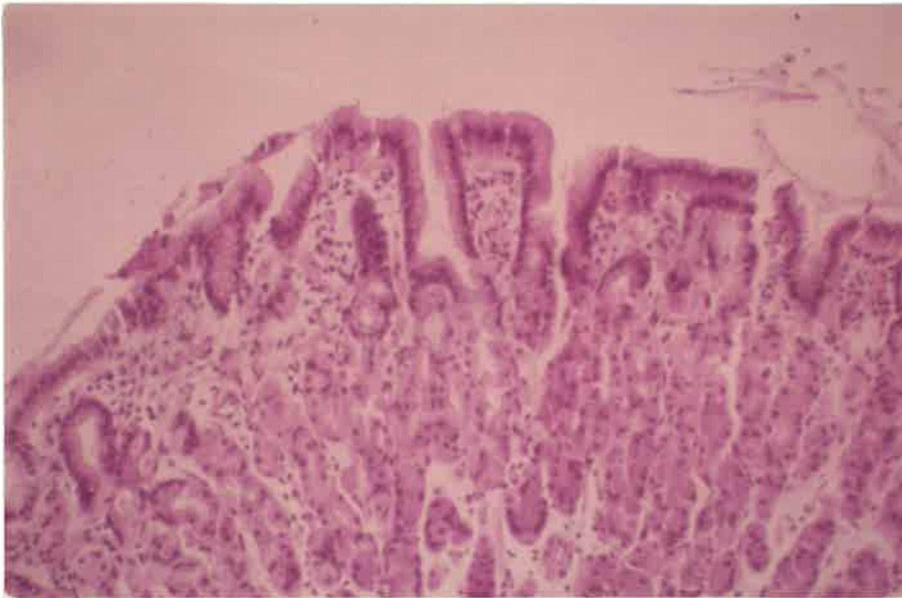
submitted for histology. The mucosal histology from patient A.E. (Table 16a), classified as moderate gastritis, is illustrated in Figure XV.

Six patients were either not suitable for carbenoxolone therapy because of their general medical condition or did not attend for a follow-up biopsy. The purine phosphoribosyltransferase activity in the fundic mucosa of these untreated patients is illustrated in Table 17.

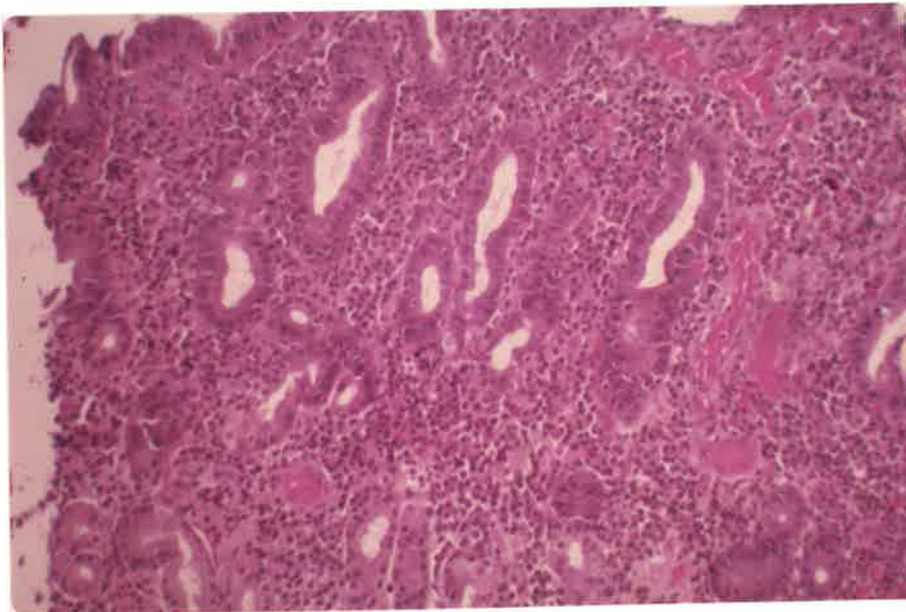
There was no demonstrable difference in P.A.S. staining of the mucosa between normal non-ulcerated individuals or those with gastric ulceration. Figure XVI illustrates the P.A.S.-positive staining in normal control gastric mucosa and that from a patient with chronic gastric ulceration.

Table 18 illustrates the enzyme values obtained for the three groups of subjects - normal; control patients with gastric ulcer; patients with gastric ulceration before and after treatment with carbenoxolone sodium. There was a significant depression of both hypoxanthine phosphoribosyltransferase ($P < 0.001$) and adenine phosphoribosyltransferase ($P < 0.002$) activity in gastric ulcer patients when compared with the levels of activity in normal control subjects. Mean levels, ranges and standard deviations are detailed in Table 18. In the smaller group of 12 gastric ulcer patients who received treatment with carbenoxolone sodium, there was a significant elevation of both hypoxanthine and adenine

FIGURE XV



(A) NORMAL FUNDIC MUCOSA. H & E STAIN X 100



(B) FUNDIC MUSOCA FROM PATIENT WITH CHRONIC GASTRIC ULCERATION. H & E STAIN X 100

phosphoribosyltransferase activity following treatment. The mean hypoxanthine phosphoribosyltransferase activity before treatment was 17.5 ± 5.1 units, and 26.5 ± 5.9 units after carbenoxolone sodium treatment ($P < 0.001$). The respective values for adenine phosphoribosyltransferase were 44.1 ± 12.3 units and 54.6 ± 16.4 units ($P < 0.05$). When the levels in patients with treated gastric ulcers were compared with the normal control values the mean values for hypoxanthine phosphoribosyltransferase were significantly different ($P < 0.001$) and there was a significant difference for the adenine enzyme ($0.05 > P > 0.02$). However these differences were less marked than before treatment.

