The Anti-Ulcerogenic Effect Of Unripe Vegetable Banana On Gastric Ulceration Induced By Aspirin In The Rat

NASSER NASSER

BSc. (Licence) Diploma (Maîtrise)

A Thesis submitted for the degree

of Doctor of Philosophy

at The University of Aston in Birmingham

Department of Pharmacy
University of Aston
in Birmingham
Gosta Green
Birmingham B4 7ET

May, 1983.

THIS THESIS IS DEDICATED TO

MY MOTHER AND FATHER

The University of Aston in Birmingham

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SUMMARY

1. The green vegetable banana was found to possess anti-ulcerogenic activity against aspirin induced ulcers in rats. The activity was present in banana imported from India and the West Indies.

2. The activity was present in unripe banana but not present in ripe banana. The activity was lost when banana or banana extract were heated above 70°C.

3. The anti-ulcerogenic activity was due to low molecular weight polar substances extractable by water or alcohol from dried banana powder. On solvent fractionation of banana extract the activity was purified 500 fold on a weight for weight basis from original banana powder. The active fraction was rich with substances that gave a positive ninhydrin reaction.

4. The anti-ulcerogenic action of banana was different to the anti-ulcerogenic activity of drugs present on the market. The anti-ulcerogenic activity of banana was due to its ability to stimulate growth of the mucosa cells. This produced protection against the effect of aspirin and also assisted the healing of ulcers already induced by aspirin. The active ingredient of banana was acting systemically to protect and heal rats from ulceration induced by aspirin.

5. An increase in mucus secretion was found in response to banana treatment. That increase in mucus secretion was a consequence of cell proliferation of the gastric mucosa.

6. The effects of banana were compared with the action of other anti-ulcerogenic agents such as prostaglandin E_2, cimetidine, aluminium hydroxide, di-butyrylcyclic AMP and 5-hydroxytryptamine. The action of banana was different from that of the above agents and is probably due to an unknown cell growth stimulating agent.

7. The present findings are discussed in relation to present theories of anti-ulcerogenic action and suggestions for further work are offered.

Key words: Banana Aspirin Ulceration Histochemistry Mucus.

ACKNOWLEDGEMENTS

First, I would like to thank Dr D.A. Lewis my university supervisor for his advice, constructive criticism, encouragement and his patience in reading through this thesis.

Many thanks are also due to Mr R. Best for his guidance and encouragement during the course of my studies.

I also wish to acknowledge the advice and constructive criticism I received from Dr M.D. Day.

I would also like to extend my grateful thanks to the following:

Dr P.W. Dettmar for his encouragement in helping me to complete the writing of this thesis.

Mrs P.A. Lynn for so expertly typing the manuscript.

Reckitt and Colman PLC for their financial support during the course of this project and in providing the samples of banana.

Finally, I would like to thank my parents for their patience, encouragement and their financial support throughout the course of the study.

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CHAPTER ONE

GENERAL INTRODUCTION

1. THE STOMACH-STRUCTURE AND FUNCTION

1.1 Human stomach

The stomach is a sac-dilation of the tubular digestive tract. The ventral and dorsal surfaces may be folded or flat and may touch when the stomach is empty. It has two borders; the concave lesser curve and the convex greater curve. These fuse at the cardia where the oesophagus enters (Figure 1). The oesophagus continues smoothly into the lesser curvature and on the opposite side there is a definite indentation known as the cardiac incisure.

The major portion of the stomach is known as the corpus or body and it blends imperceptibly with the pyloric portion except along the lesser curvature where the angular incisure marks the boundary between the corpus and the pyloric region. The latter is divided into the pyloric antrum or vestibule which narrows into the pyloric canal terminating at the pylorus.

The mammalian gastric mucosa is in part either non-glandular (squamous tissue) or glandular. Glandular mucosa is present in all species and is responsible for the secretion of gastric juice. Whereas non-glandular mucosa is found in certain species only e.g. in the fundic region of the rat, mouse and guinea pig stomach.

The stomach is characterized by many coarse fields where the mucosal surface consists of numerous shallow invaginations known as area gastricae which are pitted with numerous gastric pits or

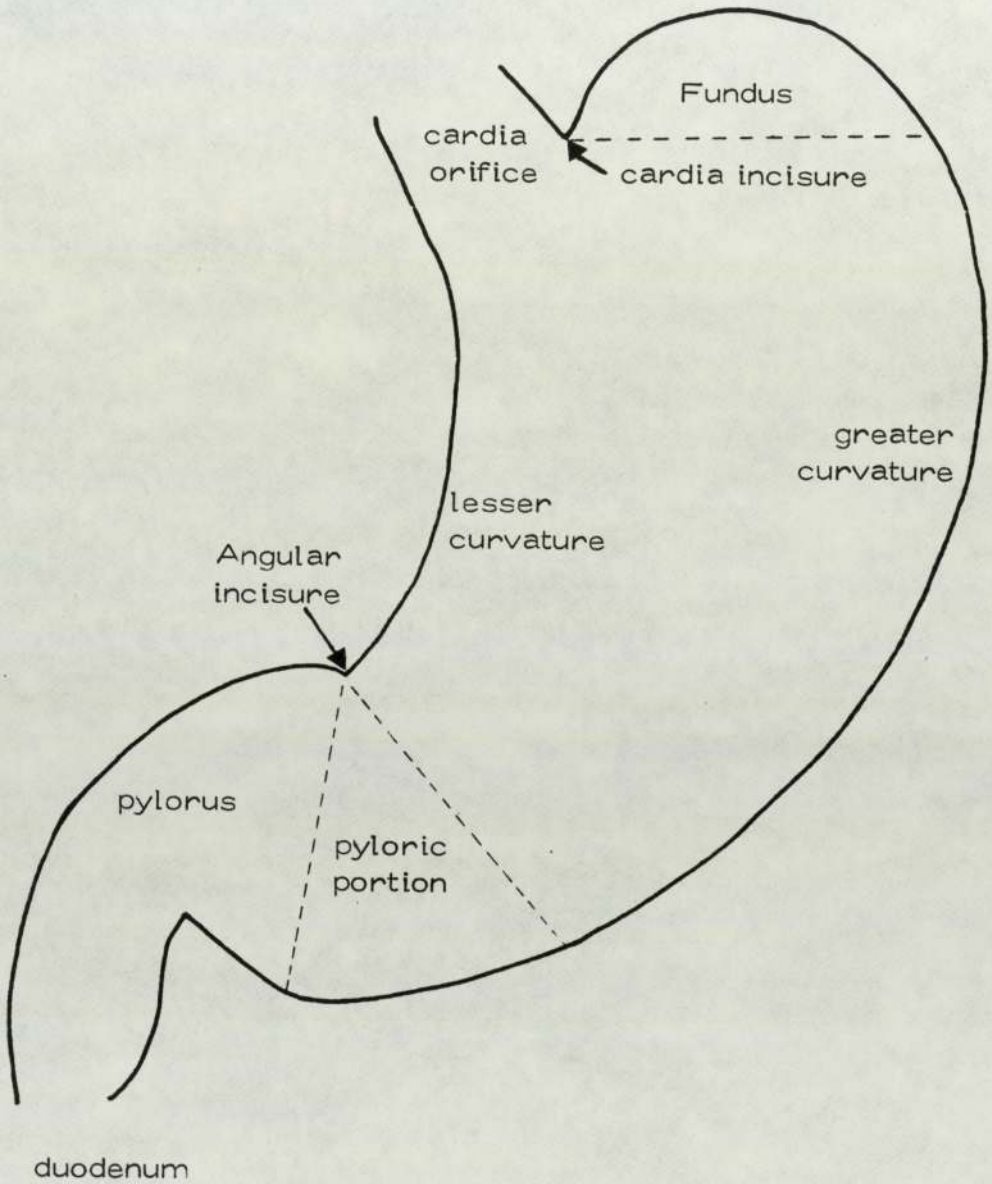


Figure 1. The human stomach

Figure 1. shows the main anatomical features of the human stomach. The differences between the human and rat stomach (used as an experimental model) are described in the text.

foveolae gastricae. It is in the lower 2/3 of these pits that gastric secretion occurs (Figure2,p.7)The gastric epithelium at the gastro-oesophageal junction consists of a single layer of columnar cells sharply demarcated from the stratified and thicker oesophageal mucosa. The glands of the stomach are tubular and can be sub-divided into three types:

a) The cardiac glands:

These are confined to narrow zones (0.5 to 4 cm in width) around the cardiac orifice. They are coiled and lined by mucus producing cells.

b) The gastric or fundic glands:

These are located in the fundus and cover the greater part of the body of the stomach. They are reasonably parallel simple branched tubules with a narrow lumen extending to the muscularis mucosa. Three types of cells are found:

i) Mucous cells:

As shown in Figure 2 (p. 7) there are two types of mucous cells. There are surface epithelial mucous cells which lie between the various glands and extend down into the neck of glands. located in all parts of the stomach. This is particularly evident in

the fundic glands and the mucous cells cover about 1/3 of the upper part of the gland. The mucous cell nuclei are rounded and located away from the base of the cell. The cells secrete a visible mucus of gelatinous consistancy which on contact with HCl,forms a dense white coagulum. The sticky coagulum adheres firmly to the underlying mucosa and forms a protective surface coating resistant to penetration by pepsin and gastric HCl. Secretion of the mucus is continuous. Therefore the loss of the surface coagulum by erosion by gastric contents is compensated by a continual supply of freshly secreted mucus from the underlying cells. The mechanism activating these cells is not clearly understood, but the practical result of this action is that the surface mucosa cells are protected from irritating substances and endogenous acid-pepsin by the mucus coating.

The second type of mucous cell are the goblets cells in the depths of the fundic glands. These cells form the mucoid cells which are prominent in the cardiac and pyloric glands, apart from the neck cells. The mucus secreted from these mucoid cells is thin and watery. This mucus is known as 'soluble' or 'dissolved' mucus. The physiological function of this juice is to add fluid to the acid food mixture in the stomach and to assist in the softening and disintegration of food masses in the stomach. The fluid also dilutes obnoxious and irritant substances in the stomach. The cells are activated by vagal impulses and secretion occurs in response to chemical and mechanical irritation of the surface epithelium.

1. Cardiac glands

2. Pyloric glands

3. Fundic glands

4. Surface epithelial cell

5. Mucous cell

6. Parietal cell between mucous cells and zymogen cells

7. Zymogen cells

8. Solitary lymph nodule

9. Argentaffin cell

10. Muscularis mucosa

11. Sub-mucosa

Figure 2. Glandular cytology of the human stomach.

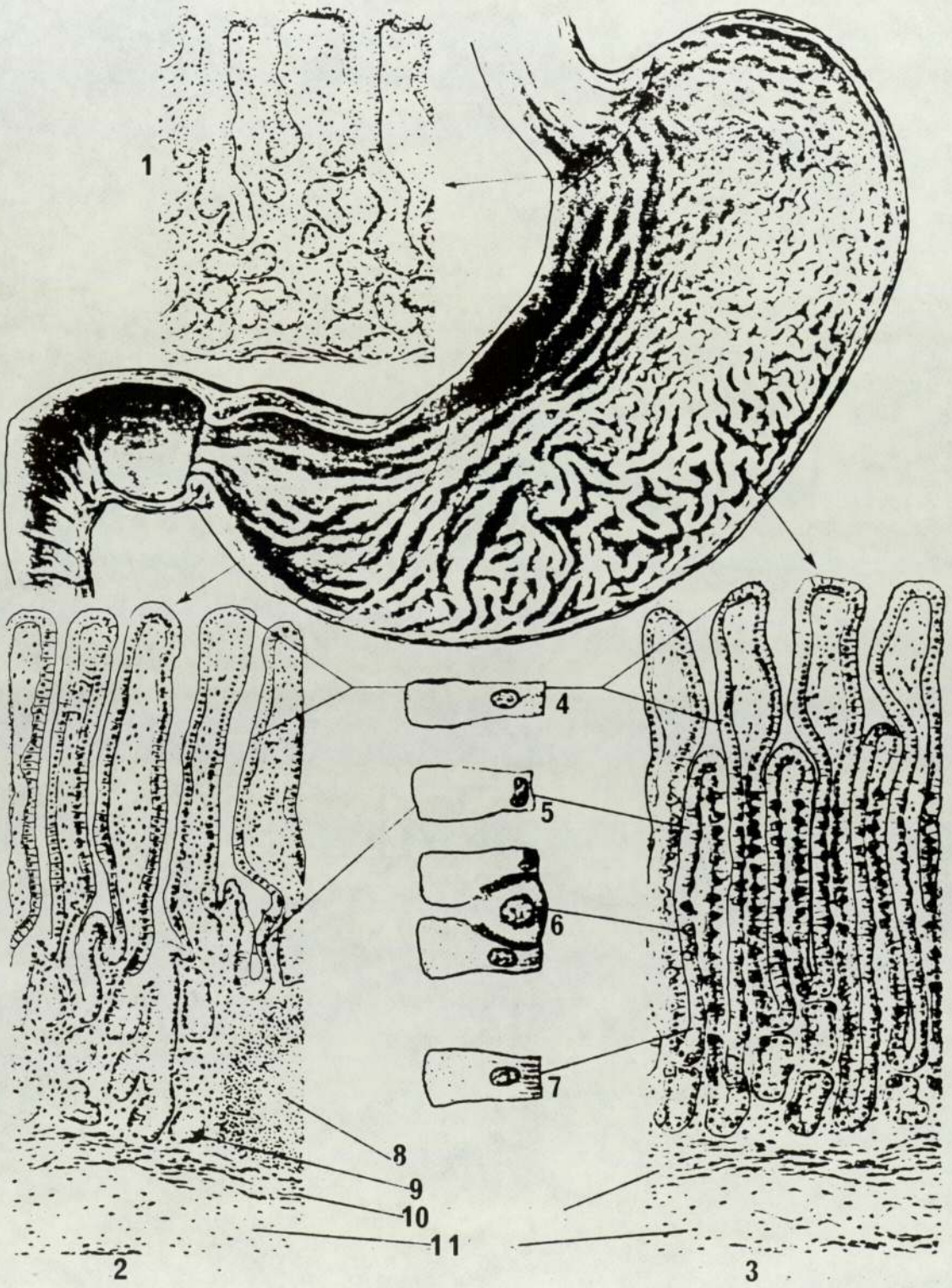


Figure 2. **Glands and cell types of the gastric mucosa**

ii) Chief cells:

These are zymogenic cells which line the lower half of the glandular tubules. They have spherical nuclei and contain dense light-refracting granules and golgi apparatus whose size and form vary with the state of secretory activity. These cells are responsible for pepsinogen secretion.

iii) Parietal cells:

These cells are usually crowded away from the lumen to which they are joined by a series of extracellular capillaries originating from the intra-cellular canaliculi.

The parietal or oxyntic cells are responsible for the production of gastric hydrochloric acid.

c) Pyloric glands:

These are located in the pyloric region buty they also extend into a transitional zone in which both gastric and pyloric glands are found and which extend diagonally and distally from the lesser to the greater curvature. The glands are lined by mucous cells which resemble closely the mucous cells of the fundic gland. Three substances are known to be produced in the human stomach "serotonin, histamine, gastrin . These substances are secreted by the argentaffin cells,argyrlophil and enterochromaffin cells . Gastric secretion is under nervous and hormonal control.

The nervous control is via the vagus nerve. Vagal stimulation and distention of the stomach wall by food stimulates secretions of acid, pepsin and gastrin. The latter stimulates the parietal cells. When stimulation fades inhibition occurs and the gastric secretion subsides. Satiety by food stops stimulation of the vagus and the fall of pH of the gastric contents regulates the further release of gastrin. Inhibitors of endocrine secretion are secretin and cholecystokinin which are released by the duodenum in response to the entry of food and gastric juice.

There is some evidence to suggest that a second messenger hypothesis for mediation of cellular responses to hormones and other agents may apply to parietal cells. It has been suggested that cyclic AMP fulfills this role (Harris et al., 1969) by an action on carbonic anhydrase (Bersimbaev et al., 1971, Salganick et al., 1972) which has an important role in the secretion of gastric juice. This enzyme is involved in the regulation of H^+ formation and its transport through the cell wall. Bersimbaev et al., (1971) have proposed a scheme to explain the effects of various secretagogues on cyclic AMP and gastric acid secretion in the rat (Figure 3). They suggest that gastrin and histamine stimulate the production of cyclic AMP which controls the production of HCl by its effect on carbonic anhydrase. This suggestion is supported by their findings that theophylline (an inhibitor of phosphodiesterase) increases the effect of the secretagogues

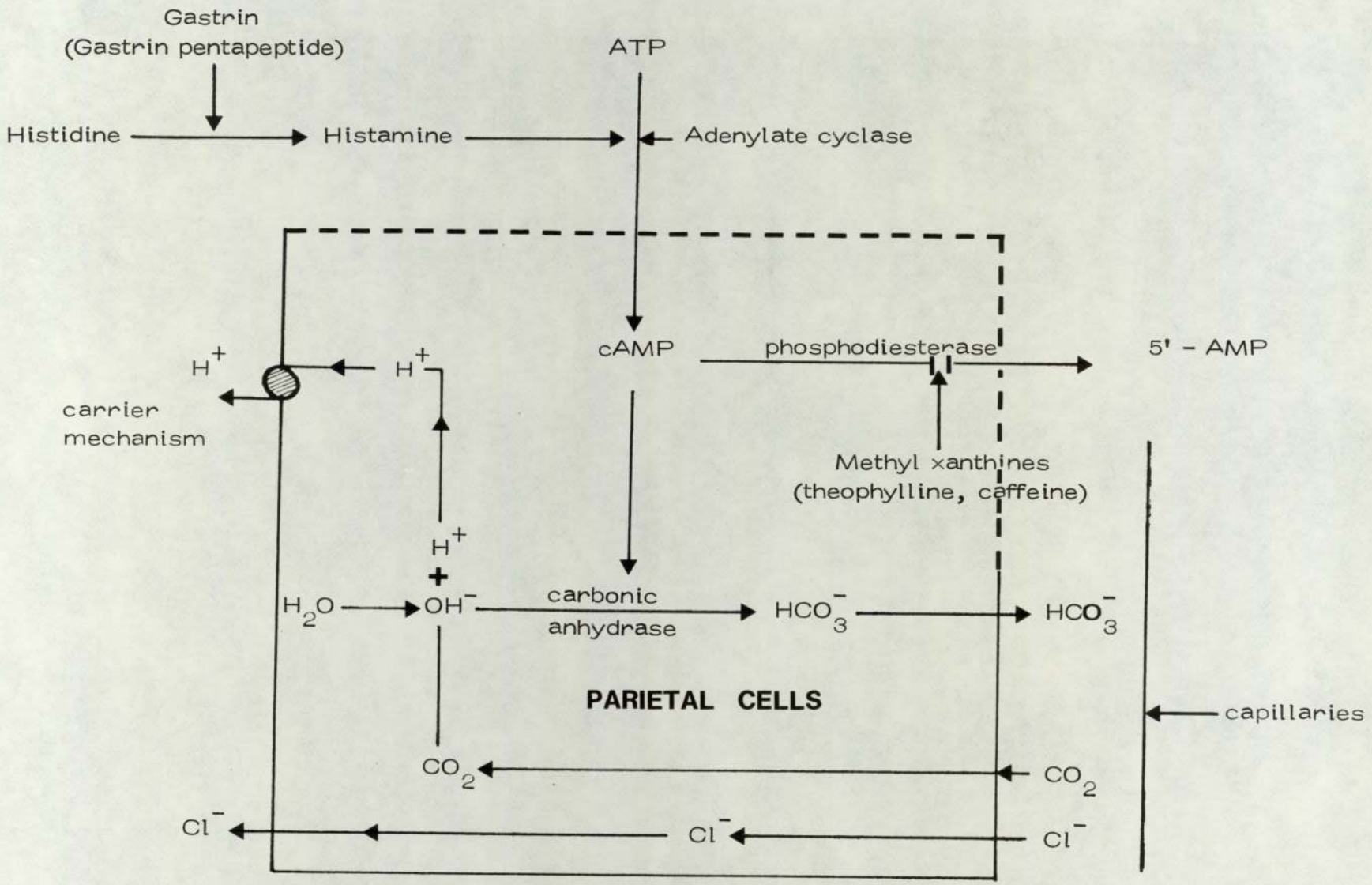


Figure 3. Scheme for the regulation of HCl secretion in the stomach.

on acid production. The role of cAMP as a second messenger in gastric acid secretion has received support in the rat (Domschke et al., 1973; Puurenen and Karppanen, 1975) the guinea-pig (Karppanen et al., 1974) and in the rabbit (Fromm et al., 1975). Conflicting results have been reported for dogs (Levine and Wilson, 1971) and in human gastric mucosa (Domschke et al., 1974, 1975).

1.2 Differences between the human and rat stomach.

Although the experiments in this thesis are relevant to human gastric ulceration the rat was used as the experimental model.

Although the rat stomach is a gastric organ like the human stomach it has some anatomical differences. In the rat the oesophagus orifice enters the body of the stomach about half way down on the lesser curvature rather than near the top as in the human. The rat stomach has a large non glandular(fundus) as well as a glandular part. The part of the stomach above the oesophageal orifice is the non glandular part. which acts as a storage organ.

2 ULCERATION

A peptic ulcer can be defined as a sharply circumscribed loss of tissue involving the mucosa, submucosa and muscular layer. Peptic ulcers occur in areas of the digestive tract exposed to acid and pepsin gastric juice. Ulcers may occur mainly in the lower part of the oesophagus, the upper duodenum and the stomach. The localisation of the ulceration in the stomach constitutes 'gastric ulcer'. Ulcers are classified according to their severity. There are three types of ulcer (Hunter, 1966):-

2.1 Acute gastric ulcers:

This type which involves only the mucosa and submucosa are usually small and may be numerous but are unlikely to haemorrhage, they usually heal without leaving a scar.

2.2 Sub-acute gastric ulcers:

They are usually fewer in number than the acute type. They extend down to the muscular coat and tend to be located on the lesser curvature.

2.3 Chronic gastric ulcers:

This type often penetrate to the muscular coat. They are usually solitary and their commonest site is on the lesser curvature or on the walls of the pyloric canal. They are larger than ulcers in the other two classes.

In experimentally induced gastric ulceration ulcers are sometimes related to muscular damage which extends across the whole glandular mucosa (Barbour and Porter, 1987; Lee et al., 1971) Some authorities consider that the term "ulcer" should only be used when the damage penetrates through the whole glandular mucosa and the muscularis mucosa. The term "erosion" was defined as damage localized only in the glandular mucosa without penetration to the muscularis part (Wilhemli and Menassé, 1972). In the present study 'ulceration' is used to describe penetration of the damaged glandular mucosa and "erosions" describe exfoliation or desquamation of the surface mucosa. Therefore ulcers can be:

a) Deep ulcer:

Where mucosal injury extends down through the whole glandular mucosa(Figure 4.A).

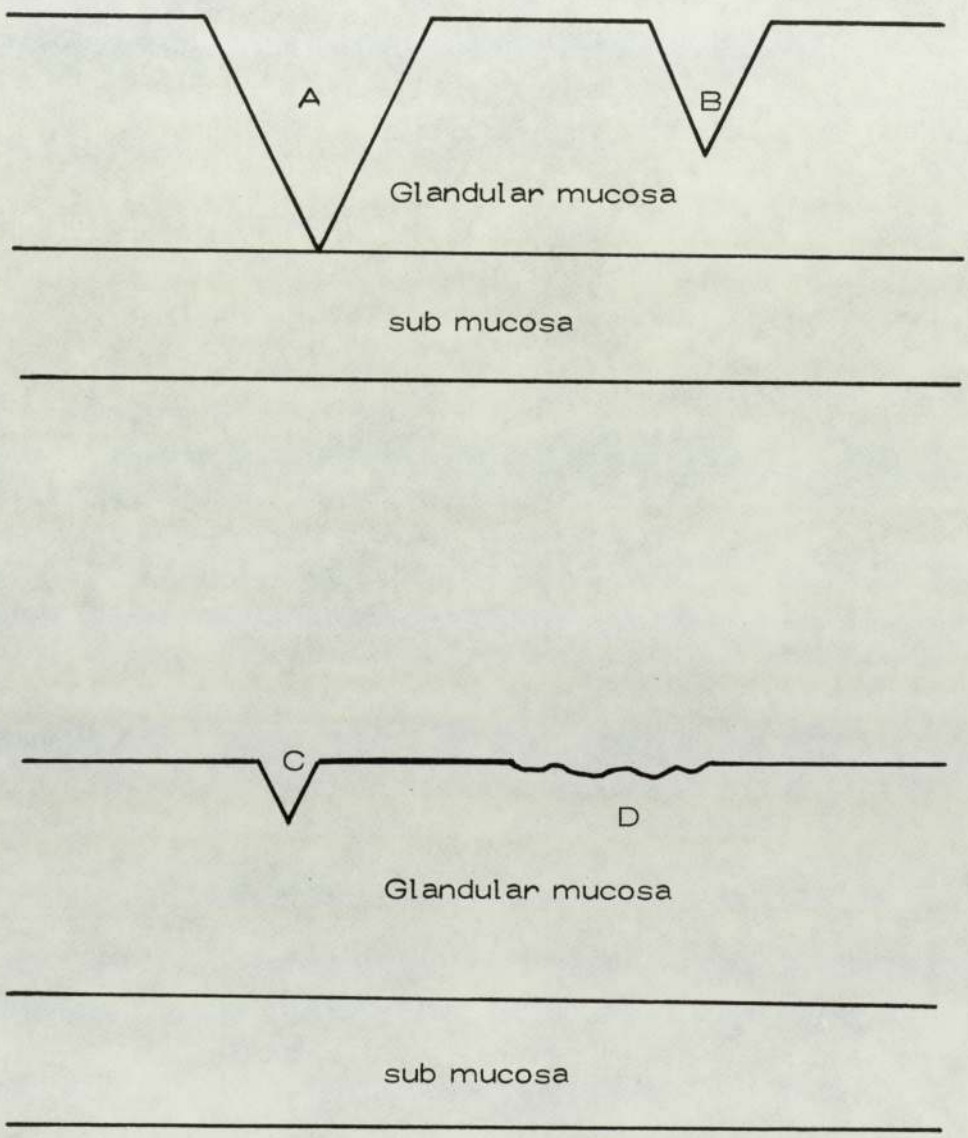


Figure 4. A definition of ulcers and erosions in the stomach.

The figure illustrates the definitions of deep(A), medium (B) and superficial ulcers (C) used in the experimental work. Also shown are surface erosions(D).

b) Medium ulcer:

Where mucosal injury extends half way through the glandular mucosa (Figure 4. B).

c) Superficial ulcer:

Which describes mild penetration of the surface mucosa (Figure 4. C).

d) Erosions:

Defined as superficial desquamation or exfoliation of the surface mucosa associated with pin point haemorrhages (Figure 4. D).

3. EPIDEMIOLOGY

Peptic ulceration is a fairly common problem in society. Our knowledge of the occurrence of gastric ulceration apparently dates from antiquity but early reports are vague until the reports of Matthew Bailie in 1793. (Cruveilhier, 1829).

Cruveilhier in 1829 described the lesion in detail and published a comprehensive account of gastric ulceration after an examination of over 7,000 autopsies. A survey of 6047 patients showed that the peak occurrence of peptic ulcers in males was in the age group 45–54 years while in females the peak occurred at age 55. Males are more likely to develop peptic ulcers than females. The ratio being 4:1 (Doll et al., 1951).

3.1 Peptic ulceration

Erosions and superficial lesions occur spontaneously in many species but chronic ulcers are rare. Before 1900 the incidence of gastric ulcers exceeded that of duodenal ulcers but this trend has now been reversed to where the occurrence of duodenal ulcers exceeds that of gastric ulcers by the ratio 10:1 (Watkinson, 1960).

Exceptions are in Japan where gastric ulcers are more common than duodenal ulcers (Rhodes, 1972) and in Finland where both are equally common. Doll et al., (1951) reported that there is a relationship between peptic ulcers and the social and economic background of patients with a higher incidence in the manual labourer classes than in the professional classes. The striking occurrence of peptic ulcers within some families and a high frequency of ulcers in living siblings of ulcer patients and in homozygous twins indicate genetic influences (Doll and Killock, 1951). An interesting relationship exists between the distribution of blood groups in patients with peptic ulcer. Individuals with blood group 0 have a 2.5 times greater risk of developing ulcers than people with blood groups A, B or AB.

Doll et al., (1958) and Monson (1970) found that ulcers heal less readily in smokers. Peptic ulceration occurs at all ages but the condition usually develops between the ages of 20 and 40. Adult men

are more prone to the disease than women but before puberty the sex distribution is the same. After artificial or spontaneous menopause the frequency of ulceration in women rises (Clark, 1953).

Active ulceration is rare during pregnancy but may occur in the last trimester or early puerperium. These observations in women suggest an endogenous factor against ulceration. However despite intensive research the cause of the disease is still largely unknown in spite of increased knowledge of the conditions necessary for ulcer formation.

Peptic ulcers are often defined in the general sense as excorated areas of the mucosa caused by the digestive action of gastric juice. Gastric ulcers are located in different regions of the stomach and as mentioned above are of much less frequent occurrance than peptic ulcers located outside the stomach. Peptic ulcers occur in the first few cm of the duodenum. This is the most frequent site of ulceration. Other sites where peptic ulcers occur are along the lesser curvature of the antral end of the stomach or more rarely in the lower end of the oesophagus, a site where gastric juices frequently reflux. A peptic ulcer called a ' stomal ulcer' can occur whenever an abnormal opening like a gastroejunostomy occurs between the stomach and some portion of the small intestine. The basic cause of peptic ulceration is too much secretion of gastric juice which overcomes the protection afforded by mucous secretion and other factors. These factors include in the duodenum the alkalinity of the small intestinal secretions including the

important pancreatic secretion. This includes large quantities of $NaHCO_3$ which normally raises the pH and inhibits pepsin attack on the mucosa.

Under normal conditions excess acid entering the duodenum reflexly inhibits gastric secretion and peristalsis (both nervously and hormonally) in the stomach. This slowing of gastric emptying allows a greater time for pancreatic secretions to neutralize gastric HCl. After neutralization the reflex naturally subsides and digestion continues. Acid in the duodenum also liberates secretin from the mucosa which after passage to the pancreas stimulates secretion of pancreatic juice rich in $Na\ HCO_3$.

Stress is an important factor in producing duodenal ulcers. Stress can result in excessive stimulation of the dorsal motor nucleus of the vagus from impulses generated in the cerebrum. The result is that vunerable patients have a high rate of gastric secretion between meals. Often patients that have gastric ulcers have a low or normal secretion rate of HCl. Usually these patients have an associated gastritis.It is probable that the stomach mucosa of these patients shows decreased resistance to the acid juice or irritants such as drugs (e.g. aspirin) or alcohol.

Reflux of duodenal contents into the stomach will also result in the detergent effect of bile acids on surface mucosal and the mucus film attached.

The work in this thesis is specifically concerned with gastric ulcers which have therefore been discussed in some detail. The relevance of the work to peptic ulcers (if any) is a separate problem.

4. FACTORS RELATED TO GASTRIC ULCER FORMATION

An ulcer develops at a circumscribed site when the resistance of the mucosa to acid and pepsin breaks down and therefore a prerequisite for the development of an ulcer is the digestive juice "no acid - no ulcer" (Schwartz, 1910). This is the only unchallenged basic fact concerning ulcer formation. The disease appears to be dependent on the interplay of a number of factors: nutritional; vascular; nervous; humoral and chemical and results from an inability, or a loss of ability, of the stomach to cope with a series of aggressive factors with which it comes into contact during normal function. Whether ulcers develop from an increase in aggressive forces or a decrease in the defensive capabilities of the stomach is not clear.

4.1 Gastric acid secretion

Early reports of the role of gastric acid secretion in the pathogenesis of peptic ulcers dates back to 1829 when Cruveilhier suggested that a high level of gastric acid secretion in the stomach overwhelms the mucus barrier protecting the mucosa. However gastric ulcer patients have acid secretion rates within the normal range (Wormsley and Grossman, 1965) and antacid therapy has been shown to be ineffective in accelerating the rate of healing of gastric ulcers (Baume and Hunt, 1969). These findings have led a number of authors to conclude that hypersecretion and hyperacidity are not in general the cause of gastric ulceration. Different observations have been reported from China by Fung (1970) who associated gastric ulcers with hypersecretion of gastric acid. A similar observation was reported by Ellison and Wilson (1964) for the Zellinger-Ellison syndrome where patients show hypersecretion of acid. The induction of achlorhydria by radiotherapy causes temporary healing of gastric ulcers (Ricketts et al., 1947), which indicates the importance of gastric acid in the development of ulcers.

4.2 Mucosal barrier

Teorell (1933) found that the gastric mucosa of cats was permeable to H^+ which were exchanged by the passive diffusion of H^+.

Davenport (1964) demonstrated that the stomach damaged by acetyl-salicylic acid or bile salts, is susceptible to H^+ back diffusion from the lumen to the mucosa. Similar results were obtained when gastric mucosa was damaged by salicylate treatment (Ivey, 1971) or bile salts (DenBesten and Hauza, 1972). It has been postulated that an abnormally permeable mucosa may exist or can be induced, in humans; a phenomenon which may explain the apparent achlorhydria frequently encountered in gastric ulcer patients (Davenport, 1965a; Overholt and Pollard, 1968; Chapman et al., 1968) and the acute haemorrhage and gastritis induced by aspirin (Davenport, 1965b). From these observations it may be concluded that the aetiology of the gastric ulceration in some patients could be due to the back diffusion of H^+ through a defective mucosal barrier. Histological studies and analysis have demonstrated that pathological conditions and drug therapy may be associated with changes in the amount and composition of mucus. Aspirin is the most widely studied drug with respect to mucus synthesis. A reduction in the quantity of sulphated and non-sulphated sugars in mucus in response to aspirin treatment has been found in rat (Menguy and Master, 1965; Ganter et al., 1969; Johansson and Lindquist, 1970). Domschke et al., (1972) have shown that N-acetyl neuraminic acid in gastric juice is reduced in patients with gastric ulcers.

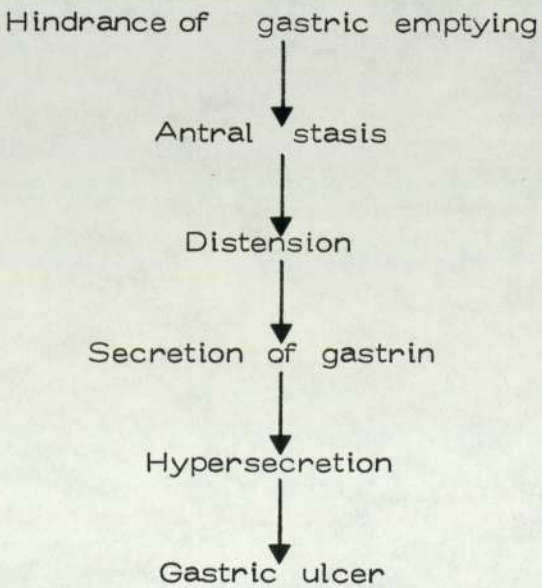
HOwever the role of mucus constituents in the aetiology of peptic ulcer requires further investigation.

4.3 Pepsin

Taylor (1959) reported that modified forms of pepsin and other enzymes may be secreted during peptic ulcer disease while in 1970 he discovered an increased secretion of pepsin in patients with duodenal or peptic ulcers. Further investigation is needed to support Taylor's observations.

4.4 Gastric motility (Dragsted theory, 1956)

This theory is summarised in the following diagram:



More recent observations suggest that this pathway, although theoretically possible, rarely occurs in practice. Delayed emptying and stasis may arise as complications from ulcers located near the pylorus but they do not affect formation and pathogenesis of ulcer formation (Mangold, 1958). Hypergastrinaemia is frequently associated with gastric ulcers but is due to hyposecretion with a high pH in the antrum (James and Pickering 1949) and is not the cause of hypersecretion (Trudeau and Guigan, 1971). Estimations of DNA content in gastric juice after aspirin administration show an increase, probably as a consequence of the exfoliation of cells (Croft, 1963). Croft, et al.,(1966) found that an increase in cell loss generally occurred in epithelial diseases, including atrophic gastritis.