COMMENT

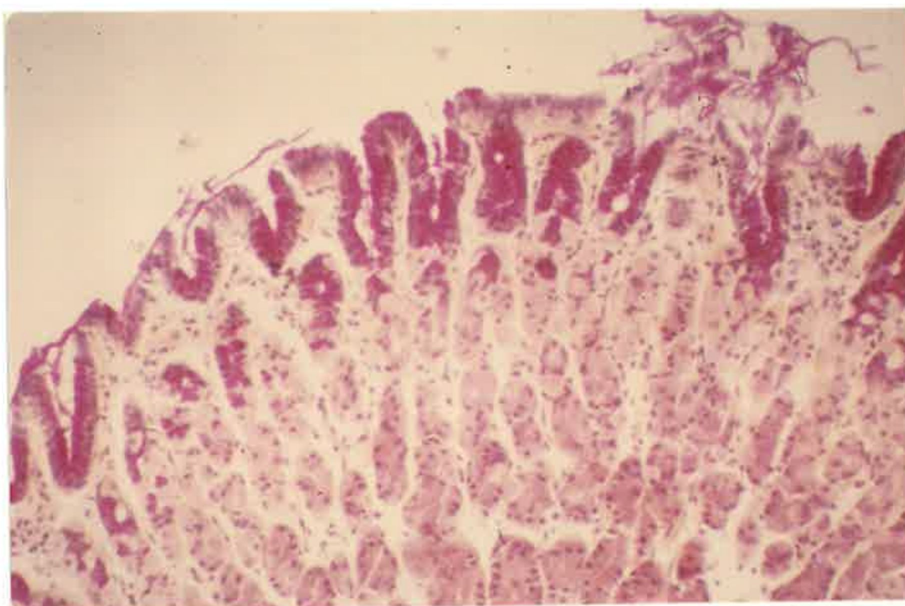
The association of extensive gastritis with chronic gastric ulceration is well established. Magnus (1952) found diffuse gastritis in 26.5 per cent of gastrectomy specimens from patients with chronic gastric ulceration, and the presence of less extensive gastritis in the remainder. Heinkel et alii (1956) confirmed the presence of extensive gastritis in association with chronic gastric ulceration by demonstrating gastritis in fundic biopsy specimens from 70 per cent of gastric ulcer patients. Marks and Shay (1959) maintained that gastritis was an invariable accompaniment of gastric ulceration, and du Plessis (1965) in a series of 65 patients with chronic gastric ulceration found that the ulcer was always situated in an area of atrophic gastritis.

The chronic gastritis extends from the pylorus for a variable distance proximally, usually further along the lesser curve than in the rest of the stomach (du Plessis, 1965). It therefore follows that the higher the ulcer in the stomach the more extensive is the chronic gastritis (Ball and James, 1961). The extent of the inflammatory process and the fact that the gastritis persists after the chronic ulcer has healed (Salupere, 1969) suggest that the gastritis is a primary and not a secondary process. Indeed, Ritchie and Delaney (1968) have demonstrated the pathogenetic importance of atrophic gastritis in an experimental situation, and confirmed that the presence of atrophic gastritis renders the mucosa more susceptible to injury.

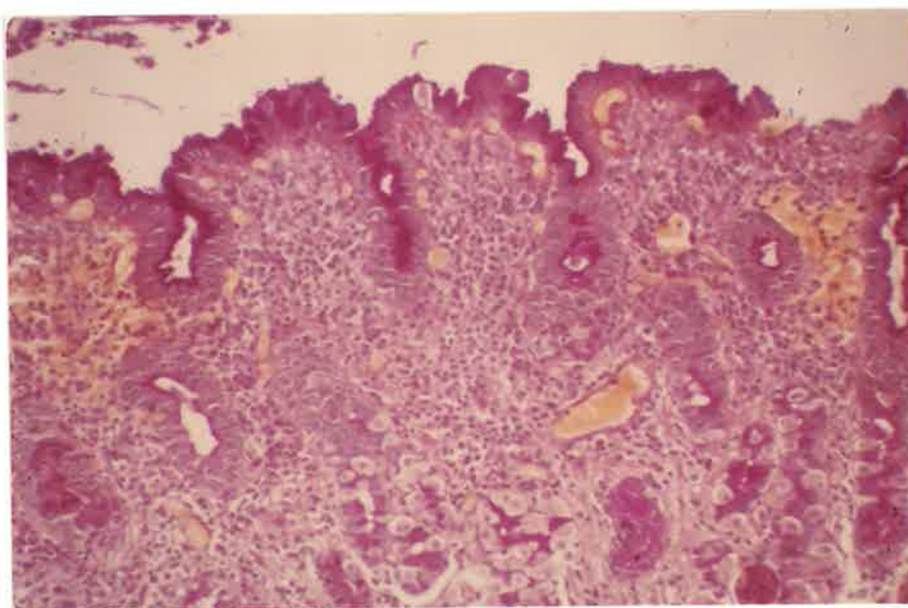
In the present study there was evidence of gastritis in 10 of the 11 fundic biopsy specimens studied. However these biopsies were from mucosa situated some distance from the ulcer, and therefore are not indicative of the degree of mucosal inflammation at the site of ulceration. In many cases the mucosa was heavily infiltrated with lymphocytes and plasma cells, and a germinal centre was noted in one specimen. In this respect the histological findings are in agreement with those of Mackay and Hislop (1966) who suggested that ulceration occurred in association with an active inflammatory type of chronic gastritis consistent with the presence of an immunological response.

No assessment of mucosal histology following carbenoxolone

FIGURE XVI



(A) NORMAL FUNDIC MUCOSA. P.A.S. STAIN X 100



(B) FUNDIC MUCOSA FROM PATIENT WITH CHRONIC GASTRIC ULCERATION. P.A.S. STAIN X 100

therapy was undertaken in the present study. However, in view of the probable glucocorticoid action of this drug it would be of particular interest to ascertain whether any change in the extent or severity of the ulcer-associated gastritis results from carbenoxolone sodium administration. One problem inherent in a biopsy study is that with subsequent biopsy localization to the previous site is not ensured, and indeed a single biopsy may not be truly representative of the surrounding mucosa. Goodier (1968) studied the histopathology of the gastric mucosa in a series of patients with chronic gastric ulceration who had been treated with carbenoxolone sodium for varied periods prior to gastrectomy. He studied 16 gastrectomy specimens and found evidence of mucosal inflammation in each case, but no assessment of pre-treatment histology was made. Furthermore, the selection of patients ultimately submitted to gastrectomy introduces a bias towards carbenoxolone-refractory ulcers, and mucosal histology in such cases may not be representative of the usual response to carbenoxolone therapy.

As observed in Chapter II, a quantitative or qualitative alteration in mucus production leading to impaired gastric mucosal resistance is a logical but as yet unproven explanation of gastric ulcer genesis. Mucus production was not examined in the present study; however P.A.S. staining of histological specimens was undertaken to assess gastric mucus content, and there was no demonstrable difference between the abnormal and the control mucosa. It should

be stressed, however, that this technique most certainly does not differentiate qualitative alterations in mucus content, and it may well be that the relative concentration of mucopolysaccharides in gastric mucus is fundamental to mucosal resistance. Furthermore the technique is not quantitative, and one must conclude that although alteration in gastric mucus is not the proven cause of gastric ulceration, it is certainly not excluded.

This study has demonstrated a reduced activity of both hypoxanthine and adenine phosphoribosyltransferase in gastric mucosa obtained from patients with chronic benign gastric ulceration - that is a functional difference between normal mucosa and that surrounding the ulcer. The most striking difference between the normal mucosa and that from the ulcer patients is the presence of mucosal inflammation in the latter group, and it is conceivable that the functional change demonstrated is a reflection of this mucosal inflammation. The argument that apparent alterations in biochemical function in inflamed tissues are a reflection of the inflammatory infiltrate and not the intrinsic function of the tissue cells can be applied to many studies of mucosal inflammation (Clark and Senior, 1969), and it is unfortunate that in many cases this is only partially resolved by indirect evidence. The factors arguing against this interpretation are that although the study was small, there was no striking correlation between the activity of the two enzymes and the degreeof mucosal inflammation. Furthermore, Clark

and Senior (1969) demonstrated elevated activity, not reduced activity, of pyrimidine nucleotide biosynthetic enzymes in the jejunal mucosa of patients with celiac disease, and this observation has been extended to the purine phosphoribosyltransferases in celiac mucosa exhibiting a comparable degree of mucosal inflammation as that reported in the present study (Mackinnon, A.M. unpublished observation). The possibility that the reduced enzyme activity demonstrated in the gastric mucosa of the ulcer patients is due to inflammatory cell infiltration is, however, not excluded, and it is possible that the carbenoxolone administered reduced this inflammation to result in an apparent increase in enzyme activity.

An alternative explanation of this phenomenon is based on the demonstration by Imondi et alii (1969) that purine phosphoribosyltransferase activity is higher in differentiated intestinal epithelial cells than in the immature crypt cells. Although this observation has not been extended to the gastric mucosa, it is conceivable that the reduced purine phosphoribosyltransferase activity in the gastric mucosa of the ulcer patients is a reflection of a reduced number of differentiated cells in the mucosa. This failure of cell differentiation may be reversed by carbenoxolone administration leading to a prolonged cell turnover time in the gastric mucosa, and a more mature population of gastric epithelial cells.

These findings may therefore be interpreted as indicative of a poorly differentiated epithelial cell population in the inflamed gastric mucosa associated with chronic gastric ulceration, possibly

resulting from an increased rate of cell migration and turnover. Any extension of this postulate must be purely speculative, but a possible corollary of this situation may be that the poorly differentiated cells do not possess the normal overall mucosal capacity for RNA, protein or mucopolysaccharide synthesis, and that either a qualitative or quantitative defect of gastric mucous secretion may be present. The increased differentiation associated with carbenoxolone therapy would be expressed at a functional level with increased synthesis of factors contributing towards mucosal resistance.

While the above postulate is undoubtedly appealing, a third explanation relating purine phosphoribosyltransferase activity to nucleic acid synthesis should be presented. The results previously reported in this thesis indicate that the level of activity of the purine phosphoribosyltransferases provides an index of the rate of purine nucleotide biosynthesis in gastric mucosa, and since purine nucleotides are utilized in nucleic acid and nucleotide coenzyme synthesis this may provide an index of the synthetic rate of these compounds. In the current state of knowledge it is not possible to comment on the normal distribution between nucleic acid and coenzyme synthesis. While a reduced rate of DNA synthesis and a reduced rate of cellular proliferation in the gastric mucosa would impair gastric mucosal resistance by reducing the second line of defence against aggressive factors, it is unlikely that the finding of reduced purine phosphoribosyltransferase activity is indicative of impaired DNA synthesis. However, purine nucleotides are essential precursors of

both DNA and RNA, and it is conceivable that the depression of enzyme activity demonstrated in this study indicates impaired RNA synthesis in the inflamed gastric mucosa. Certainly, this hypothesis would be in keeping with that of the poor differentiation and functional immaturity of the gastric epithelial cells in this situation, as presented above.

This study has demonstrated a functional difference between normal and inflamed gastric mucosa, with a reduced activity of the purine phosphoribosyltransferases in the gastric mucosa obtained from patients with chronic benign gastric ulceration. The most likely explanation of this observation is that cell differentiation, and RNA synthesis are impaired in the inflamed gastric mucosa.