4.5 Reflux of bile salts:

According to Duplessis (1965) the following occur:

i) Pyloric incompetence followed by a reflux of bile leading to damage to the mucosal barrier.

ii) Chronic gastritis spreads upwards from the pylorus leading to the development of ulcers in the damaged mucosa. The evidence in favour of this theory is based on findings that bile reflux occurs in the majority of gastric ulcer patients (Duplessis, 1965) but is rare in normal subjects.

Accumulated data is in favour of this theory. Davenport (1968) and Geall et al., (1970) discovered that bile damaged mucosa becomes freely permeable to H^+. Where massive exposure to bile occurs e.g. after gastric resection, intra-cellular mucus disappears from the superficial cells (Van Geertruyden, 1961) and accelerated desquamation occurs (Castrup and Fuchs, 1974; Rhodes, 1972). It was observed that ulcers were more easily produced in mucosa after exposure to bile rather than in healthy mucosa (Duplessis, 1965; Stadelmann, et al., 1971).

In addition to bile induced damage to the mucosa it has been suggested that duodenal reflux plays an important role in the pathogenesis of gastric ulceration. The ulcerated site is also exposed to pancreatic secretions including proteases such as trypsin and chymotrypsin which are active at both neutral and alkaline pH. Therefore autodigestion continues at pH levels where peptic digestion ceases. Pyloric deformity may arise from duodenal ulceration (Johnson, 1957) and gastric ulcer usually follows other submucosal fibrosis (Rhinds, 1959) or carcinoma of the pylorus. However all patients with peptic ulcers do not have bile reflux and the healing of ulcers appears to be independent of the amount of material refluxed (Black et al., 1971). It appears that bile reflux is an associated factor in gastric ulcer development rather than the main aetiological factor.

4.6 Vascular impairment

It is now acknowledged that an adequate vascularisation is essential for the integrity and function of the mucosal membrane and its absence or decline is a fundamental factor for ulcerogenesis. Virchow in 1853 stated that an altered circulation was responsible for ulcer development. Evidence suggests that vascular disturbances by vasoactive agents such as histamine (Kowalewski, 1967, Watt, 1959) adrenaline or noradrenaline Sethbhakdi et al., 1970a, b) actively contribute to the pathogenesis of ulceration. There is insufficient evidence to confirm that ulcerogenesis is a direct consequence of vascular alteration. It does appear that vascular impairment is associated with other ulcerogenic factors that induce the disease. Several theories relating to the role of vascular impairment with ulcer formation were reviewed by Pfeiffer and Sethbhakdi (1971) and a significant amount of evidence showed that alterations in blood flow by vasoactive agents are a primary factor in peptic ulcer disease. Such alterations in blood flow induce stasis and capillary congestion with thrombosis and ischaemia (Underhill and Freikeit, 1928; Bishton, 1950, Basu, 1955). Clinical evidence of vascular impairment in the aetiology of peptic ulcer is inconclusive and most of the available data is derived from autopsies. Some reports claim that there is a greater vascular impairment in people with peptic ulcers compared with ulcer-free patients. However the data is contradictory (Peiffer and Sethbhakdi, 1971).

4.7 Nervous system

Balo (1963) reported that discernable lesions were present in the central or peripheral nervous system in the majority of peptic ulcer cases studied. It is well known that severe physical stress (e.g. injuries, burns or major operations) particularly when associated with blood loss are frequently followed by the development of acute gastric mucosal lesions (Harjola and Sivala, 1966; Goodman and Frey, 1968; Flowers et al., 1970; Cushing, 1932; Curling, 1842). Psychological factors appear to play an important role in the induction of peptic ulcer. The pathogenesis of the lesion is not clear but it was stated that chronic anxiety and stress can induce gastric hypersecretion which may predispose the patient to ulceration (Richman, 1971). The role of the parasympathetic nervous system, and the adrenal gland appear to be considerable in the pathogenesis of ulcer formation.

5. TREATMENT OF PEPTIC ULCER

There are three aims in the management of peptic ulcer: - the relief of symptoms; the healing of the ulcer and the prevention of the recurrence of ulceration. Whilst there are effective methods for relieving symptoms and assisting healing there are no means available for preventing the recurrence of ulcers.

Methods have been used in the past to alleviate symptoms and accelerate healing which were only marginally effective in healing but effective in relieving symptoms. These include alterations in diet, cessation of smoking and bed rest. Undoubtedly the most effective measure for the relief of pain was bed rest. It has been shown that the reflux of duodenal contents back into the stomach is markedly lowered in recumbent patients (Capper, 1967; Flint and Grech, 1970). There is also evidence that cessation of smoking accelerates the healing of gastric ulcers (Doll et al., 1958). Doll (1964) carried out a trial to investigate the effect of diet in healing peptic ulcers. He concluded that agents in diets traditionally associated with healing ulcers such as low fat, milk, ascorbic acid, cabbage juice and bran were ineffective. In general the avoidance of diets containing irritant foods were beneficial in assisting the healing of ulcers.

5.1 Surgical intervention in gastric ulceration

Surgical treatment is only used when all other measures fail. Modern surgical treatment has much to offer to the patient with intractable peptic ulceration. Surgery is necessary when haemorrhage or pyloric stenosis occurs. Partial gastrectomy is the operation of choice for gastric ulcer. Preferably with a "Billroth" type II anastomosis in which the ulcer itself and the adjacent area of the stomach are resected. However major surgery does have the disadvantage in that it is irreversible and is prone to complications such as perforation, anaemia and nutritional impairment. Drug therapy on the other hand provides relief and promotes healing in many cases.

5.2 Drug treatment of peptic ulcers

drug treatment of peptic ulcer has in the past relied mainly on procedures designed to decrease acid activity (neutralisation or blocking secretion) in the stomach involving the use of alkali and anti-cholinergic therapy . Recently this traditional approach in the management of peptic ulcers has been reinforced by the use of a wide range of newly developed drugs with other modes of action.However antacids are still the most important class of drug used in the medical treatment of peptic ulcer.

a) Antacids

Antacids are employed extensively to neutralize the hydro chloric acid in the stomach . They are popular in gastric ulcer therapy and studies have shown accelerated healing of ulcerated tissues in pati ents on antacid therapy (Butler and Gersh,1975; Hollander and Harlan, 1973 ; Peterson et al., 1977).

These authors found that antacids are useful for treating peptic ulcers if given in seven to eight post prandial doses that are sufficient to neutralize about 1,000 mEQ H^+ ion per day . However, other reports deny that antacids affect the healing rates of ulcers(Doll, 1964;Baum and Hunt, 1969).Antacids are still used in combination with other drugs since they have a significant effect in the relief of pain .The use of antacids over along period may induce adverse reactions such as alkalosis, nausea, constipation or diarrhoea (McMillan and Freeman, 1965; Hava and Hurwitz,1974).

The antacids agent may be divided into two groups(Stewart,1970):

i) Systematic antacids:

Systematic antacids are midly alkaline substances which after absorption, are capable of altering the acid-base balance of the body. The chief ones are sodium and potassium bicarbonate which are rapid but short-lived in their action. When these substances are taken orally, the gastric contents are neutralized in the stomach and CO_2 is formed . Any excess of the bicarbonate remains unchanged, but in the intestine both the intestinal bicarbonate and exogenous bicarbonate are reabsorbed.The cocentration in blood is therefore increased (alkalosis) and the kidneys excrete an alkaline urine to restore the "balance".Excess also leads to an alkaline pH in the stomach which leads to further acid secretion "acid rebound" since high pH in the antrum is a strong stimulus to gastrin release.

ii) Non-systemic antacids:

Non-systematic antacids react to remove hydrogen ions from solution at the acid pH of the gastric contents. However ,in the alkaline medium of the small intestine, hydrogen ions are again released and the antacid is either restored to its original insoluble state or they are converted to other compounds: examples

- Aluminium hydroxide: it reacts with gastric acid to form aluminium chloride, and this in turn reacts with the intestinal secretions to form insoluble salts,

the chloride being reabsorbed.

$$Al(OH)_3 + 3HCl \underset{\text{in intestine}}{\overset{\text{in stomach}}{\rightleftharpoons}} AlCl_3 + 3H_2O$$

- Magnesium trisilicate: it neutralizes the acid with the formation of magnesium chloride, in the intestine where the insoluble magnesium carbonate is formed, and the chloride is reabsorbed.

$$Mg_2Si_3O_8 + 4H^+ \longrightarrow Mg^{++} + 3SiO_2 + 2H_2O.$$

- Magnesium oxide,
- Magnesium carbonate,
- Calcium carbonate,
- Calcium hydroxide.

Magnesium salts have an additional action in causing diarrhoea, whereas calcium and aluminium salts cause constipation. Finally, the indiscriminate use of antacids is inadvisable and may even be deleterious, since excessive neutralization of the gastric contents inhibits the digestive enzymes and also interfere with the acid-base balance in patients whose kidney function is inadequate. These conditions may call for special treatment to restore fluid and electrolyte balance.

b) Anticholinergic drugs:

Anticholinergic drugs have been prescribed in peptic ulcer therapy with the object of producing a chemical vagotomy . The results from clinical trials are not convincing and show a broad spectrum of adverse effect such as dry mouth, blurred vision and impotence (Hurwitz et al.,1977; Bieberdorf et al.,1975).Antispasmodics such as dicyclomine and and propantheline are still in use but require high doses to be effective .

c) Anti-histamine blocking agents:

Various other approaches to medical therapy have been tried such as inhibiting endogenous substances active in stimulating gastric secretion. Xylamide , Burimamide(Figure 5)were reported to inhibit histamine and gastrin stimulated gastric secretion in man (Wyllie et al., 1973;Danhof,1967, 1969; Glordano and Comi,1967; Debas,1977). However these drugs have serious side effects such as production of gastric carcinoma and agromlocytoma. A new histamine H_2-receptor antagonist(Cimetidine) was reported to have a similar action to the above histamine antagonists but with greater activity compared to burimamide, xylamide and metiamide and with fewer side effects (see Chapter 4,section1.2).

d) Other compounds:

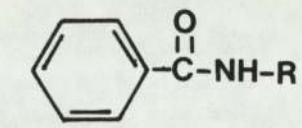
Other compounds have been used to enhance the resistance of mucosa to gastric juice and to accelerate the rate of healing .The major drugs of this group are carbenoxolone, prostaglandin and gefarnate and are alternatives to anti-secretory drugs . The role of prostaglandines in peptic ulcer treatment will be discussed in Chapter4.

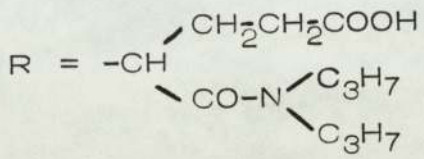
Carbenoxolone is a sweet tasting substance present in liquorice root and was synthesized by Turner and Wattion (Brown, et al., 1959). (Figure 5). It is a large highly lipophilic molecule extensively and rapidly absorbed in man (Baron et al., 1975) which binds strongly to plasma proteins (Parke and Lindup, 1973). The first controlled trial with carbenoxolone to treat gastric ulcer was reported by Doll et al., (1962) who found a significant acceleration of the healing process.

Further trials with a large number of patients (reviewed by Piper and Heap, 1972; Pinder, et al., 1976) has confirmed the earlier findings. The precise mode of action of carbenoxolone is unknown. If, however ulcers result from an inbalance between aggressive and defensive factors a number of possibilities are apparent. There is insufficient evidence to suggest that carbenoxolone affects gastric acid secretion (Berstad et al., 1970; Ivey and Gray, 1973) but carbenoxolone was found to prevent an increase in the back diffusion of H^+ caused by large doses of prednisolone (Domschke et al., 1975). Convincing evidence was published by Berstad (1972); Walker and Taylor (1975a, b) showing that carbenoxolone was capable of reducing the peptic ulcer activity of human gastric juice. Their results suggest that carbenoxolone inactivates pepsinogen in mucosal cells and in the lumen. This action is feasible since carbenoxolone

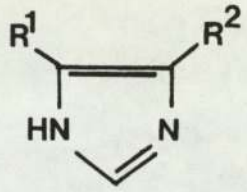
inactivates pepsinogen in mucosal cells and in the lumen and was reported to have a strong affinity for proteins and peptides (Parke and Lindup 1973). Carbenoxolone induced a significant increase in mucus secretion in human subjects(Hausmann and Tarnoky, 1966).Disadvantage of carbenoxolone treatment is that it has aldosterone-like side effects which seriously limits its clinical usefulness. One-fifth of patients treated with carbenoxolone show hypokalaemia and hypertension (Hausmann and Tarnoky,1966). To minimize these side effects spironlactone was administered with carbenoxolone (Doll et al.,1968) but this largely abolished the ulcer-healing effect.

Geranyl Farnesyl Acetate (Gefarnate) was discovered in 1958 in Italy by Adam (Bass,1974). This compound (Figure 5) is present in white-headed cabbages (Cheney, 1940). Gefarnate is soluble in fats. Clinical investigations have shown that the drug is active in curing gastric ulcers. The mechanism of healing by gefarnate is possibly due to an increase in mucus secretion (Takagi and Okabe,1968).The use of gefarnate in treating ulceration has the advantage that it is devoid of other unwanted pharmacological activity and has a very low toxicity .A comparative trial between carbenoxolone and gefarnate was performed in patients with gastric ulcers. The results measured by radiological examination and the ulcer healing rate, was an average improvement of 80.6% for carbenoxolone treatment and 56.7% for gefarnate treatment (Langman et al .,1973). This may explain why carbenoxolone was more extensively used than gefarnate .





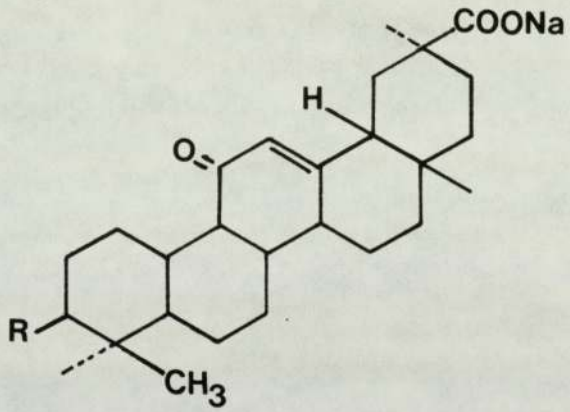
Xylamide
(Proglumide)



$R_1 = H$

$R_2 = CH_2-S-CH_2-CH_2-NH-C(=S)NH-CH_3$

Burimamide



$R = O-CH_2CH_2-C-ONa$

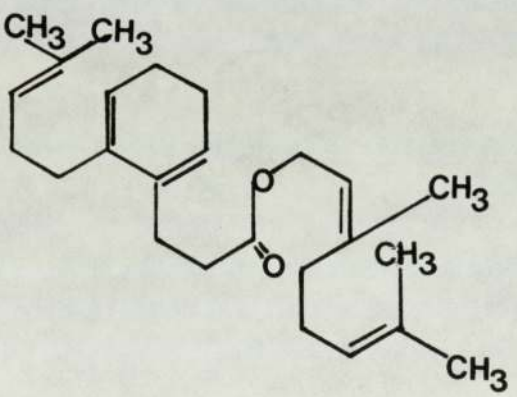
Carbenoxolone sodium

$R_1 = CH_3$

$R_2 = CH_2-S-CH_2-CH_2-NH-CNH-CH_3$

N-C N

Cimetidine



Geranyl Farnesyl acetate
(Gefarnate)

Figure 5. Structural representation of some anti-ulcerogenic drugs.

6. METHODS OF INDUCING ULCERS AND EVALUATING ANTI-ULCEROGENIC AGENTS IN EXPERIMENTAL ANIMAL MODELS

The main purpose of this section is to discuss methods of evaluating ulcers induced by different ulcerogenic agents and to evaluate the anti-ulcerogenic properties of certain substances.

The methods of evaluating ulcers are numerous, this diversity is due to different procedures used for inducing ulceration. However the methods can be classified as:-

i) Evaluation of anti-secretory activity.

ii) Estimations of mucosal damage (e.g. ulcer index).

The variety of methods used for inducing ulceration is an indication of the lack of basic knowledge of the relative roles of various factors in the pathogenesis of peptic ulceration. It is evident that methods of both evaluating and producing ulcers are closely related to each other. Therefore both methods of producing and for evaluating ulcers will be discussed together.

6.1 Evaluation of the anti-secretory properties of an anti-ulcerogenic agent

The rat stomach continuously secretes gastric juice at constant rate, which increases on stimulation. Several models were used to evaluate the anti-secretory properties of different agents. Shay et al.,

(1945) ligated the pyloric end of the stomach for four to five hours in anaesthetized rats. The gastric juice was collected, its volume was noted, and the concentration of H^+ was determined by titration. The peptic activity was evaluated as described by Benjonine and William (1943). In later work the above model was used to test anti-secretory action by drugs administered orally to nineteen hours ligated pylorus rats. It was used to test the anti-secretory properties of SC 15290 and Probanthine after the rats had been fasted for seventy two hours (Sun and Chen, 1963). Ulcers produced in these experiments were localised in the squamous and glandular regions of the stomach. Acute and chronic gastric fistulae were used in dog and rats by Bellmont (1959) to evaluate anti-secretagogues and secretagogue agents. This technique was also used by Choh and Aschild (1958); Lai (1964); Lee and Thompson (1969); to evaluate the secretagogue properties of gastrin, insulin and histamine.

Both the innervated "Pavlov pouch" and chronically denervated "Heiden hain" gastric pouch have been used to study the effect of anti-secretagogues (Markovity et al., 1959).

6.2 Estimation of mucosal damage

A visual examination of the mucosa of the gastric wall after dissection of the stomach can be either used to assess the anti-ulcerogenic properties of agents or to study the ulcerogenic activity of ulcerogenic promoters.