CHAPTER VIII

DISCUSSION

For many years gastric and duodenal ulceration have not been adequately differentiated but have been considered as a common entity with only minor differences. It is becoming increasingly apparent, however, that the collective term peptic ulceration leads only to confusion and that gastric and duodenal ulceration should be considered as separate diseases (Nyhus, 1970). The differences between gastric and duodenal ulceration are emphasized by the differences which have emerged in their apparent aetiology. Present evidence suggests that the pathogenesis of duodenal ulceration is primarily related to what has been described (Menguy, 1964) as the acid peptic factor. This author cited eight clinical or experimental associations demonstrating the role of the aggressive or acid-peptic factor in duodenal ulcer genesis. On the other hand hypersecretion of acid or pepsin is not a factor in the aetiology of the majority of gastric ulcers (du Plessis, 1965; Vesely et alii, 1968), and consequently the assumption that these ulcers result from lowered mucosal resistance has developed. Although the mucosal barrier is aetiologically incriminated, the mechanism leading to impaired mucosal resistance remains unresolved. Indeed, it may be wrong to invoke a unified concept for the pathogenesis of any such disease, and in this respect the classification of chronic gastric ulceration into three distinct clinical categories (Johnson, 1957) is particularly relevant for it reinforces the possibility of a multifactorial aetiology for gastric ulceration.

The mucosal barrier consists of a layer of mucus secreted by

and covering the epithelium, and the epithelial cell layer itself (Hollander, 1954). Conceivably an alteration in either the mucus or cellular component could result in the impaired mucosal resistance which is postulated to be the causal factor in chronic gastric ulceration. Johnson (1957) maintained that a deficiency of mucus secretion was found in association with many gastric ulcers. Nevertheless, the attractive hypothesis that a quantitative or qualitative alteration in mucus production is the primary mechanism in gastric ulcer genesis remains to be proven. Indeed, both Piper et alii (1965) and Robert and co-workers (1967) were unable to demonstrate that mucus production in patients with chronic gastric ulceration differed from normal. Furthermore there is no indisputable evidence that gastric mucus has any more than a lubricant function. Cooke (1967) in a review of the relevant literature found little to suggest that the mucus layer contributed significantly to mucosal resistance. Thus, on present evidence alteration in mucus production leading to reduced mucosal resistance is an unlikely explanation of gastric ulcer genesis.

A number of workers (du Plessis, 1965; Lawson, 1966; Capper, 1967) believe that the duodenal reflux demonstrable in many cases of chronic gastric ulceration is primarily responsible for the mucosal defect. Reflux of duodenal contents may loosen the mucus covering of the gastric epithelium and render the mucosa more susceptible to acid peptic digestion (du Plessis, 1965) or may

produce chronic gastritis and thus a damaged mucosa which is more susceptible to injury (du Plessis, 1965; Lawson, 1966). Capper, Airth and Kilby (1966) demonstrated significant regurgitation through the pylorus in two thirds of the gastric ulcer patients they studied, but also found significant duodenal reflux in 50 per cent of patients with duodenal ulceration in whom there was no evidence of a gastric lesion. While many patients with chronic gastric ulceration have demonstrable duodenal reflux, it remains to be established whether this is of significance in the pathogenesis of gastric ulceration. Indeed, the low incidence of gastric ulceration consequent on the reflux following gastric surgery (Small, 1964) and the generally long interval between occurrence of reflux and gastric ulceration in this situation suggest that regurgitation of alkaline intestinal content is of minor importance in the causation of chronic gastric ulceration.

A further theory of gastric ulcer genesis has been advanced by Oi, Ito, Kumagai, Yoshica, Tanaka, Yoshikawa, Miho and Masamura (1969). These workers maintain that chronic gastric ulcers develop adjacent to anatomical muscle and mucosal boundaries. They postulate that gastric motility imposes a particular strain, possibly ischaemic, on the mucosa in these areas rendering them particularly liable to gastric ulceration.

The main purpose of the present study was to examine the hypothesis that chronic gastric ulceration in humans results from

a functional abnormality of the gastric mucosa, and that the functional integrity of the mucosa is of the utmost importance in maintaining resistance to ulceration. Magnus (1952) drew attention to the epithelial cell layer in chronic gastric ulceration when he reported the presence of chronic gastritis in all of 284 gastrectomy specimens from chronic gastric ulcer patients. The high incidence of extensive chronic inflammation in the gastric mucosa of patients with chronic gastric ulceration is now well established (Heinkel et alii, 1956; Marks and Shay, 1959; du Plessis, 1965; Mackay and Hislop, 1966). Indeed, Marks and Shay (1959) maintained that chronic gastritis invariably accompanied chronic gastric ulceration and that such ulcers were commonly situated in an area of severe atrophic gastritis. The persistence of the gastritis after the ulcer has healed (Palmer, 1951; Mackay and Hislop, 1966; Salupere, 1969) suggests that it is a primary phenomenon and as such is of obvious aetiological significance. This was implied by Wood and Taft (1958) when they stated that "..gastritis in its superficial and atrophic forms may be related to gastric ulcer, but not to duodenal ulcer which may have a different aetiology."

The present study was established to examine the gastric mucosa from patients with chronic benign gastric ulceration at a functional level. Purine nucleotide biosynthesis was selected for study because of its relation to nucleic acid metabolism and so indirectly to RNA and protein metabolism, it being considered possible that the fundamental defect leading to gastric ulceration may be a disturbance

of RNA metabolism, mucoprotein and protein secretion.

The initial studies presented in this thesis established the importance of the purine salvage pathway in gastrointestinal tissue. The important finding in these early studies was the absence of demonstrable activity of the rate controlling enzyme of the de novo purine biosynthetic pathway, phosphoribosylpyrophosphate amido-transferase, in gastric or intestinal mucosa. The enzyme was, however, active in guinea pig liver. The demonstration of the absence of de novo pathway activity in rapidly proliferating and highly metabolic tissue is not without precedent, for current evidence indicates that the bone marrow lacks de novo activity (Lajtha and Vane, 1958) and that these cells are dependent on other tissues or possibly dietary sources for supply of purines. The present concept, recently supported by the work of Pritchard et alii (1970), is that the liver is the major source of supply of purine to such tissue as bone marrow and possibly gastrointestinal mucosa. Thus even in the absence of a dietary source of adenine, de novo synthesis of purines by the liver should maintain an adequate supply of purine to dependent tissues. Therefore, since a supply of purine nucleotide is of absolute and fundamental importance to the body tissues, the absence of demonstrable de novo activity and presumed inactivity of the de novo pathway in gastrointestinal mucosa emphasizes the importance of the purine salvage pathway in this situation.

The assay of the rate limiting enzyme of de novo purine synthesis

is not easy, and as emphasized in the comment to Chapter II, the absence of de novo activity in intestinal mucosa may be relative rather than absolute. Nevertheless, previous studies (Murray, 1967; Davidson and Winter, 1964; Price and Murray, 1969) have demonstrated that the activity of the purine salvage enzymes hypoxanthine and adenine phosphoribosyltransferase is an index of the rate of purine nucleotide biosynthesis and nucleic acid synthesis, probably RNA, in tissues. It is probable therefore, since gastrointestinal mucosa is relatively dependent on the salvage pathway that measurement of phosphoribosyltransferase activity should provide a reliable index of purine nucleotide synthesis in this tissue.

The model of stress induced gastric ulceration was selected for study because of the documented changes that occur in nucleic acid metabolism in this situation (Lipkin et alii, 1966; Ludwig and Lipkin, 1969). The present study confirmed the efficacy of the immobilization technique in inducing gastric mucosal damage, and in addition an apparent susceptibility of the antral mucosa in the guinea pig was noted. Moreover a marked reduction in purine phosphoribosyltransferase activity was demonstrated in the non-ulcerated mucosa of the restrained animals. This suggests that the synthesis of purine nucleotide is reduced in the gastric mucosa of these animals, and that the demand for nucleic acid synthesis and possibly nucleotide coenzyme synthesis is reduced. However, as was discussed when the experimental data was presented, the combination

of circumstances was such that it is probable that the restraint studies indicate a reduction of RNA synthesis in this situation. Nevertheless, both DNA and RNA synthesis are reduced by the stress imposed by restraint (Ludwig and Lipkin, 1969), and it is conceivable that the reduced rate of cell proliferation in combination with reduced RNA and protein synthesis gives rise to a gastric mucosal population of poorly differentiated cells. Imondi et alii (1969) demonstrated that purine phosphoribosyltransferase activity was lower in immature than in mature differentiated intestinal cells. Therefore it is possible that the reduced enzyme activity demonstrated in the restrained animals is a reflection of an increased population of poorly differentiated gastric epithelial cells.

Although the administration of both aspirin and prednisolone gave rise to gastric mucosal injury there was a marked difference between the effect of these two drugs on mucosal phosphoribosyltransferase activity. Aspirin did not apparently affect mucosal purine nucleotide metabolism, while prednisolone administration was associated with a marked reduction in purine phosphoribosyltransferase activity. It is difficult to explain this reduced activity in terms of the concept of cellular immaturity or impaired differentiation. Prednisolone reduces the rate of cellular proliferation and DNA synthesis in gastric mucosa (Räsänen, 1963; Max and Menguy, 1970), and since mucosal architecture is preserved this presumably results in an increased cell migration time in the gastric mucosa.

The individual epithelial cells would therefore be on average older and more mature than normal, and one could predict if anything higher activity of the purine phosphoribosyltransferases than normal. Therefore, since purine nucleotides are utilized in the synthesis of DNA, and it is established that corticosteroids reduce DNA synthesis in gastric mucosa (Max and Menguy, 1970), it is suggested that the reduced phosphoribosyltransferase activity in this situation reflects the impaired synthesis of DNA.

Carbenoxolone administration resulted in a reduction of activity of both purine phosphoribosyltransferases in guinea pig and human gastric mucosa. However, it is of interest that only the antral mucosa of the guinea pig was apparently affected suggesting that this area of guinea pig stomach is more susceptible to the action of the drug. Nevertheless, although the dose of carbenoxolone administered to the guinea pigs was low, the enzyme changes in antral mucosa parallelled those observed with prednisolone administration, and one may speculate that these drugs were acting through a common mechanism.

The mineralocorticoid side effects of carbenoxolone therapy are well established (Doll et alii, 1962; Turpie and Thomson, 1965), and indeed the ulcer healing benefit of this drug is closely associated with its mineralocorticoid action (Doll et alii, 1968; Cocking and MacCaig, 1969). It is possible that the mineralo-corticoid effect is exerted through the intact adrenal cortex of

the patient receiving the drug (Elmadjian, Hope and Pincus, 1956; Boorst, ten Holt, de Vries and Molhuysen, 1953). Although Baron and Nabarro (1968) maintained that there was little evidence that carbenoxolone sodium exerted a glucocorticoid action, the work of Khan and Sullivan (1968) indicated that some glucocorticoid activity may result from adrenocortical stimulation by this drug. The findings presented in this thesis provide some support for the concept that carbenoxolone administration may stimulate glucocorticoid production. Thus, when considered with the finding of Mattingly et alii (1970) that carbenoxolone administration results in an acute elevation of plasma 11 hydrocorticoid levels in patients with functioning adrenal tissue, there is evidence that further investigation of the possible glucocorticoid action of carbenoxolone may prove rewarding.