The mucosal damage is evaluated by different methods of 'scoring'. Some methods consist of scoring only for the number of ulcers (Madden and Ramsburg, 1951). Others include the severity of the ulceration (Robert and Nezamis, 1958a, b). The degree of haemorrhage is another scoring parameter. The differences between these types of scoring depends in part on the pathogenesis of ulceration which differs from one ulcerogenic agent to another. The distribution of ulceration in the stomach depends on the ulcerogenic agent employed.

Chemicals which occur naturally in the body, i.e. endogenous agents have been used to induce ulceration in animal models as compared to exogenous factors (e.g. stress, drugs or thermocautery).

6.3 Experimental methods for producing ulcers.

a) Endogenous agents:

i) Adrenergic substances:

Catecholamines are reported to induce ulceration in animals (Baronofsky and Wangensteen, 1945).

ii) Cholinergic substances:

Over secretion of acetylcholine is claimed to induce ulceration in animals by miming the action of vagal stimulation (Hanke, 1934). The use of adrenergic and cholinergic agents is very limited since they induce systemic changes in the body rather than a localized action on the gut.

iii) Steroids:

Steroids have been used extensively in animals to produce ulceration. Robert and Nezamis (1958a, b) have used cortisol and Δ-cortisol to induce ulceration in rats. The animals were fasted during the experiments. Steroids were injected subcutaneously at a dose of 40 mg kg^{-1}. The animals were killed on the 8th day of the experiment and the ulcers were examined with a glass magnifier. The ulcers ranged in size from 0.5 to 3 mm and were located in the pyloric portion of the stomach. The severity of the lesions was expressed according to an arbitrary scale of 0 to 3 and the number of ulcers per animal were also recorded. The maximum ulcer index was 20 (100% incidence) which corresponded to a score of 3 for the severity and 7 marks for the number of ulcers per animal. The total score was 10 which was doubled to give 20 marks.

Δ-Cortisol was three times as ulcerogenic as cortisol. The ulcers produced by cortisol healed leaving a scar. Prolonged treatment with cortisol also induced ulcers in non-fasted rats (Ingle et al., 1951; Baker, 1952); Kelly and Robert (1969) induced ulcers by the subcutaneous injection of prednisolone at a daily dose of 25 mg kg^{-1} for four days. The animals were killed four days after the last injection. The ulcers were found in the corpus region of the glandular part of the stomach but were not found in the antrum.

iv) Histamine

Watt and Eagleton (1964) reported on the ulcerogenic effect of a single intraperitoneal injection of histamine (4 mg kg^{-1}) in the guinea-pig after fasting for 24 hours. The animals were sacrificed at six hours after histamine injection and an arbitrary method of scoring was employed to estimate the ulceration:

0 = Normal stomach

0.5 = Grey discolouration of the stomach

1 = Petechial haemorrhage

2 = One or two small ulcers

3 = Many ulcers

4 = Perforated ulcers

The same model was used by Alphin and Ward, (1969) in a preventative test to study the anti-histamine properties of various drugs. The compounds under test were introduced daily into the stomach for four days and then histamine was injected 6 hours before the animals were killed. Histamine induced ulcers are linear in form and distributed around the body of the stomach.

v) 5-Hydroxytryptamine (5-HT):

Intraperitoneal injection of 5-HT (50 mg kg^{-1}) or 5-hydroxytryptophan (300 mg kg^{-1}) were shown to be ulcerogenic in rats previously fasted for 24 hours. The animals were sacrificed 3 hours

after injection and a high incidence of ulceration was found (Haverbach and Bogdanski, 1957). The same effect of 5-HT was reported by Blackman et al., (1959) and Wilhelmi (1957) in rats at doses between 30-50 mg kg^{-1}.

5-HT releasers such as reserpine were also reported to induce ulceration in fasted rats after intraperitoneal injection at a dose of 2 mg kg^{-1} (Benditt and Rowley, 1956). The same system was used by Lee and Bianchi (1971) to evaluate the protective anti-ulcerogenic effect of Probanthine (anti-cholinergic agent) at doses of 12-15 mg kg^{-1} orally administered 24 hours before reserpine (2 mg kg^{-1}).

b) Exogenous factors:

i) Stress:

Stress is a common stimulus used to produce gastric ulceration in animals. Selye (1936) was the first to use a restraint method when he immobilized rats by a spinal cord transection which induced ulceration in the glandular part of the stomach.

Rossi et al., (1956) introduced a restraint method for rats in which they tied the fore and hind limbs together and wrapped the rats in a towel. The rats were deprived of water and food for 20 hours. Ulcers were also induced by stress in mice, rats and guinea-pigs by immobilizing the animals for 24 hours in a wire mesh envelope without food and water. The ulcers were localized in the

corpus of the stomach. This method was ineffective in the rabbit and monkey. The ulceration was assessed by an arbitrary method (Brodie, 1966) as follows:-

0 = no damage

1 = blood in lumen

2 = pin point erosions

3 = 1-5 small ulcers < 2 mm

4 = >5 small ulcers

5 = 1-3 large ulcers > 2 mm

6 = >3 large ulcers

The importance of the psychological stress in gastric ulceration was demonstrated by Sawrey et al., (1956) when rats were subjected to an electrical conflict situation for 30 days in which each time an animal approached a food or water container it received an electrical shock.

ii) Non-steroidal anti-inflammatory drugs:

Non-steroidal anti-inflammatory drugs are commonly used in man for their analgesic and anti-pyretic effects. However the uncontrolled use of such agents can cause gastrointestinal damage (Katz et al., 1965).

Aspirin

Barbour and Dickerson (1938) described a method of producing ulcers in rats. The animals were maintained on a low quantity of food and the ulceration was induced by daily oral administration of aspirin at a dose of 150 mg kg^{-1} over a period of ten days on empty stomachs. The incidence of ulceration was 66% and these were localized in the glandular part of the stomach. Gastric ulceration was also induced by subcutaneous injection of aspirin at a dose of 300 mg kg^{-1} daily for ten days (Barbour and Dickerson, 1938). This method also produced a great deal of oedema at the injection site which presumably produced pain, therefore the ulceration could be due to stress as well as to aspirin.

Mortality was high in this model mainly due to gastric haemorrhage. The ulcer index was estimated simply as either ulcerated or not. (Barbour and Porter, 1937; Barbour and Dickerson, 1938).

Guth et al., (1979) studied the action of aspirin on pyloric ligated rats. The aspirin was introduced orally to the stomach at a dose of 200 mg kg^{-1}, 0.5 h after ligating the pylorus. Ninety minutes later the animals were killed and the ulcer index was estimated as follows:

SCORE

Petechial lesion	=	1
Erosion < 1 mm	=	2
Erosion 1-2 mm	=	3
Erosion 2-4 mm	=	4
Erosion > 4 mm	=	5

MacDonald (1976) induced ulceration in rats by oral administration of aspirin at a dose of 200 mg kg^{-1} in 18 h fasted rats. The animals were killed and the stomachs were filled with 70% alcohol. The assessment of the ulcer index was carried out by a similar method to that of Brodie, 1966.

Phenylbutazone:

Phenylbutazone has been reported to be an ulcerogenic drug. This activity was demonstrated by oral administration of phenylbutazone at a dose of 2 x 100 mg kg^{-1} in 24 h fasted rats. The ulcers were localized in the glandular part of the stomach (Wilhelmi and Menassé, 1972). Subcutaneous injection of phenylbutazone was also ulcerogenic at a dose of 100 mg kg^{-1} daily for 12 days in non-fasted rats. The mortality was high and the ulceration was also localized in the glandular part of the stomach (Bonfils et al., 1954).

Indomethacin:

Indomethacin was reported to induce ulceration in rats. This activity was demonstrated by the intraperitoneal injection of

indomethacin at a dose of 15 mg kg^{-1} in previously fasted animals for 24 h . The animals were sacrificed 5 h after the indomethacin was administered. The number of ulcers were counted in each rat as an estimate of ulcer severity (Lee et al., 1971).

Djahanguiri et al., (1973) determined that 10 mg kg^{-1} was the effective ulcerogenic dose of indomethacin and the maximum values for ulceration appeared within 5 h using the same procedure of Lee et al., (1971). The ulcers were distributed in the glandular part of the stomach.

iii) Caffeine:

The ulcerogenic effect of caffeine was reported in rats by Roth and Ivy (1944). This ulceration was induced by i.p. or i.v. injection of caffeine at a dose of 125 mg kg^{-1} in 24 h fasted rats The animals were sacrificed and ulceration was localized in the lesser curvature, the antrum and the pre-pyloric regions of the stomach.

6.4 Chronic models of ulceration:

One of the main disadvantages of most experimental acute ulcer models is that the ulceration is fairly superficial and heals without scarring. There have been relatively few studies which attempt to produce a situation similar to that found in man where the chronicity of peptic ulcers is one of the major problems of the disease.

Acetic acid model:

Takagi et al., (1969) developed a method for inducing chronic ulceration in rats by injecting acetic acid into the submucosal layer of the stomach in the interdigestive phase. Small erosions were noted on the fifth day after the operation and the ulceration persisted for more than 150 days after the operation. The ulcers were oval or round and the ulcer index was assessed by calculating the total surface area of the ulcer animal.

Okabe et al., 1970 produced the same results by applying orally a cylindrical metal mould previously immersed in acetic acid directly to the antrum of the stomach for thirty seconds in an anaesthetised rat

MacDonald (1976) also confirmed Takagi's results (Takagi et al., 1969) in rats using injections of acetic acid under the mucosa to form blisters.

Clamping - cortisone method:

Umehara et al., (1963) have reported a method of inducing chronic gastric ulcers in rats which consists of clamping the stomach 5 mm below the border of the forestomach with an aluminium plate for 24 h and administering cortisone daily for seven days after the operation. The induced ulcers took up to 120 days to heal. The same model was used by Umehara et al., (1971) to evaluate the anti-ulcerogenic properties of some drugs.

Thermocautery model of ulceration:

Skoryna et al., (1958) has reported a method of inducing chronic ulceration in rats which consisted of opening the stomach and applying a sharp thermocautery needle to the submucosal part of the glandular stomach. The ulceration was aggravated by the injection of cortisone (Khan and Phillips, 1963).

The previous three techniques have advantages in that the ulceration was persistent and can be localized.

Having described the methods of inducing and evaluating ulceration in animal models the next section deals with some of the different factors involved in ulcerogenesis.

7. POSSIBLE CAUSATIVE FACTORS IN ULCEROGENESIS.

7.1 The Shay rat:

- The digestive action of accumulated gastric juice Brodie (1966).
- The breakdown of the mucosa barrier and decrease of mucosal resistance (Ishii, 1970).

7.2 Histamine:

- An angiotoxic effect (Anderson and Soman, 1965).
- The excessive and prolonged secretion of gastric juice (Hay et al., 1942).

7.3 5-Hydroxytryptamine:

- Increased blood coagulation (Milne and Cohn, 1957)
- Cholinergic stimulation (Haverbach and Bogdanski, 1957).
- Enhanced motility of the stomach (Hori, 1962; Yano et al., 1977).

7.4 Reserpine:

- Interference with the metabolic process in the gastric mucosa and the disruption of proteins. (Anichkov and Zavodskaya, 1968).
- Excessive gastric secretion and stimulation of vaso-motor activity (Lambert, 1963; Refshy and Fein, 1954).

7.5 Corticosteroids:

- Increased secretion of acid and pepsin (Vanov and Mildosevic, 1962).
- The breakdown of the protective mucosa barrier (Robert and Nezamis, 1958a, b).
- Vascular and metabolic disturbance (Segal, 1960).
- Decreased secretion of mucus (Rober and Nezamis, 1963).
- An impairment of the arterial supply to the gastric mucosa by microembolisms (Weinshelbaum and Ferguson, 1963).

- A reduced rate of regeneration of epithelials cells (Max and Menguy, 1970).

7.6 Stress:

- The back diffusion of H^+ (Skillman et al., 1970).
- Mast cell degranulation (Rasanen, 1963).
- Adrenal insufficiency (Bonfils et al., 1959).
- Secretion of pepsinogen (Ader, 1967).
- Bile reflux (Hamza and Denbesten, 1972).
- Acid hypersecretion (Guth and Kozbur, 1969).
- Vascular changes (Guth, 1972).
- Alterations in mucus synthesis (Ludwig and Lipkin, 1969).
- Vagal hyperactivity (Goldman and Rosoff, 1968).
- Decrease in cell regeneration (Kim et al., 1967).

7.7 Acetylsalicylic acid:

- The back diffusion of H^+ by breaking down the mucosal barrier (Davenport, 1967; Bunce et al., 1982).
- Lowered gastric blood flow (Takagi and Kawashima, 1969).
- An increase in the rate of shedding of epithelial surface (Max and Menguy, 1969, 1970).
- Decrease in mucus secretion (Menguy and Masters, 1965).
- Inhibition of prostaglandin synthesis (Vane, 1971).

7.8 Indomethacin:

- Alterations in the rate and composition of gastric mucus secretion (Menguy and Desbaillets, 1967; Piper and Stiel, 1962).
- Lowered mucosal blood flow (Buckingham et al., 1977).
- Stimulation of the cholinergic pathway (Gregory, 1962).
- Inhibition of prostaglandin synthesis (Tarun et al., 1978).

7.9 Phenylbutazone:

- No variation in gastric acid secretion (Menguy and Desbaillets, 1967).
- Lysosomal damage (Lewis et al., 1971).
- A decrease in mucus production (Menguy and Desbaillets, 1967).
- Increased shedding of surface epithelial cells (Max and Menguy, 1969, 1970).
- Decrease in mitotic activity of the antrum cells (Bucciarelli et al., 1968).
- Vascular system is involved (Barroy et al., 1970).
- Inhibition of prostaglandins synthesis (Main and Whittle, 1975).

CHAPTER TWO

BANANA AND PEPTIC ULCER

1. INTRODUCTION

1.1 Banana and peptic ulcers:

There are consistent reports concerning the anti-ulcerogenic activity of banana.

The Indian green vegetable banana has been reported to have an anti-ulcerogenic activity in rats as shown by post-mortum examination (Hanszen,1934). This activity was believed to be due to the buffering effect of banana pulp in the stomach.

Killian (1959) confirmed the anti-ulcerogenic activity of banana, but he attributed its activity to the neutralizing effect of basic salts in banana on gastrichydrochloric acid. Sanyal et al., 1961 reported that banana is rich in 5-hydroxytryptamine, and suggested that this substance was responsible for the inhibition of gastric acid secretion in guinea-pigs. Furthermore they showed that a neutralized suspension of banana powder administered orally to anaesthetized guinea-pigs reduced histamine-induced gastric acid secretion. They also found that guinea-pigs fed entirely on a banana diet after daily intra-muscular injections of histamine (1 mg kg^{-1}), developed fewer ulcers than controls (non-banana diet) after histamine treatment.

Banana powder has been reported to have protective anti-ulcerogenic properties against gastric ulcers produced by restraint in rats (Sanyal et al., 1963a) and by phenylbutazone in guinea-pigs and rats (Sanyal et al., 1963b, Sanyal et al., 1964).

Banana was not active in reducing the incidence of gastric ulcers induced by prednisolone in rats (Sanyal et al., 1965). Clinical trials in the U.S.A. (Rider, et al., 1967) have shown that the ripe fruit banana has anti-secretory activity in man. This activity was assumed to be due to the low protein content of banana since protein is a potent stimulant for the secretion of gastrin.

Sanyal (1980, personal communication) has described a clinical trial in India in which raw fruit banana has anti-ulcerogenic activity in man.

Although the mode of action of banana has been associated with an anti-secretory or neutralizing effect of gastric acid, recent work (Elliot and Heward, 1976) has shown that the anti-ulcerogenic action of banana on histamine-induced gastric ulcers in mice is associated with an increase in fresh stomach weight. They suggest that the effect of banana diet may be due to a promotion of gastric mucus secretion.

1.2 Medicinal aspects of banana

There are many reports on the medicinal and pharmacological properties of banana. Ripe bananas are alleged to be beneficial in the treatment of anaemia due to their high iron content and it is reported to be a valuable remedy for chronic dysentery and diarrhoea (Nadkarni, 1960). Juice from the green banana skin has alleged beneficial medicinal properties against eczematous skin eruptions and when incorporated into an ointment has been used to treat wrinkles (Boismare et al., 1970, Hakim, 1962). Juice squeezed from the petioles and the banana stalk causes contraction of guinea-pig isolated ileum, rat duodenum and a rise in blood pressure in the dog and rat. These effects are antagonised by atropine, phentolamine and 5-hydroxytryptamine (Bustos, 1963). The juice also has been used medicinally to treat otalgia and haemoptysis (Col et al., 1958). The banana flower contains three hypoglycaemic agents with activity in the rat. These three compounds have been isolated and the most potent of these has the empirical formula $C_{30} H_{50} O$. The chemical studies of this compound indicate that it could be either a steroid or a triterpene (Jain, 1969). Extracts of the flowers mixed with curds have been used to treat dysmenorrhoea and menorrhagia (Nadkarni, 1960). The root is used in India to treat bronchocela and strumous disorders (Nadkarni, 1960).

Linaweaver et al., (1976) have reported on a case of a girl, who suffered from an allergic disorder, developing anaphylactic shock after eating a banana. Radioimmunoassay of a serum sample showed an elevated IgE titre. A second case similar to the above has been reported (Ritz et al., 1969).

Shaper (1967) and McKinney and Crawford (1965) attributed the high incidence of endomyocardial fibrosis in East and West Africa to a high consumption of plantain banana which is supported by experimental work where fibrosis was induced in guinea-pigs on a plantain diet (Antia et al., 1967). However, McKinney (1976) failed to demonstrate a similar effect in the dog and monkey. Ojo (1970) has reported that there is no correlation between 5-hydroxytryptamine and endomyocardial fibrosis in man since he found no difference in the incidence of disease between patients on a high plantain diet and a non plantain diet. Although some of the above effects may be associated with the presence of 5-hydroxytryptamine, others must be treated with caution. The frequency of reports attributing medicinal properties to the banana may illustrate the prevalent established beliefs, particularly in third world countries that banana is a medicinal panacea.