The biopsy study of patients with chronic benign gastric ulceration demonstrated a high incidence of chronic mucosal inflammation and confirms the association between chronic gastric ulceration and chronic gastritis described by other workers (Magnus, 1952; Heinkel et alii, 1956; Mackay and Hislop, 1966). Although it was recognized that the method was neither qualitative nor quantitative, there was no demonstrable difference in P.A.S. positive mucosubstance staining between the normal and the inflamed gastric mucosa. Nevertheless it is stressed that this does not

exclude the possibility that the fundamental defect leading to chronic gastric ulceration is impaired gastric mucus secretion.

The enzyme study ef biopsy material from patients with chronic benign gastric ulceration demonstrated a marked difference in activity of the purine phosphoribosyltransferases between the control and ulcer populations. The most probable explanation of this observation is that the chronically inflamed gastric mucosa associated with ulceration is composed largely of undifferentiated, immature cells with a low intrinsic activity of the purine phosphoribosyltransferases. In this respect the study of Imondi et alii (1969) demonstrated that the activity of both phosphoribosyltransferases is lower in undifferentiated than in differentiated intestinal cells.

Furthermore, this concept of low enzyme activity resulting from impaired cell maturation is consistent with current knowledge of cellular kinetics in chronic gastritis. Is is more than likely that the rate of cell proliferation in the chronically inflamed gastric mucosa is greater than normal (Teir and Räsänen, 1961; Winawer and Lipkin, 1969), and it is probable that the rate of cell migration in this situation is increased and the cell turnover time decreased. Thus it is conceivable that the

time available for cellular maturation would be less than normal, and since the differentiation and functional maturity of intestinal cells is a function of cell age (Padykula, 1962) the overall functional maturity of the gastric mucosa would be reduced in this hypothetical situation. The lower activity of the phosphoribosyltransferases in the mucosa from patients with chronic gastric ulceration may well be a reflection of functional immaturity and a reduced synthesis of RNA.

This study furthermore demonstrated that purine phosphoribosyltransferase activity was significantly increased after a therapeutic course of carbenoxolone sodium. This observation presents an apparent paradox, for it was previously demonstrated that carbenoxolone, acting on normal gastric mucosa resulted in depression of enzyme activity. However, this apparently paradoxical situation is explicable in terms of the possible corticosteroid action of the drug carbenoxolone. Glucocorticoids depress the rate of cell proliferation in gastric mucosa (Räsänen, 1963; Max and Menguy, 1970), and one could predict that the rate of cellular migration would be comparably reduced. Should this be the case, the average life span of the individual epithelial cells would be increased, allowing more time for cellular

differentiation and maturation. It is suggested, therefore, that the observed increase in phosphoribosyltransferase activity associated with carbenoxolone therapy indicates that this drug alters mucosal cell kinetics to allow greater cell differentiation.

It is conceivable that the basic defect in the gastric mucosa of patients with gastric ulceration is one of cellular immaturity. This immaturity may impair either or both factors theoretically contributing to mucosal resistance. The cells may be less resistant to the action of acid and pepsin, or conversely the immature cell may produce less mucus, or qualitatively abnormal mucus less resistant to the passage of aggressive factors. Since glucocorticoids could theoretically affect mucosal maturity and function, one could speculate that theoretically at least prednisolone may be beneficial in cases of established chronic benign gastric ulceration; however much more scientific evidence is required before this heretical speculation could be justifiably examined in a clinical situation!

An alternative explanation of the findings reported in this study of biopsy specimens from patients with benign gastric ulceration is that the apparent epithelial cell enzyme activity is

a reflection of the diluting effect of the infiltrating inflammatory cells. Certainly the mucosa obtained from the ulcer patients was, overall, chronically inflamed, and it is unfortunate that no assessment was made of post treatment histology. Indeed there has been no controlled study of the effect of carbenoxolone on the ulcer associated chronic gastritis. Goodier (1968) studied the histopathology of the gastric mucosa in a series of patients who had been treated with carbenoxolone for varying periods prior to gastrectomy. Mucosal inflammation was demonstrable in every case, and in a number of cases the inflammation was marked. However, no assessment of pre-treatment histology was made, and furthermore, the selection of patients ultimately submitted to gastrectomy introduces a bias towards carbenoxolone refractory ulcers, and mucosal histology in such cases may not be representative of the usual response to carbenoxolone therapy. Although the inflammatory change may not be uniformly distributed throughout the gastric mucosa (du Plessis, 1965), it is suggested that a carefully controlled study to assess the effect of carbenoxolone on mucosal histology is indicated and warranted.

While the aetiology of chronic gastric ulceration remains unresolved any classification of gastric ulcers must be

unsatisfactory. Johnson (1957) divided gastric ulcers into three main types: those in the body of the stomach, those in the prepyloric area, and a combined type with both gastric and duodenal lesions. He suggested that the former type was uniformly associated with defective mucus production. However, it is probably not pathogenetically correct to classify all prepyloric ulcers together for it is apparent that there are two categories of prepyloric ulcer. One type is associated with acid hypersecretion and blood group O non-secretor status (Johnson et alii, 1964), while many prepyloric ulcers are associated with gastric hypochlorhydria (Vesely et alii, 1968) and therefore resemble the more commonly encountered gastric body ulcers. Therefore, from a consideration of the available literature, and from the studies reported in this thesis, an amended classification of chronic gastric ulceration based on differing pathogenesis is proposed.

1. Combined duodenal and gastric ulceration, where there is evidence of active duodenal ulceration or scarring and the gastric ulcer is secondary to the duodenal lesion.

2. Duodenal-type gastric ulceration, usually prepyloric in which there is demonstrable acid hypersecretion.

3. 'Primary' gastric ulceration, occasionally prepyloric, associated with acid hyposecretion.

It is suggested that, in this latter group alone, the chronic mucosal inflammation that precedes frank ulceration is of fundamental pathogenetic significance, and is associated with an increased rate of mucosal cell turnover and functional immaturity of the epithelial cells.