1.3 Banana species and occurrence:

The family Musaceae is a member of the order Scitimineae which also include the Cannaceae, Marantaceae, Zingiberaceae,

Streliziaceae and Lewiaceae (Simmonds, 1966).

Cytologists and taxonomists at the Imperial College of Agriculture in Trinidad have classified both the wild and cultivated banana by their system. The family Musaceae consists of two genera, the first is Ensete, and the second is Musa (Table 1).

Table 1. The number of banana species after Simmonds (1966).

Genus	Basic section	distribution	species	uses
Ensete	9	W. Africa to New Guinea	7-8	fibre vegetable
Musa	10 Australimusa	Queensland to the Philippines	5-6	fibre fruit
	10 Callimusa	Indochina and Indonesia	5-6	ornamental
	11 Eumusa	South India to Japan and Samoa	9-10	fruit, fibre, vegetable
	11 Rhodochlamys	India to Indonesia	5-6	ornamental

a) The genus Ensete is composed of monocarpic herbs, none of which bear edible fruit.

b) The genera Musa consists of four sections of subgenera.

The section Australimusa contains M.textilis abaca, which yields the Manila hemp of commerce. The section Australimusa also contains the Fe'i banana of the Pacific, which bears edible fruits.

The section Callimusa contains only M.coccinea of any economic significance since its scarlet bracts are of ornamental interest.

The majority of the edible banana belong to the section Eumusa which is the largest and geographically the most widespread section of the genus.

The section Rhodochlamys is mainly of ornamental interest with few edible species. Therefore the edible banana is of limited origin in that it is present in only two of the principal sub-divisions of the family and one of these is only of minor importance due to its limited localisation (Simmonds, 1966).

i) The species of Musa:

Musa contains about thirty species, most of which are diploid and seeded and the two important species are M.balbisiana and M.accuminata, which are both species of the section M.Eumusa (Cheesman, 1948).

M.balbisiana appears to be the most widely distributed species of Musa occurring in Sri Lanka, India, Burma, Malaysia and Indonesia. M.balbisiana has a characteristic 'starch' taste.

M.accuminata has been recorded in Burma, Thailand, the Malay Pensinula and in Java. It is sweet when ripe (Cheesman, 1948). Simmonds and Shepherd (1955) suggest that edible bananas have developed in one or both of the edible species and cross hybridation between edible and wild species. Today's varieties are

the result of numerous crossings between species over a long period of time. Research at the Imperial College of Agriculture of Trinidad (Cheesman, 1949) has confirmed that M.accuminata and M.balbisiana are related in their acernity to the actual triploidy varieties of banana, and they suggest that Mysore, silk, and Pome are varieties resulting from crossing M.balbisiana while Gross Michel and Rajah appears to be derived from M.accuminata.

Linnaeuse (1783) classified M.paradisiaca, as an 'edible species' distinguishing it from non 'edible species'.

Later Linnaeuse (Simmonds, 1966) included M.sapientum as an edible species.

Cheesman (1947) has tried to relate M.paradisiaca and M.sapientum to M.balbisiana and M.accuminata and he suggests that M.paradisiaca has been derived from the wild species M.accuminata and M.sapientum from banana of hydrid origin. Present opinion suggests that such a solution is no longer adequate. Simmonds and Shepherd (1955) suggested that the two names M.paradisiaca and M.sapientum must be discarded from the nomenclature and they have advised botanists to refer always to M.accuminata and M.balbisiana. At present the difference between M.sapientum and M. paradisiaca is the starchy fruit that requires cooking in order to be edible (Simmonds, 1966).

The interest of this work is focussed on the Indian banana where the anti-ulcerogenic property was first reported (Sanyal et al., 1961, Sinha et al., 1961).

Nadkarni (1960) has reported the presence of at least eleven varieties of edible banana universally cultivated throughout India not including the wild types;

1. Tambdi kel Rajhel Ramkel
2. Bengaliguji, Cavendish, Hirvi, Basrai
3. Motheli
4. Rajeli
5. Sonkela or Safed Elchi; Shasrafali, Yalakki-bali, Sugandhibali
6. Bankel, Ambel (M.paradisiaca)
7. Lalechi, Karanjali, Sonkel
8. Mhaskel, Basrai
9. Govekari
10. Pattemadaraangabali
11. Yellaybali or Lokhandi of Poona

It is obvious that there is much confusion in the nomenclature identification of banana varieties in India. A variety of banana is identified in different localities by different names (Nadkarni, 1960). The most reliable method of identifying banana species appears to be by referring to the locality of the source from which the banana can be obtained.

1.4 Conditions required for screening for the anti-ulcerogenic properties of banana

Since there is no universal model for screening all types of anti-ulcerogenic agents acting with different mechanisms one must compromise and select a model with a broad spectrum of activity particularly when the anti-ulcerogenic agent involved is crude material:

a) The model must be simple and easy to use particularly when a large number of animals are required in the experiment for statistical purposes.

b) The model must be reproducible.

c) The model must be achieved with low mortality

d) The model must be similar to human gastric ulceration.

e) The ulcers must be induced in the glandular areas of the stomach.

f) The model must allow quantitative estimation of the ulceration.

g) The ulcers must not heal spontaneously during the experimental investigation of the properties of the anti-ulcerogenic agent.

Aspirin-induced ulceration appears to fulfil the above requirements and this model was employed in the experiments described in this thesis.

1.5 Aims of project:

The aims of the present studies were:

1. To study the anti-ulcerogenic activity of the different samples of banana.

2. To extract and purify the anti-ulcerogenic activity of banana.

3. To study the mode of action of banana and compare it with that of standard anti-ulcerogenic agents.

2. MATERIALS

2.1 Banana, aspirin and rats used in this project.

a) Banana

Dried banana powder was supplied by Reckitt and Colman (PLC) who obtained the samples from two sources:-

i) India where the anti-ulcerogenic activity was first reported by Sanyal et al., (1961).

ii) St. Lucia supplied by Geest Ltd. (Cutts, 1981, personal communication) when a shortage in Indian supplies occurred and a variation in the samples activities were noted. The following Table(2) gives the origin of the samples used in this project and their characteristics.

Table 2. Banana samples, origins and characteristics

Sample	Origin	Physical appearance of the samples
A	Varanasi district of India, by Professor Sanyal	fine powder, dark grey
B		fine powder, grey
C		fine powder, dark grey
D		fine powder, grey-white
G		fine powder, white-grey
Cavendish		gritty granules, pale-yellow
Mondan		gritty granules, pale-white
E	St. Lucia (Windward Islands) by Geest Ltd	banana pulp powder, very light grey
E-1		whole banana powder, dark grey
F		whole banana powder, dark grey
0	Birmingham market	fine powder, yellow

All bananas were picked unripe with the exception of sample O which was a fruit banana used for comparison.

The Indian samples A, B, C, D, G, Cavendish and Mondan were supplied at different times between March, 1979 and April, 1981 by Professor Sanyal. Samples A, B, C, D and G were picked green from the tree and each banana was peeled by making six longitudinal cuts. The pulp was sliced, sun dried and then powdered. Their taste was bitter.

Cavendish and Mondan samples were also picked green from the tree but the drying process was by hot air (180°C) for three minutes. The taste of these latter two samples was slightly sweet. The West Indian samples E and E_1 were also collected green for marketing (4–5 weeks before ripening). Sample E was peeled in a similar manner to the Indian samples. The pulp was sliced into two cm thick strips and dried at 50°C for 12 hours in a previously sterilized oven. Sample E_1 was sliced without peeling and dried by the same process. Table (3) shows the percentage of the fresh and the dried banana pulp referred to as whole fresh banana.

Table 3. Percentage of the fresh and the dried banana pulp compared to whole fresh banana.

Whole banana (fresh)	100 g
Fresh banana pulp	54 g
Dried banana pulp	15 g

A sample claimed to be identical to sample (E) was supplied by Geest and mixed by them with sample (E) previously tested. The sample so produced was designated as sample (F).

b) Aspirin was purchased from Sigma Ltd., and kept in stoppered bottles.

c) Male Wistar strain albino rats (Bodyweight 150 g) supplied by Bantin and Kingman Ltd., were used throughout this project. Animal food was supplied by Labsure.

3. METHODS

3.1 Different methods used to evaluate the anti-ulcerogenic activity of banana:

a) Prophylactic method; measured by ulcer index.

b) Curative method; measured by ulcer index.

c) Mucosa weight method: measured by weighing the gastric mucosal tissue (dry weight) which was scraped from the stomach.

a) Prophylactic method:

Male albino rats were housed in separate cages. A banana diet was administered either, ground into paste with the normal diet or as a separate paste from the normal diet or sliced banana. The

amount of diet given during the treatment period was just sufficient to satisfy the normal appetite of the animal. Aspirin was administered orally at a dose of 150 mg kg^{-1} in a water suspension (5 ml kg^{-1}) using a glass syringe to ensure the total dose was delivered. Two groups of rats were employed, i.e. with and without treatment. The rats were caged individually and allowed water ad libitum. All animals were allowed 48 h to adapt before commencing treatment. The following experimental design was adopted:

0–48 h	48–96 h	96–144 h	144 h	149 h
Acclimatization food and water ad libitum	14 g * normal diet	fast	aspirin oral dose 150 mg kg^{-1}	animals killed

NB. For control groups aspirin was omitted at 144 h. For the test groups the diet given from 48–96 h and was *14 –xg of normal diet plus xg of test banana. Water was available throughout the experiment.

The animals were killed by a blow to the neck and the stomach removed immediately, cleaned from serosal plexus, and dissected along the greater curvature. The stomachs were washed with water and placed in labelled beakers containing cold saline solution (0.9% w/v^{-1}). The ulcers were scored 'blind' using a binocular microscope. The ulcer index was evaluated by a modified version of the Robert and Nezamis (1958 a, b) method as follows:-

1. 1 Mark for each deep ulcer, up to a maximum of 7, where each ulcer did not exceed 1 cm in length.

2. 0.5 Marks for each ulcer of medium depth.

3. 0.25 Marks for each superficial ulcer.

4. For ulcers exceeding 1 cm in length, 2 marks were given to that ulcer.

5. 1 Mark was added for the severity of the haemorrhage.

6. 1 Mark was added for evidence of microscopic observable erosions (up to 24 erosions cm ^{2}).

7. 1 Mark was added when the tissue appeared thin (transparency degree) when compared to normal tissue.

This gave a maximum score of 10. To compare with Robert's method of scoring the final total was doubled.

Example:

3 deep ulcers (less than 1 cm length)		3 marks
4 medium ulcers	4 x 0.5 =	2 marks
1 superficial ulcer	1 x 0.25 =	0.25 marks
14 microscopical erosions		0.55 marks
70% haemorrhages		0.7 marks
40% transparency evident		0.4 marks

6.9 x 2 = 13.8

The ulcer index for this animal was therefore 13.8, which represents ulcerated animal at the level of 70%.

b) Curative method:

There are two differences between the prophylactic model and the curative one. The first difference is in the procedure. In the prophylactic model the treatment with banana precedes the administration of the ulcerogenic agent whereas in the curative model, the aspirin was given before the treatment with banana as follows:

0-48 h	48-96 h	96 h	101-149 h	149 h
Acclimatization food and water ad libitum	fast	aspirin oral dose 150 mg kg^{-1}	14 * g normal diet	animals killed

NB. For the control groups aspirin was omitted at 96 h. For the test groups, the dose given from 101-149 h consisted of 14 * - xg of normal diet plus xg of test banana. Water was available throughout the experiment.

The second difference was in the scoring. In the prophylactic method, the small erosions were clearly visible in the mucosa whilst in the curative model these erosions were not apparent. This was due either to the effect of food or treatment over the two days following aspirin administration. Therefore the mark which corresponded to the erosions in the prophylactic model was used to indicate the general severity of gastric damage in the curative model.

c) Mucosa weight method:

After scoring, the stomachs were stored at $-20^{o}C$ for 48 hours which made the mucosa much easier to scrape away from the underlying layers. The entire glandular mucosa was scraped from the underlying muscularis mucosa with a glass microscope slide. Care was taken not to remove muscle tissue. All the mucosa was removed when further scraping produced a clean edge to the slide. The mucosal samples were freeze-dried and then weighed.

4. EXPERIMENTS CARRIED OUT ON BANANA SAMPLES

4.1 Curative anti-ulcerogenic tests using Indian green vegetable banana powder sample (A).

Twenty-one rats were divided into three groups each of seven rats. The curative model of ulceration was used to evaluate the protective anti-ulcerogenic action of banana sample A (Indian). Of the 14 g diet, 9 g was normal rat pellets ground to a powder and 5 g banana powder. The two constituents were mixed together as a paste. All diets were totally consumed.

On the seventh day of the experiment the rats were killed and the ulcer indices were assessed and the mucosa weighed.

4.2 Curative anti-ulcerogenic test using samples A, B and D of the Indian green vegetable banana powder.

Thirty-two rats were used in this experiment divided into four groups each of 8 rats.

The curative procedure was used. In the diets 9 g of ground rat pellets were mixed with 7 g of the banana powders (A, B and D). This made a total of 16 g of diet. This increase over the standard 14 g diet was to allow an increase in the amount of banana used. This would ensure that activity in samples B and D would be detected. The 16 g of diet was totally consumed.

The curative method of the ulcer index and the mucosa weights were used to evaluate the extent of ulceration.

4.3 Curative anti-ulcerogenic test using Cavendish banana, Mondan banana powder and banana sample (B).

The curative model of ulceration was used in this experiment to evaluate ulceration. Twenty-eight rats were used divided into four groups each of seven rats.

The procedure employed in section 4.2 was used e.g. 16 g of diet including 7 g of banana .

The curative ulcer index and the mucosa weights were used to evaluate the ulceration.

4.4 A long-term treatment with Cavendish and Mondan banana samples compared to sample (B) using the curative method of ulceration.

Although the curative procedure was employed in this experiment the format was somewhat different to the procedure described earlier in the text. It is therefore described in full.

The banana dose was increased in order to detect activity which might be missed at a lower dose. The dosing period was extended for the same reason.

Tewenty-eight rats were used divided into four groups as follows:

Treatment	Notes
Aspirin	Oral dose of aspirin 150 mg kg^{-1} plus 16 g of normal diet per 48 h, per rat, 5 h after aspirin administration.
Aspirin with banana (B)	Aspirin as above plus 9 g of normal diet with 7 g of banana sample (B); per 48 h, per rat, 5 h after aspirin administration.
Aspirin with banana Cavendish	Aspirin as above plus 9 g of normal diet with 7 g of banana Cavendish as above.
Aspirin with banana Mondan	Aspirin as above plus 9 g of normal diet with 7 g of banana Mondan as above.

On the seventh day of the experiment instead of killing the animals, the 14 g normal diet with and without banana was given for a further 14 days and the animals were killed on the twenty-first day of the experiment. The curative ulcer index and the mucosa weights were used to evaluate the ulceration.

4.5 The curative anti-ulcerogenic test using samples E and E-2 compared to the Indian sample C

Twenty-eight rats were used in this experiment divided into four groups each of seven rats.

The curative procedure was used but employing 16 g (7 g of banana powder) of diet (cf 4.2) instead to 14 g. The object of this experiment was to see if differences occurred when peel was included in the diet (E-1) compared to pulp alone (E) in the same banana. The curative ulcer index and the mucosa weights were used to evaluate the activity of these samples.

4.6 An evaluation of the anti-ulcerogenic activity of sample F, G and ripe market banana compared to the activity of banana sample (A).

The curative method of ulceration was used to investigate the activity of banana sample (F), (G) and (O) (ripe banana) compared to the activity of sample (A)

Sixty rats were used in this experiment divided into six groups of ten rats. In this experment 14 g of diet was used .The amount of banana contained in these diets was sample A(3 g),ripe banana (sample O) 7 g, sample F,7 g and 14 g and sample G,14 g.

5. RESULTS

5.1 Aspirin-induced ulceration:

The oral administration of aspirin(150 mg Kg^{-1}) produced a 100% incidence of ulceration in rats previously fasted for 48 h

The ulcers varied from pin point lesions to deep linear lesions which were occasionlly 20 mm long. The edges of these ulcers were clearly demarcated and the craters were frequently full of blood.

The severity of the haemorrhage noticeably decreased 24 h after aspirin administration and this was particularly evident when a comparison was made between the curative and the prophylactic methods. However persistent haemorrhage was observed in the curative method but was was less severe. Traces of old haemorrhages were found in the stomach 16 days after the induced ulceration by aspirin . After aspirin treatment the gastric tissue became very fragile and thin. This was clearly noted in the body and the antrum of the stomach the majority of ulcers were located

Ulcers occurred only occasionally in the pyloric and never in the squamous part of the stomach. The mucosa in the pyloric region and in the lesser curvatures was transparent when lit with back lighting compared to the normal stomach or to those treated with banana. This could also be seen during autopsy when the sjpport on which the opened stomach was pinned could be clearly seen through the mucosa. some oedema was observed in the squamous part of the stomach 48 h after aspirin administration.

In preliminary experment aspirin failed to induced ulceration in non-fasted rats at a dose 150 mg Kg^{-1}. Small pin-point haemorrhage were only found in rats previously fasted for 48 h. These erosions disappeared completely in the curative method possibly due to the buffering effect of food against gastric secretions. These were localized mainly in the antrum and in the body of the stomach.

5.2 The prophylactic activity of banana sample (A).

Banana sample (A) was significantly active in protecting rats against ulceration induced by a dose of 150 mg kg^{-1} of aspirin. The decrease in ulcer index (Figure 6) was due to a decrease in ulceration (both number and severity), a decrease in the severity of haemorrhage and an increase in the thickness of the mucus coating on the mucosa surface after banana treatment. The increase in mucus secretion made the mucosa less transparent than in the aspirin treated rats. The low ulcer index found in untreated fasted control animals is a natural consequence of fasting where the gastric secretions are in direct contact with the mucosal surface.