There are a number of similarities between the mucosal lesion associated with celiac disease and that associated with primary gastric ulceration. There is a heavy inflammatory cell infiltration in both situations (Magnus, 1952; Stewart, Pollock, Hoffbrand, Mollin and Booth, 1967); there is an increased rate of cell proliferation in each case (Teir and Räsänen, 1963; Booth, 1970); and it is possible to demonstrate associated, probably non-specific

antibodies in each disease (Doniach and Roitt, 1964; Wangel, 1970; Alp and Wright, 1971).

It is of some interest therefore, that Doe, Henry and Booth (1972) have recently demonstrated the deposition of immune complexes in the jejunal mucosa of treated celiac patients challenged with an oral dose of gluten, and this finding suggests that the pathogenesis of celiac disease may be on the basis of an immunological reaction. Furthermore, Mackay and Hislop (1966) maintained that the histological characteristics of the gastric mucosa in chronic gastric ulceration were very suggestive of an underlying immunological process. Therefore, it is not improbable that the mucosal defect associated with primary gastric ulceration evolves on the basis of an immunological reaction. Although this hypothesis is unproven, it is suggested that further studies of the mechanism of action of the drug carbenoxolone sodium in the treatment of primary gastric ulceration are clearly indicated.

Revision of part of the discussion in Chapter VIII has resulted in the deletion of pages 139-142.

SUMMARY

The current concept of the pathogenesis of gastroduodenal ulceration is that the mucosal defect arises from an imbalance between aggressive and defensive factors acting on the gastroduodenal mucosa. The indices of aggression such as production of acid and pepsin are reduced in the majority of patients with chronic gastric ulceration, and for this reason gastric ulceration has been presumed to arise from a reduction in the mucosal resistance to normal trauma.

The capacity of the mucosa to resist acid-peptic digestion is dependent on two components; gastric mucus and the functional integrity of the epithelium. An attractive but as yet unproven explanation of gastric ulcer genesis is that a quantitative or qualitative alteration in mucus production leads to increased susceptibility to mucosal trauma. However, there is no evidence at present that mucus production in patients with chronic gastric ulceration differs from normal; indeed the role of gastric mucus in affording protection against acid-peptic digestion remains unproven. It is probable that the functional integrity of the mucosa is the ultimate determinant in providing resistance to acid-peptic digestion and other mucosal trauma.

The aim of the present investigation was to establish a method of assessing the function of the gastric mucosa utilizing mucosal specimens of comparable size to those obtained with peroral suction biopsy, and to apply this method to an examination of the gastric

mucosa in patients with proven chronic benign gastric ulceration. The enzymic control of purine nucleotide biosynthesis was selected for study because of the established relationship between nucleotide metabolism, nucleic acid synthesis and the rate of cellular proliferation and protein synthesis. The activity of selected enzymes was studied in normal guinea pig and human gastrointestinal tissue.

The de novo pathway of nucleotide biosynthesis was considered to be inactive in gastrointestinal mucosa as there was no appreciable activity of the rate controlling enzyme phosphoribosylpyrophosphate amidotransferase detected. This contrasted to the situation in guinea pig liver where appreciable activity of this enzyme was detected. On the other hand the purine salvage pathway enzymes hypoxanthine and adenine phosphoribosyltransferase were active in all areas of the gastrointestinal tract studied, with regional differences in the ratios of activity of these two enzymes. These results suggested that gastrointestinal mucosa is dependent on the salvage pathway to maintain purine nucleotides at levels adapted to the rate of nucleic acid synthesis.

Both DNA and RNA metabolism are known to be depressed in the gastric mucosa of restrained animals, and accordingly the experimental model of restraint induced acute gastric ulcer in guinea pigs was selected to test the hypothesis that activity of the purine salvage enzymes hypoxanthine and adenine phosphoribosyltransferase reflected the rate of nucleic acid synthesis. Acute

gastric ulceration developed in all animals restrained for 40 hours, while a lower incidence of mucosal injury was evident in guinea pigs retrained for 10 and 20 hours. The amount of gastric mucus, assessed visually and by P.A.S. and Hales colloidal iron staining, was reduced in each of the restrained animals. The antral mucosa appeared particularly susceptible to the stress imposed by immobilization as the mucosal lesions were concentrated in the distal portion of the animals' stomachs. Phosphoribosyltransferase activity was depressed in the restrained group of animals particularly in the antral mucosa where a significant depression of both phosphoribosyltransferase enzymes was recorded after 10, 20 and 40 hours of immobilization. The reduction in enzyme activity and the degree of mucosal injury in the antral area were both most marked in the animals which had been restrained for 40 hours. These studies suggest that purine nucleotide biosynthesis is reduced in the gastric mucosa of animals subjected to immobilization. It is conceivable that the reduced purine nucleotide synthesis is a reflection of reduced RNA synthesis and a reduced demand for purine nucleotides, and that in this situation measurement of purine phosphoribosyltransferase activity provides an index of RNA biosynthesis.

The ulcerogenic potential of a number of anti-inflammatory drugs has been established. However, the mechanism of the mucosal injury induced by these agents has not been clarified.

There is convincing experimental evidence that corticosteroids depress the rate of cell proliferation and DNA metabolism in the gastric mucosa. Therefore to test further the hypothesis that purine phosphoribosyltransferase activity is a valid index of nucleic acid metabolism the effect of prednisolone on gastric mucosal enzyme activity was studied in guinea pigs. A further anti-inflammatory drug, acetylsalicylic acid (aspirin) was also tested. Both drugs produced gastric mucosal injury following prolonged oral administration, but the distribution of the injury differed. Aspirin administration was associated with an haemorrhagic gastritis confined to the proximal or fundic portion of the stomach, while prednisolone produced circumscribed antral erosions with no macroscopic involvement of the proximal mucosa. The effect of these drugs on purine salvage enzyme activity was also dissimilar. A marked depression of both hypoxanthine and adenine phosphoribosyltransferase activity in the fundic and antral mucosa resulted from prednisolone administration, while aspirin produced no change in enzyme activity. These studies indicate that aspirin and prednisolone produce gastric mucosal damage through different mechanisms. Since purine nucleotides are utilized in both DNA and RNA synthesis, and it is established that corticosteroids reduce the rate of DNA synthesis in gastric mucosa, these studies suggest that reduced phosphoribosyltransferase activity in this situation may possibly be a reflection of impaired synthesis of DNA.