5.3 The curative activity of samples A, B and D of the Indian green vegetable banana powder.

The ulcer index and the mucosa weight (Figure 7 and 8) showed that banana sample (A) supplemented diet was not only protective against ulceration induced by aspirin but, also was capable of curing established ulcers. In addition to the observed prophylactic effect of banana in the previous section (5.2), banana sample (A) and(B) treatment for 48 h followed by aspirin administered had a considerable effect in increasing the scraped mucosa weights. Stomachs of animals treated with aspirin followed by banana treatment were much

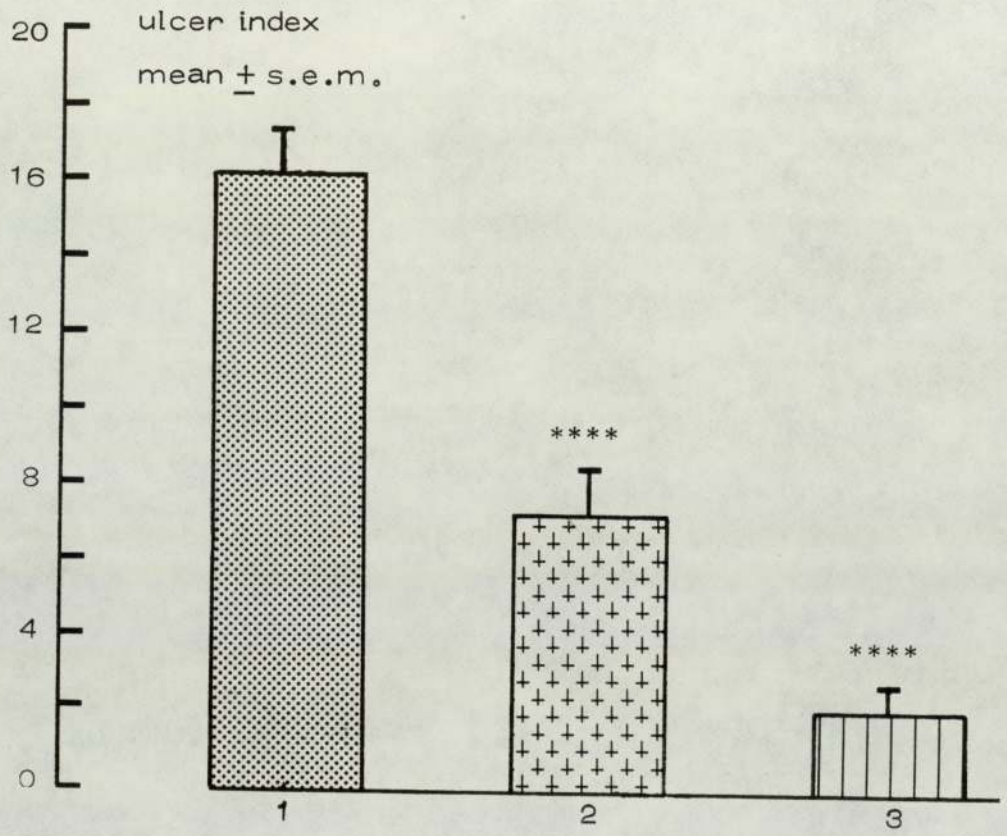


Figure 6. The prophylactic effect of banana sample(A) on the ulcer index

This histograms show the effect of banana sample (A) (2) on aspirin induced ulceration (1) where the prophlactic procedure was used. Clearly 5 g of banana sample (A) suppressed the induction of ulcers by aspirin. The small positive value in non-treated rats(3) was probably the result of fasting the animals .The results are discussed in the text (cf. 5.2 p . 74)

***$p<0.001$; (Wilcoxon rank sum test)

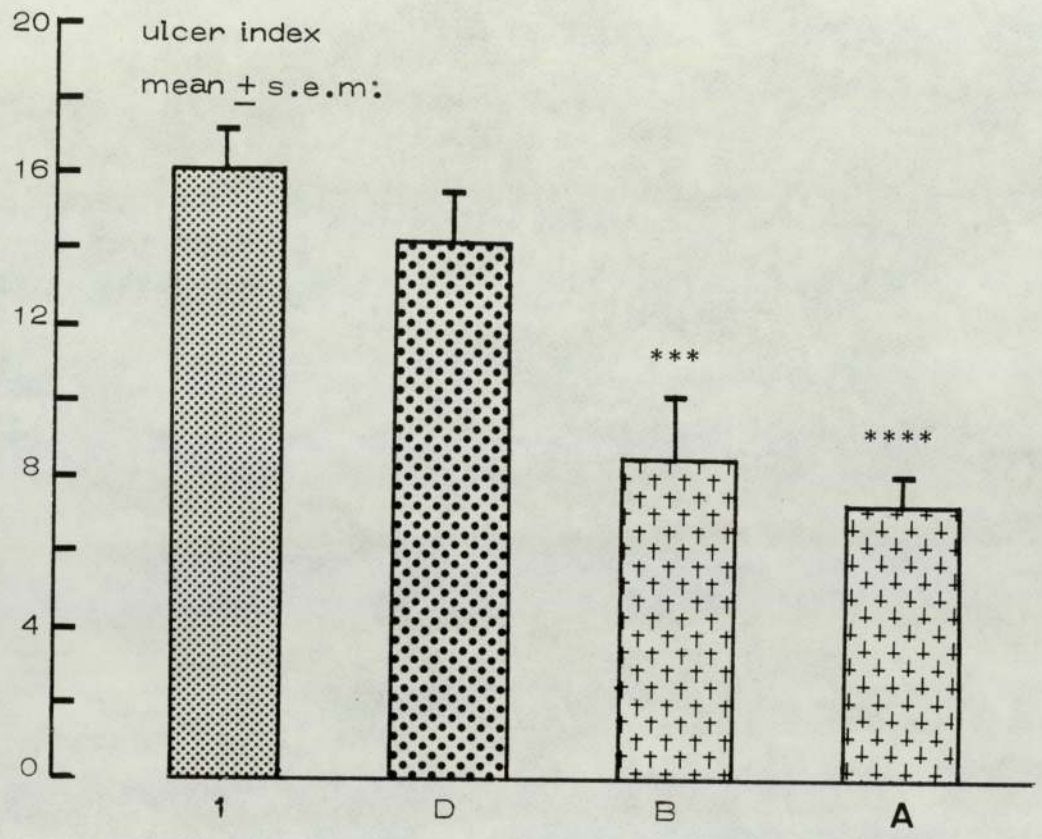


Figure 7. The curative effect of banana samples (A),(B) and (D) on ulceration-induced by aspirin:

The Figure above shows that banana sample(D) was inactive in healing ulceration-induced by aspirin compared to the activity of banana samples (A) and (B)

***$p<0.01$, ****$p<0.001$(Wilcoxon rank sum test)

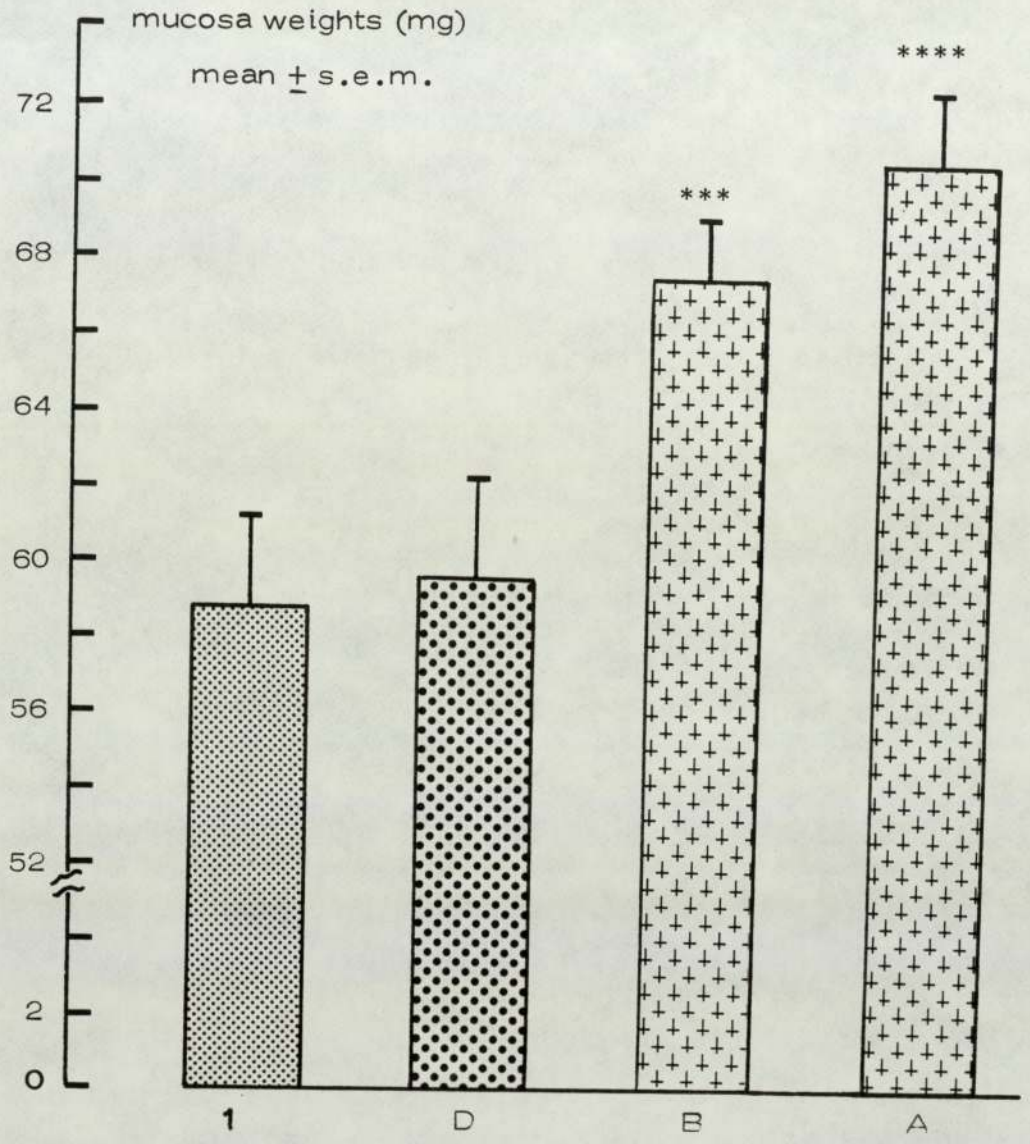


Figure 8. The mucosa weights of rats' stomachs treated with banana samples (A),(B) and (D):

The mean weights are given as histograms which represent the curative effect of banana samples (A),(B)and(D) onaspirin-induced ulceration(1) The experiment is discussed in 5.3 (p.74)

***p<0.01; ****p<0.001 (Student's t-test)

less fragile than those treated only with aspirin. The failure to obtain improvements both in the ulcer index and in the mucosa weights in animals treated with banana sample (D) compared to improvements obtained with banana samples (A) and (B) suggest that the anti-ulcerogenic activity of the Indian banana varies from one sample to another.

5.4 The effect of Cavendish banana and Mondan banana in healing ulceration compared to that of banana sample (B).

The ulcer index and the mucosa weights (Table 4) showed that neither Cavendish nor Mondan has any effect on the ulcer index or the mucosa weights when compared to the activity of sample (B) which induced healing and an increase in the mucosa weights.

5.5 Effect of 16 days treatment with Cavendish and Mondan banana compared to banana sample (B).

The ulcer index and the mucosa weights (Table 5) indicated that chronic treatment with Cavendish and Mondan banana has no effect on ulceration or the mucosa weights when compared to banana sample(B)which again showed a significant improvement.

Table 4. Effect of Cavendish banana and Mondan banana in healing ulceration compared to that of banana sample(B).

Treatment	Ulcer index	Mucosa weights (mg)
Aspirin treatment	16.77 ± 0.5	56.4 ± 2.57
Aspirin treatment with banana (B) (7 g)	8.31 ± 1.25 ****	69.04 ± 1.64 **
Aspirin treatment with Cavendish banana (7 g)	16.85 ± 0.52	56.32 ± 2.42
Aspirin treatment with Mondan banana (7 g)	14.68 ± 1.02	59.31 ± 2.19

** $p<0.02$ (Student's t test); **** $p<0.01$ (Wilcoxon rank sum test).

Table 5. Effect of 16 days treatment with Cavendish and Mondan banana compared to banana sample (B).

Treatment	Ulcer index	Mucosa weights (mg)
Aspirin treatment	9.71 ± 0.67	72.62 ± 3.25
Aspirin treatment with banana (B) (7 g)	5.11 ± 0.62 ***	83.04 ± 2.98 **
Aspirin treatment with Cavendish banana (7 g)	10.44 ± 0.94	72.57 ± 2.69
Aspirin treatment with Mondan banana (7 g)	7.41 ± 4 N.S.	74.7 ± 3.4 N.S.

** $p<0.02$ (Student's t test); *** $p<0.01$ (Wilcoxon rank sum test).

5.6 The curative activity of banana (E) and banana (E-1) compared to the Indian sample C.

The ulcer index and the mucosa weights (Table 6) showed that Geest samples E and E.1 were effective in healing ulceration although not as active as the Indian sample C.

5.7 The curative activity of Geest sample F compared to sample A, G and to sample O (Market banana).

The ulcer index (Table 7) indicated that sample F at 7 g was not significantly effective in healing ulceration. The significance was $p<0.02$ when 14 g of sample F were given to each rat. The Indian banana sample (G) given at a dose of 14 g per rat was inactive in healing ulceration compared to the activity of 3 g of banana sample(A).The ripe market banana was also inactive in healing ulceration.

Table 6. The curative effect of banana (E) and (E-1) compared to the Indian sample C.

Treatment	Ulcer index 't'	Mucosa weights mg 'tt'
Aspirin treatment	15.83 ± 0.77	61.73 ± 2.63
Aspirin treatment with banana(E) (pulp only)	10.56 ± 1.82***	70.00 ± 2.07**
Aspirin treatment with banana(E-1) (pulp and skin)	10.66 ± 1.2***	70.02 ± 1.94**
Aspirin treatment with banana (C).	8.13 ± 1.82***	72.28 ± 1.99***

*** p<0.01; **p<0.02; t - after Wilcoxon rank sum test;

tt - Student's t test. (banana diet = 7 g)

Table 7. The activity of Geest sample F compared to sample A, G and to sample O (Market banana).

Treatment	Ulcer index
Aspirin treatment	14.42 ± 0.67
Aspirin treatment with 3 g of banana (A)	7.02 ± 0.54****
Aspirin treatment with 14 g of ripe banana (O)	12.35 ± 2
Aspirin treatment with 7 g of banana (F)	11.61 ± 1.39
Aspirin treatment with 14 g of banana sample (F)	10.82 ± 1.42**
Aspirin treatment with 14 g of banana (G)	12.10 ± 1.20

****p<0.001; **p<0.02 (Wilcoxon rank sum test).

6. DISCUSSION

6.1 Ulcer index and aspirin induced ulceration:

The use of the ulcer index to screen for anti-ulcerogenic effects of banana has some advantages over the Shay rat model. The Shay rat is particularly useful when the anti-ulcerogenic properties of crude material are due to an anti-secretory mechanism. However, the ulcer index gives a quantitative estimation of the anti-ulcerogenic action of materials with a wide spectrum of action or when the mode of action is unknown.

The survival of animals using aspirin as the ulcerogenic agent was 100% in these experiments compared to the high incidence of mortality reported for the Shay rat model. (Anichkov and Zavodskaya, 1968). The ulceration induced by aspirin was localized in the glandular area of the stomach. This is similar to human ulceration. In the Shay rat model of ulceration the induced ulcers are localized mainly in the squamous tissue of the stomach which is dissimilar to the situation in the human stomach. The need for surgery in the Shay rat is an additional complication particularly when a large number of animals were involved in the experiments.

Dietary protein is known to increase gastric acid secretion (Code, 1962) and this is difficult to control in the Shay rat, in which

protein containing material is being assessed (e.g. banana), since the protein content of the normal diet and that of the crude material will vary. This may alter gastric acid secretion which is not due in total to potential anti-secretory agents. The severity of ulceration induced by aspirin was reproducible compared to the variation on the ulcer severity induced by stress (MacDonald, 1976).

The ulcers induced by aspirin were distributed in most areas of the glandular region of the stomach. This may involve different susceptibilities of cells to aspirin. Steroid induced ulcers are localized in one area or the other of the stomach depending on the type of steroid used to induce ulceration (Robert and Nezamis, 1958 a and b., Kelly and Robert, 1969). Aspirin induces ulceration in most of the glandular part of the stomach which may involve different cells in the pathogenesis of gastric ulcer. This is of interest particularly when the anti-ulcerogenic properties of the crude materials have an unknown mode of action. Some ulcers induced by aspirin at an oral dose of 150 mg kg^{-1} in 48 h fasted rats extended to 20 mm length and the ulceration was more severe than in rats treated with the same dose of aspirin but were fasted for only 24 h (preliminary experiment) and no ulceration exceeded 4 mm even with higher dose of aspirin at a dose of 200 mg kg^{-1} (MacDonald, 1976, Guth et al., 1979). The severity of ulceration 48 h after aspirin administration in

48 h fasted rats showed a slow healing process. This and the general severity of the ulceration allowing differentiation to be made between deep, medium and superficial ulcers which made this an excellent model for assaying the activity of banana.

The chronic model of ulceration (Okabe et al., 1970, Takagi, et al., 1969) is a suitable model for investigating the anti-ulcerogenic properties of new agents because of its similarity to chronic human ulceration. However the use of chronic ulceration to investigate the anti-ulcerogenic effect of banana would have some disadvantages if it was used when particularly large numbers of animals are involved in the experiment. Also the period of assessing the anti-ulcerogenic activity is much longer than in the aspirin model of ulceration. A chronic model of ulceration would be useful when the active agent has been isolated and identified since although the acute model identifies its action the chronic model is more relevant to the human situation. The chronic model would also be useful to study the toxic effects associated with the new agent during healing of ulceration. The fixation of tissue by formalin (Sanyal et al., 1965) or preservation by alcohol (MacDonald, 1976) to score the stomach at a later time is not desirable since these agents increase the folding in the tissue and dehydrate the mucosa. In preliminary experiments using formalin to preserve the tissue it was impossible to differentiate between real

ulceration and 'cracks' in the tissue due to mechanical or solvent damage. Alcohol also causes dehydration and increases the folding of the stomach which made the scoring process more difficult. It was difficult to distinguish between deep ulcers, medium ones and superficial erosions.

It was found that the severity of aspirin-induced ulceration depended on the length of the fasting period. Therefore a short fasting period (e.g. a few h) would allow investigation of the early factors involved in the development of ulcers e.g. drug-induced mucosal erosions and bleeding. Such erosions heal very quickly.

The vasodilation and the oedema observed in the squamous tissue is an indication of vascular involvement in the pathogenesis of peptic ulcer induced by aspirin. This agrees with the finding of Augur (1970).

6.2 The prophylactic and the curative effect of banana on ulceration.

The use of the prophylactic and the curative models of ulceration showed that banana is not only prophylactic against aspirin-induced ulceration in the rat but is also curative. The prophylactic

effect of banana observed is in agreement with reports by Sanyal et al., (1961) and Elliot and Heward (1976) where histamine-induced ulceration was used in guinea-pigs and mice and phenylbutazone-induced ulceration in guinea-pigs (Sanyal et al., 1963 b).

The prophylactic effect was also reported by Sanyal et al., (1965) against stress-induced ulceration in rats. These reports combined with the present work shows that the anti-ulcerogenic activity of banana has a broad spectrum of activity in terms of species and ulcerogenic agents.

The healing effect of banana was confirmed by Professor Sanyal, (1980, personal communication) who found that banana was also active in healing human ulceration. The protective and healing effects by banana on ulceration were due, at least in part, to increases in the mass of the mucosa associated with increases in mucus secretion. It was these factors which made the stomach wall on examination appear much less transparent than in rats treated with aspirin alone.

The anti-ulcerogenic activity of banana probably is not due to its buffering effects as reported by Hanszen, (1934). This follows since samples A, B, C and E were active in protecting and healing ulceration .while samples D, F and G were inactive . All were green banana samples and it is unlikely that the buffering action between samples varied widely. It is more likely that samples D, F and G were

inactive due to the absence or loss of activity of a specific factor

The anti-ulcerogenic property of banana was unlikely to be due to 5-hydroxytryptamine (5-HT) (Sanyal et al., 1961; Sinha et al., 1961) for several reasons. Banana peel has been reported to contain 56 $\mu g\ g^{-1}$ of 5-HT whilst the pulp content of 5-HT was 20 $\mu g\ g^{-1}$. The concentration of 5-HT in the pulp increases by 50% during ripening (Waalkes et al., 1958) and ripe banana (sample O) was inactive against ulceration. There was no difference in the activity found when rats were fed with whole banana (sample E-1) or with pulp from the same banana (sample E). Therefore it is unlikely that the anti-ulcerogenic activity of banana was due to its 5-HT content.

The observed increase in mucus secretion and mucosa mass is in agreement with the findings of Elliot and Heward (1976) in mice. They observed increases in stomach weights by weighing the whole stomach. They concluded that the increase in mucosa weights was due to stimulation of mucus secretion. This suggestion will be examined in detail in Chapter 4.

6.3 Variations in the activity of banana:

The variation in activity between samples of banana presented difficulties in the present project. This variation in activity was always between samples but never within the same sample. The

anti-ulcerogenic activity of banana was stable since no variation was observed in the activity of sample (A) when it was used after one year's storage at room temperature.

The preparation of the Indian samples A, B, C and G was identical (Sanyal, 1981, personal communication), however, sample G demonstrated no anti-ulcerogenic properties. Therefore the variation in activity was not due to the method of preparation of the samples. Geest samples E, E-1 and F were also prepared by identical methods, but F showed no activity (at 7 g per rat). The Indian and the Geest samples were known to be different variaties of banana (Cutts, 1981, personal communication). Therefore the variation in the anti-ulcerogenic activity of the samples was not due to variety differences.

The anti-ulcerogenic activity is not restricted to a single variety but may be common to several closely related varieties, the growth cycle of banana is between 11-18 months (Simmonds, 1966) and the Indian samples were collected at different times of the year. A wide range of substances appear and disappear during banana growth (see chapter 3, section 1.1). Therefore it is very likely that the variation in the anti-ulcerogenic activity of banana was due to variations in banana composition during the growth cycle. This suggestion was supported by Sanyal in late, 1981 (personal

communication) who stated that active bananas are collected between October and January. Banana sample F which was alleged to be identical to sample E was received from Geest Ltd., three months after sample E had been tested. However the slight activity present in sample F (new sample mixed with original sample E) was due to a dilution of the original activity. It was found that higher doses than 14 g per rat over 48 hours did not give any improvement in the anti-ulcerogenic activity.

The absence of the activity in Cavendish and Mondan banana may be due to a different method of preparation being used which involved the use of high temperatures (180^{o}C).

Overall, Table (8) gives a summary of the activity of different banana samples.

Table 8. Summary of different banana activity

sample	origin	ulcer index	mucosa weight	activity
A	Varanasi	+++	++	+++
B	district of	++	++	++
C	India by	+++	++	+++
D	Professor	-	-	-
G	Sanyal	-	-	-
Cavendish		-	-	-
Mondan		-	-	-
E	Saint Lucia	++	+	+
E-1	by Geest Ltd.	++	+	+
F		$\bar{+}$		-
O	Birmingham market	-	-	-

+++ very strong activity ; ++ strong activity ; + moderate activity;

($\bar{+}$) weak activity ; - no activity

Since the anti-ulcerogenic activity of banana is prophylactic in different species (section 1.1) and reported to be curative in rat (present study) and man (Sanyal, personal communication, 1981) it was of interest to attempt to fractionate banana and to study the mode of action of banana with that of other established anti-ulcerogenic agents. These will be studied in Chapters 3 and 4.

CHAPTER THREE

EXTRACTION AND FRACTIONATION OF THE ANTI-ULCEROGENIC AGENTS OF BANANA

1. INTRODUCTION

1.1 The composition of banana:

For commercial purposes banana is usually harvested green four to five weeks before it is fully ripe (Simmonds, 1962).

A marked change in composition occurs during the ripening period, in particular the pulp composition and its variation will be of interest as the anti-ulcerogenic activity of banana was reported to be in the pulp (Sanyal, et al., 1961). Banana contains 25% (w/w) of water four to five weeks before harvesting (Simmonds, 1962). Solids consist of; carbohydrates, proteins, lipids, organic acids, phenolic substances, pigments, volatile substances and vitamins.

a) Carbohydrates:

Carbohydrates are the major constituent of banana. The fresh pulp contains 20-25% (w/w) of starch and the optimum concentration is reached four to five weeks before ripening (Barnell and Barnell, 1947). During the final ripening stage the starch level falls extensively due to hydrolysis to free sugars. Only 1-2% of starch remains in the fully ripe fruit whilst at this time it contains 15-20% of simple sugars. There is a loss in sugar during the ripening process which is due to respiration (Loesecke, 1950).

Plantains differ from sweet bananas in having a higher starch content in the pulp. At harvest plantains contain 30% starch in the pulp which falls to 5-10% when ripe. The sugar content of plantain is similar to that of sweet bananas. Poland et al., (1937) found 66% sucrose, 20% glucose and Palmer (1971) found 14% fructose as the major sugar constituents of banana pulp. Banana also contains cellulose and hemi-cellulose which decrease during ripening (Loesecke, 1950). Cutts (personal communication) established a relationship between the average sugar content of the pulp and the colour of banana peel in which the free sugar content increased as the peel turned yellow.

b) Proteins:

The protein content of green banana pulp varies from 0.5 to 1.6% fruit weight and there are no quantitative changes in protein mass during ripening (Brady et al., 1970). Brady et al., (1970) reported the presence of the following amino-acids at different concentrations in the pre-climacteric.

Amino-acid	Micromoles g^{-1} fruit weight
Aspartic acid	2.44
Glutamic acid	1.35
Serine	0.55
Glycine	0.55
Asparagine	3.33
Threonine	0.36
α-Alanine	0.56
Glutamine	4.27
Histidine	6.09
Lysine	1.07
Proline	0.18
Valine	0.11
Leucine	0.20
Isoleucine	0.12
Tyrosine	0.07
Arginine	1.25

Also in the pre-climacteric pulp Buckley (1962) reported that histidine concentration increases at the expense of glutamic and aspartic acid and their amides. Valine and leucine also increase and these two amino-acid substances contribute to precursors of banana flavour.

c) Amines:

Waalkes et al., (1958) reported that banana contains 5-hydroxytryptamine (5-HT), L-noradrenaline and dopamine at the following respective concentrations 28 $\mu g\ g^{-1}$ fruit weight; 2 $\mu g\ g^{-1}$ fruit weight and 8 $\mu g\ g^{-1}$ fruit weight in green banana pulp. Higher concentrations were reported in the peel 56 $\mu g\ g^{-1}$, fruit weight of 5-HT; 122 $\mu g\ g^{-1}$ fruit weight of L-noradrenaline and 700 $\mu g\ g^{-1}$ fruit weight of dopamine.

The 5-HT content of the peel increased sharply during ripening to 161 $\mu g\ g^{-1}$ fruit weight and also an increase in the pulp was found up to 50% of the pre-climacteric value of the pulp.

Griffiths (1961) reported that dopamine in banana has a fundamental role in browning banana during ripening.

d) Lipids:

Lipids constitute between 0.2 and 0.5% of the fresh weight of banana at all stages of ripeness (Grobois and Mazliak, 1964). Goldstein and Wick (1970) examined the lipids of green and ripe banana pulp using a gas chromatography technique and found the following fatty acids in mg 10 g^{-1} of dry weight.

Acid		unripe banana	ripe banana
Palmitic acid	(16:0)	10.89	11.92
Palmitoleic	(16:1)	2.21	0.84
Stearic	(18:0)	0.63	1.68
Oleic	(18:1)	4.44	4.08
Linoleic	(18:2)	12.85	4.88
Linolenic	(18:3)	6.08	6.84

There was a tendency during ripening toward a loss of unsaturated acids (particularly linoleic acid) and an increase in saturated acids.

e) Organic acids:

The pH of banana pulp fell during ripening from 5.4 ± 0.4 in green banana to about 4.5 ± 0.3 in ripe banana (Loesecke, 1950).

The acidity of green banana is due to oxalic, malic and citric acid (Palmer, 1963). Steward et al., (1960) found that shikimic acid, quinic acid, glycolic acid, glyceric acid, pyroglutamic acid, succinic acid and tartaric acid were minor constituents of green banana pulp.

f) Phenolic substances:

Tannins and other phenolic substances (serotonin, noradrenaline, dopamine) have been confirmed to be present in banana (Waalkes et al., 1958). Tannins are responsible for the astringent taste of green banana. The tannins content of banana decreases sharply during ripening by polymerisation. Dopamine is one of the phenolic

compounds which contributes a major role in browning during the ripening of bananas.

g) Pigments:

The change in colour of the peel from green to yellow is an indication of pigment changes in the green banana. The chlorophyll content drops from 50–100 $\mu g\ g^{-1}$ fruit weight to zero, while the xanthophyll increases sharply. Banana also contains carotene (Looney and Patterson, 1967; Brady et al., 1970).

h) Volatile substances:

Banana fruit contains at least 200 individual volatile components which contribute to the flavour and aroma of banana (Wick et al., 1966).

McCarthy and Palmer (1964) found that most of these volatile compounds originate from L-leucine, L-isoleucine and L-valine. The flavour appears during ripening and does not exist in the green state of banana.

i) Vitamins:

Banana contains vitamins and is considered a very good source of vitamins A, C and E with lower amounts of vitamin B1, B2, D and H (Palmer, 1971).

2. MATERIALS

2.1 Materials used in extraction and fractionation procedures

The following materials were used for reporting and identifying substances present in the active fractions of banana powder.

a) Sephadex (G.10):

Sephadex (G.10) was supplied by Pharmacia Fine Chemical Limited. This size range of sephadex (G.10) is suitable for fractionating substances of 700 Dalton molecular weight.

b) Dextran Blue:

($PM 2.10^6$). This polymer was used to determine the void volume of the column.

c) Glucose, fructose and sucrose:

These sugars were supplied by B.D.H. Limited and used as a reference for the T.L.C.

d) Silica gel thin layer chromatography (T.L.C.).

Silica gel plates type 60 f$_{254}$, 0.25 mm thickness were supplied by B.D.H. Limited, and used for the separation and identification of the active fractions of banana.

e) Solvent:

All solvents in this project were supplied by B.D.H. Limited and organic solvents were dried before use. The following reagents were used for detecting the different substances on the T.L.C.

f) Aniline hydrogen phthalate reagent:

This reagent consists of mixing 0.92 ml of aniline, 1.6 g of phthalic acid, 49 ml of butanol, 49 ml of ether and 2 ml of water. The T.L.C plates were sprayed with the reagent and heated at 110°C for 10 min. This reagent detects all reducing sugars as yellow-brown spots.

g) Modified Dragendorff's reagent:

i) Bismuth subnitrate (850 mg) was dissolved in water (10 ml) and glacial acetic acid (10 ml).

ii) Potassium iodide (8 g) was dissolved in water (20 ml)

The solutions (i) and (ii) were mixed and glacial acetic acid (20 ml) and water (100 ml) were added to the mixture (10 ml). The reagent must be used fresh. The T.L.C. plates were sprayed with this reagent and heated at 130°C for 10 min. This reagent is very sensitive to small amounts of alkaloids which show as orange spots.

h) Phosphomolybdic acid reagent:

15% Phosphomolybdic acid in methanol. The plates were heated at 90°C for 5 min after being sprayed with this reagent. This reagent gives blue colours with strongly reducing compounds, polyoxygenated steroids, most terpenoids and fatty acids.

i) Ninhydrin reagent:

0.1% (w.v.$^{-1}$) In acetone. This reagent was used to locate peptides, amino sugars and amino acids. The T.L.C. plates were heated at 100°C for 5 min . The colour of the spots varied from red to violet.

j) Anisaldehyde reagent:

This reagent consists of mixing concentrated H_2SO_4 (1 ml) and acetic acid (100 ml). 0.1 ml of anisaldehyde was added to the above mixture before use. The sprayed plates were heated at 130°C for 10 min., red to brown spots appear on the plates. This is a test for sterols and steroids.

k) Antimony trichloride reagent:

4 g Of antimony trichloride was dissolved in 100 ml of chloroform. The plates were heated at 120°C for 15 min. This reagent locates terpenes with a colour varying from brown to red.

l) 15% Phosphoric acid:

This reagent locates sterols, steroids and terpene compounds but it is not specific. The sprayed T.L.C. plates were heated at 120°C for 15 min and the colour varied from blue to brown and red.

m) Diazotized sulphanilic acid:

It consists of a 0.3% solution of sulphanilic acid in 8% HCl (25 ml) added to 5% Na NO_2 solution (1.5 ml) directly before use. The plates were heated at 200°C for 15 min and were then sprayed with 20% Na_2CO_3. This reagent detects phenolic compounds.

n) Iodine vapour:

The plates were incubated at room temperature in iodine vapour. This vapour reagent is especially for unsaturated compounds. The colour varying from brown to orange.

o) Ultra-Violet (U.V.) light:

The T.L.C. plates were examined by U.V. light at 283 nm. Many, but not all phenols fluorescese and aromatic compounds absorb and appear as dark spots on a blue back ground.

3. METHODS

3.1 Animal models:

Animal weights, conditions of housing, food and banana diets administered were described in Chapter 2. Both the prophylactic and curative models of evaluating anti-ulcerogenic activity were used and they were described in Chapter 2, but they were:

a) Prophylactic method:

The rats were maintained on a normal diet for 48 h under restful conditions. The treatment with the banana extracts was administered orally at three doses at 8 h intervals during the first day of the treatment period. The animals were then fasted for 48 h and then aspirin was administered orally at 150 mg kg^{-1} and the rats killed 5 h later. The stomachs were removed and assessed for ulcerogenic activity.

b) Curative method:

Again the animals were allowed to stabilize on a normal diet for a 48 h fasting period. Aspirin was then administered orally at 150 mg kg^{-1} and treatment with the extracts commenced 5 h later. Treatment was maintained by three oral doses of the extract at 8 h intervals during the treatment period after which the animals

were killed and the stomachs were removed and evaluated for ulceration. In both methods the animals were allowed free access to water over the experimental period.

c) Evaluation of severity of ulceration:

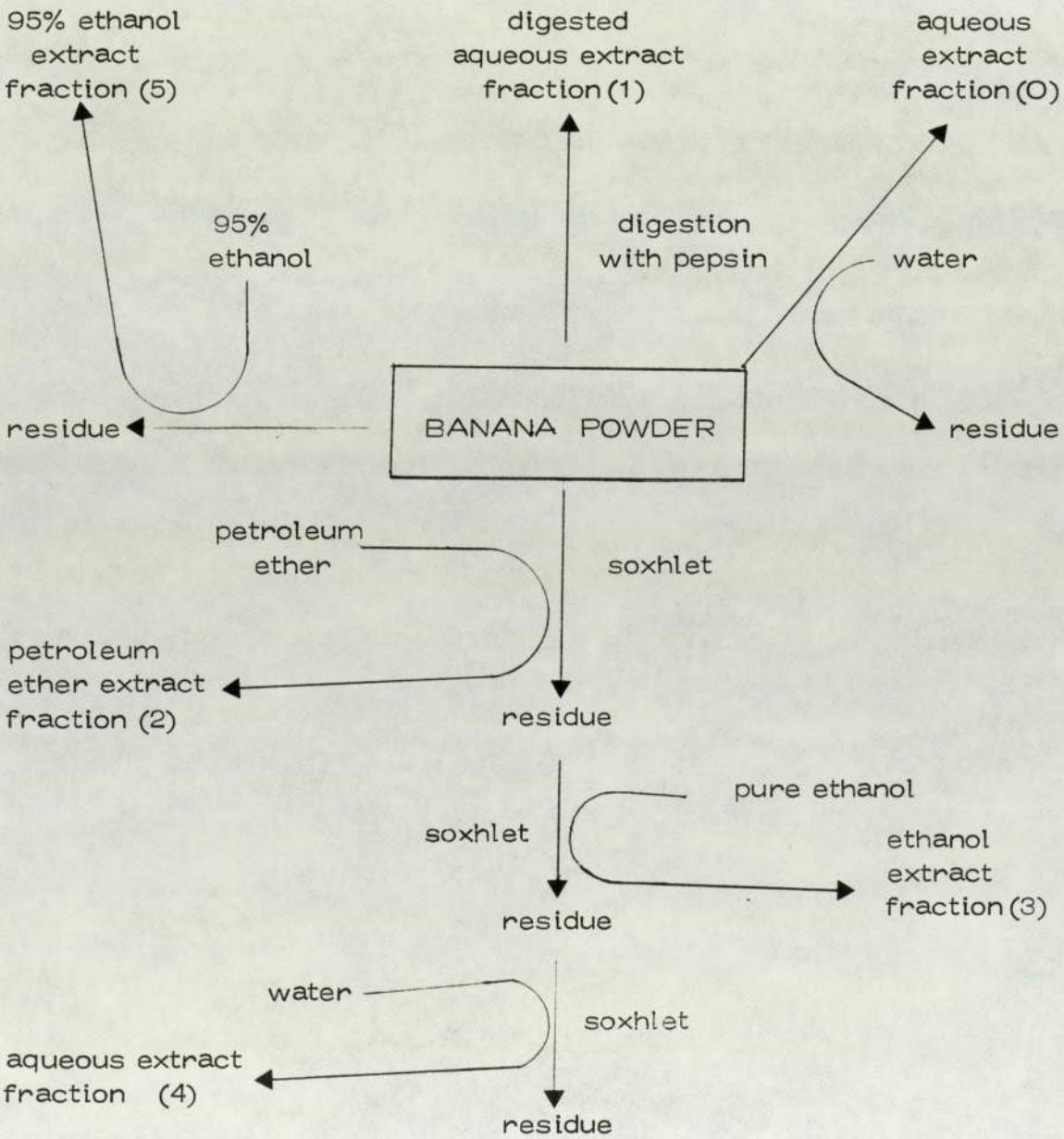
The effect of treatment on the induction of ulcers by aspirin in both the prophylactic and curative methods described as above was evaluated either by:

i) Determination of the ulcer index (Chapter 2, section 3.1. a, b).

ii) Determination of the mucosa weights (Chapter 2, section 3.1.c).