The efficacy of the drug carbenoxolone sodium in accelerating the healing of chronic benign gastric ulcers has been established by a number of clinical trials, yet its mechanism of action in this situation remains obscure. Carbenoxolone administration was associated with a significant depression of purine phosphoribosyltransferase activity in the antral mucosa of guinea pigs, and a similar reduction in activity was observed in a small human study. Although the effect from carbenoxolone sodium in guinea pigs was not as pronounced as that from prednisolone, these studies suggest that both drugs may be acting on the gastric mucosa through a common mechanism. Further study of the mechanism of action of carbenoxolone sodium is clearly indicated.

Purine phosphoribosyltransferase activity was studied in gastric biopsy specimens from 19 patients with proven chronic gastric ulceration. These included 5 patients with prepyloric ulcers and 13 with a primary type of ulcer. A significant depression of both hypoxanthine and adenine phosphoribosyltransferase activity in the fundic mucosa of these patients was demonstrated. In 12 patients treated

with a four week course of carbenoxolone sodium, purine phosphoribosyltransferase activity was demonstrably increased above pre-treatment levels when assessed on completion of the therapeutic trial. Furthermore this study confirmed the high incidence of chronic gastritis associated with chronic gastric ulceration.

It is conceivable that the reduction in enzyme activity observed in the gastric mucosa of the gastric ulcer group is a reflection of the diluting effect of the inflammatory cell infiltrate demonstrable in the mucosal biopsies. However, current evidence indicates that the rate of cellular proliferation is increased in chronically inflamed gastric mucosa, and it is possible that the increased rate of cell turnover leads to an increased population of poorly differentiated cells in the gastric mucosa. An alternative explanation of the findings reported in this thesis is that the depressed levels of purine phosphoribosyltransferase activity in the inflamed gastric mucosa provide an indication of a poorly differentiated gastric mucosa. Furthermore, it is suggested that carbenoxolone therapy, by reducing the rate of cellular proliferation, results in a more mature, better differentiated gastric mucosa.

It is concluded that the functional immaturity of the epithelial cells in the gastric mucosa of patients with chronic gastric ulceration is of pathogenetic importance, and that the initiating factor leading to a susceptibility to gastric ulcer genesis is the development of chronic gastritis.

APPENDICES

APPENDIX A

REAGENTS USED IN THE METHODS ADOPTED IN THIS THESIS

Tris (hydroxymethyl) aminomethane (Sigma)

Magnesium chloride 6 H_2O (Ajax)

Adenine (Koch-Light)

Hypoxanthine (Koch-Light)

Adenosine monophosphate (Sigma)

n-Butanol (B.D.H.)

Glacial acetic acid (B.D.H.)

Sodium carbonate (anhydrous) (B.D.H.)

Sodium hydroxide (B.D.H.)

Cupric sulphate 5 H_2O (Ajax)

Hydrochloric acid (Adelaide and Wallaroo Fertilizers Ltd.)

Folin and Ciocalteu's reagent (By-Products and Chemicals Ltd.)

Phenolpthalein (Analytical and Research Chemical Co.)

Sodium-potassium tartrate

Bovine serum albumen (Sigma)

Trichloracetic acid (B.D.H.)

Ether (B.D.H.)

Ethanol (Univar)

Naphthalene blue-black (Gurrs C.I.)

Methanol (B.D.H.)

Dithiothretol (P-L Biochem.)

L-Glutamine (Sigma)

L-Glutamate (Sigma)

Propan-2-ol (Baker)

Formic acid (May & Baker)

POPOP (Packard)

PPO (Packard)

Toluene (Ajax)

Ninhydrin aerosol spray (Sigma)

Whatman chromatography paper 3 mm

APPENDIX B

EQUIPMENT USED IN THE METHODS ADOPTED IN THIS THESIS

Eppendorf pipettes (5, 10, 20, 50, 100, 200 µl)

U.V. light source (Camag TL 900)

pH meter (E.I.L.)

Water bath (Pacific Electric Co. Pty. Ltd. type 4FS)

Spectrophotometer (Carl Zeiss, PMQ II)

Packard Tricarb scintillation counter

(Spectrometer settings were optimised for C^{14}, and quench correction was performed using the channels ratio method).

Glassware: washed in Diversey Pyroneg detergent, rinsed in tap water then three times in glass distilled water.

APPENDIX C

STATISTICAL METHODS

The statistical methods employed for the analysis of results have been outlined by Bailey (1959).

STATISTICAL FORMULAE

A. Calculation of mean and standard deviation

mean $(\bar{x}) = 1/n \quad \Sigma x$

standard deviation $(S) = \sqrt{\dfrac{\Sigma (x - \bar{x})^2}{n - 1}}$

B. Comparison of mean of two samples

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{S_1^2}{n_1} + \dfrac{S_2^2}{n_2}}}$$

List of abbreviations used in statistical formulae:

- n number of observations in sample
- x observed measurements in a sample, independent variables in a regression
- $\bar{x}$ mean of sample of measurements x
- Σ summation symbol
- S estimated standard deviation
- t t test
- P significance level actually achieved by data

All normal ranges are stated as mean ± one standard deviation.

The significance levels were obtained from published statistical terms (Documenta Geigy, 1962).

The following notations have been utilised in this thesis:

$P > 0.05$ not significant

$P < 0.01$ significant

$P < 0.001$ highly significant

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