3.2 Plan of banana extraction with solvents.

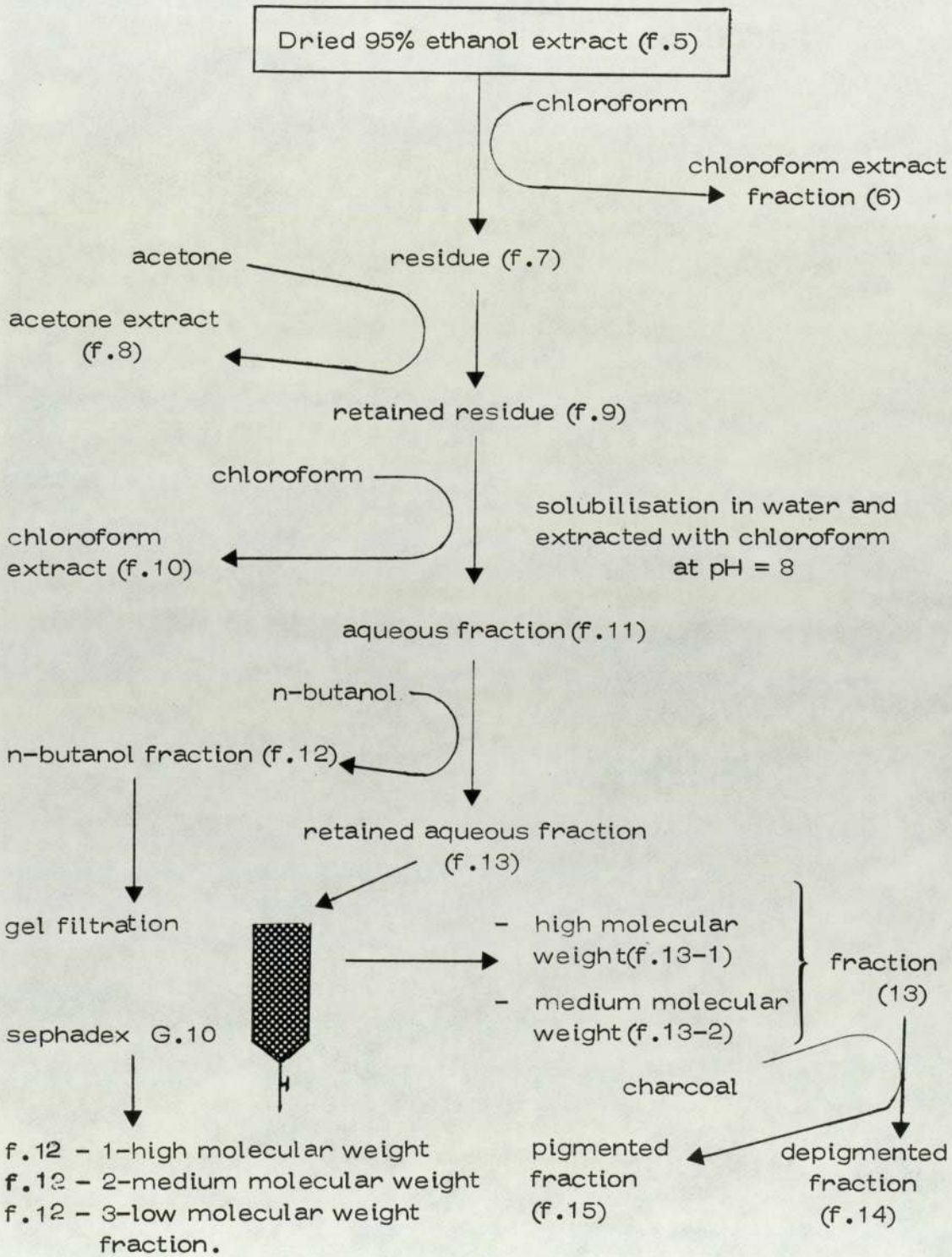
Plan 1: Extraction scheme of banana with different solvents



3.3 Plan of fraction (5) extraction with solvents.

After finding that the 95% ethanol extract was active and represented a purification of the aqueous extract (f.O) the 95% ethanol extract was dried at low temperature and submitted to further fractionation. (Plan 2).

Plan 2: Extraction scheme of the 95% ethanol extract.



3.4 Anti-ulcerogenic test of water soluble compounds and of the retained residue (prophylactic model)

50 g of banana sample (A) (treatment for ten rats) was extracted with 200 ml of H_2O at 20°C for 30 min with continuous stirring. The supernatant was obtained by centrifugation at 10,000 for ten minutes and was freeze-dried and the weight of the residue was determined (f.O). The residue was retained. The aqueous fraction was solubilized in distilled water (0.88 g ml^{-1}). Both of the aqueous fraction and the retained residue were tested against the prophylactic animal model using 40 rats divided into 4 groups of ten.

In the control group the animals received 14 g of normal diet plus 3 oral doses of H_2O at 8 h intervals during the treatment period. The banana treated group received 9 g of normal diet + 5 g of banana sample (A) plus 3 oral doses of H_2O at 8 h intervals during the treatment period. The animals treated with banana aqueous extract received 13.3 g of normal diet plus 3 oral doses of the aqueous extract (4.4 g of banana.Kg^{-1}) during the treatment period .

The final group of animals received banana residue (4.3 g per animal) plus 9.7 g of normal diet with 3 oral doses of H_2O.

3.5 The anti-ulcerogenic activity of pepsin-digested suspension (f.1) of banana powder sample (A)(prophylactic model).

50 g of banana was incubated with stirring in a water bath at 37°C with 125 ml of H_2O and 25 ml of pepsin solution (1% w.v.$^{-1}$). The pH was initially adjusted to 2, but 15 h later the pH was raised to 6 and the supernatant was obtained by centrifugation as described above in section (3.4) and freeze dried (f.1). As a control a solution of pepsin (1% w.v.$^{-1}$) was incubated for 15 h at pH 2 and then the solution adjusted to pH 6 prior to the recovery of the supernatant, as before. The freeze dried fraction was solubilized in distilled water (0.9 g ml^{-1}) and tested against the prophylactic animal model using 20 rats divided into 2 groups.

The same treatment principle as in 3.4 (p.110) was used. One group was given 13.1 g of normal diet with 3 oral doses of digested banana extract. The second group was given 14 g of food and 3 doses of pepsin solution.

On the 7th day when the animals were killed the prophylactic model of evaluating the ulcer index was used to assess the activity.

3.6 The anti-ulcerogenic activity of successive extractions with petroleum ether, ethanol and water using soxhlet extraction. (curative model)

35 g Of banana sample (A) was extracted with 200 ml of petroleum ether (A.R., 60-80^{o}) by soxhlet extraction (25 cm length, 4 cm diameter) at 4^{o}C, 15 h later the percolation was stopped and the petroleum ether fraction was evaporated to dryness under reduced pressure at 35^{o}C (f.2). The insoluble petroleum ether residue was dried in a stream of warm air and then extracted in a soxhlet extractor with 200 ml of ethanol at 74^{o}C for 15 h . The obtained ethanol soluble extract was evaporated to dryness under reduced pressure (f.3) and the insoluble residue was dried and then submitted to water extraction (200 ml) in the soxhlet extractor at 100^{o}C for 15 h . The aqueous fraction(4) was evaporated to dryness under reduced pressure at 80^{o}C. The residue retained after water extraction was dried in a low temperature oven for 24 h at 40^{o}C and the weight of the dried residue was determined. An aqueous solution was made with the ethanol fraction(3) (360 mg ml^{-1}) and a saturated solution (1 g ml^{-1}) of the aqueous fraction (4) was made in H_2O. An aqueous suspension (50 mg ml^{-1}) was made with the petroleum ether fraction using tragacanth powder as a dispersing agent due to the insolubility of the extract in water. The same quantity of tragacanth was added to the

other fractions (viz: fractions 3 and 4). Thirty-five rats were divided into groups of 5 and each group was used to test the activity of one of the previous fractions using the curative model of ulceration as follows:

Treatment	Notes
Aspirin plus 3 oral doses of tragacanth suspension	14 g of normal diet plus 3 oral doses of tragacanth suspension at 8 h intervals. 5 h after aspirin administration.
Aspirin plus 3 oral doses of petroleum ether fraction (2)	14 g of normal diet with 3 oral doses of petroleum ether fraction (equivalent to a total dose of 250 mg kg^{-1}) at 8 h intervals. 5 h after aspirin administration.
Aspirin plus 3 doses of ethanol fraction (3)	13.25 g of normal diet with 3 oral doses of the ethanol fraction (equivalent to a total dose of 1.8 g kg^{-1}) administered as above.
Aspirin plus 3 oral doses of aqueous extract fraction (4)	13 g of normal diet with 3 oral doses of the aqueous fraction (equivalent to a total dose of 5.1 g kg^{-1}) administered as above.

On the seventh day, the animals were killed. The curative method of the ulcer index and the mucosal weights were used to assess the activity of the previous fractions.

3.7 The anti-ulcerogenic activity of successive cold ethanol extraction (f.5), aqueous extraction (f.5́) and the retained residue of banana sample (A). (curative model).

35 g of banana sample (A) was extracted with 200 ml of 95% ethanol by stirring for 8 hours at room temperature. This operation was repeated 4 times with 200, 150, and 100 ml of 95% ethanol. The supernatants were obtained as described in section (3.4). All the ethanol extracts were combined and filtered using filter paper. The filtrate was reduced in volume by evaporation under reduced pressure and then freezed dried (f.5). The retained residue was extracted with 200 ml of H_2O with stirring over 3 h . This operation was repeated twice. The supernatants were combined and after filtering as described above they were freeze dried (f.5́). The residue was retained and dried in a low temperature oven at 40°C for 24 hours. The weights of all fractions were determined. An aqueous solution (240 mg ml^{-1}) was made with the ethanol fraction (5) and a strong aqueous solution (660 mg ml^{-1}) was made with the aqueous fraction (5́). The ethanol, the aqueous and the residual fractions were all tested biologically against the curative model of ulceration using 28 rats divided into four groups as follows:

Treatment	Notes
Aspirin with 3 oral doses of H_2O	14 g of normal diet with 3 oral doses of H_2O (equivalent to a total dose of 5 ml kg^{-1}) at 8 h intervals.
Aspirin with 3 oral doses of ethanol fraction (5)	14 g of normal diet with 3 oral doses of the ethanol fraction (5) (equivalent to a total dose of 1.2 g kg^{-1}) administered as above.
Aspirin with 3 oral doses of aqueous fraction (f.5)	13.5 g of normal diet with 3 oral doses of the aqueous fraction (equivalent to a total dose of 3.3g kg^{-1}) administered as above.
Aspirin with 3 oral doses of H_2O plus retained residue	9.75 g of normal diet with 4.25 g of retained residue plus 3 oral doses of H_2O administered as above.

The curative model of evaluating ulceration and the mucosa weights were used to assess the healing.

a) The identification of the nature of the substances in the 95% ethanol extract (f.5) by thin layer chromatography (T.L.C.).

A solution of the 95% ethanol fraction (10 mg ml^{-1}) was prepared in distilled water and 5 µl samples were spotted on T.L.C. plates (silica gel, type 60, 5 x 20 cm). One unique mobile phase was used to develop the T.L.C. plates which consisted of chloroform, methanol, acetic acid and water: 45:15:2:3 v/v. The following detecting systems were used to identify the substances present in the 95% ethanol fraction (5) system.

System 1 : Aniline hydrogen phthalate

System 2 : Dragendorff's reagent

System 3 : Phosphomolybdic acid

System 4 : Ninhydrin

System 5 : Anisaldehyde

System 6 : Antimony trichloride

System 7 : 15% phosphoric acid

System 8 : Diazotized sulphanilic acid

System 9 : Iodine vapour

System 10 : Ultra-violet (U.V.) light at 283 nm.

b) Determination of 5-hydroxytryptamine (5-HT) content of fraction (5) of banana samples (A) and (F).

The 95% ethanol fraction (5) was prepared as described in section 3.7, from sample A and F., and solubilized in 1% (v/v) formic acid. The concentration of 5-HT content in fraction (5) was determined by high pressure liquid chromatography as follows:

Column A : Spherisorb column was used 5 µm ODS, 10 x 0.46 cm i.d.

Mobile phase : 1% (v/v) formic acid in water, delivered at 1 ml min^{-1}.

Detection : The detection was achieved by fluorescence with excitation at 294 nm and emission at 313 nm.

Injection : 20 µl, via a rheodyne 7120 valve.

Solvents : All standard solutions were prepared in the mobile phase.

Retention time : The retention time is in the order of 5 minutes.

Standard solutions : 2 µg of 5-HT was solubilized in 1 ml of the mobile phase.

3.8 Activity of the chloroform extract (f.6) and the residue retained (f.7) following extraction with 95% ethanol (f.5) (Plan 2).(curative model)

The dried fraction (5) (equivalent to 35 g of banana sample A) was extracted with 2 x 25 ml of distilled chloroform by stirring for 3 h. The chloroform fraction (6) was evaporated to dryness at 35°C under reduced pressure. The weight of the chloroform fraction was determined, and an aqueous suspension (66 mg ml^{-1}) was made out of this fraction using tragacanth powder.

The retained residue after chloroform extraction of the 95%, ethanol extract was solubilized in water and then freeze-dried to eliminate any trace of chloroform. The freeze dried fraction (7) was then solubilized in distilled water (170 mg ml^{-1}) and the same quantity of tragacanth powder which was used for the suspension of the chloroform fraction was added to fraction (7).

Both of fractions (6) and (7) were tested against the curative model of ulceration, using 28 rats as follows:

Treatment	Notes
Aspirin plus 3 oral doses of tragacanth suspension.	14 g of normal diet plus 3 oral doses of tragacanth suspension.
Aspirin plus 5 g of banana (A) plus 3 oral doses of tragacanth suspension.	9 g of normal diet plus 5 g of banana sample (A) plus 3 oral doses of tragacanth suspension (equivalent to a total dose of 5 ml kg^{-1}).
Aspirin plus 3 oral doses of chloroform fraction (6)	14 g of normal diet plus 3 oral doses of the chloroform fraction (6). (equivalent to a total dose of 330 mg kg^{-1}).
Aspirin plus 3 oral doses of retained residue fraction (7)	14 g of normal diet plus 3 oral doses of the retained residue fraction (7). (equivalent to a total dose of 850 mg kg^{-1}).

On the seventh day, the animals were killed and the curative model of ulceration and the ulcer index were used to assess the activity of fraction (6) and (7).

3.9 Anti-ulcerogenic test on the acetone extact (f.8) and the residue retained (f.9) after the extraction of fraction(7) (curative model).

Fraction (7) was obtained from 49 g of banana sample (B) using the method described in section (3.8). Each rat in the experiment was given either extract or residue equivalent to 7 g of original banana sample (B).

The freeze-dried fraction (7) was extracted twice by stirring with 25 ml of distilled acetone for 3 h at room temperature. The acetone fraction was filtered using filter paper and evaporated to dryness at 40°C under reduced pressure (f.8). The weight of the fraction was determined and a suspension (62.5 mg. ml^{-1}) was made using tragacanth powder . The retained residue was solubilized in water and freeze-dried (f.9) and then solubilized in distilled water (92.3 mg ml^{-1}).

Both of the fractions (8) and (9) were tested using the curative model of ulceration by using 28 rats divided into 4 groups, each of 7 rats as follows:

Treatment	Notes
Aspirin	16 g of normal diet during the treatment period.
Aspirin with banana (B)	9 g of normal diet with 7 g of banana during the treatment period.
Aspirin with fraction (8)	16 g of normal diet plus 3 oral doses of fraction (8). (equivalent to a total dose of 312 mg kg^{-1}), at 8 h intervals, 5 h after aspirin administered.
Aspirin with fraction (9)	Aspirin treatment plus 16 g of normal diet plus 3 oral doses of fraction (9). (equivalent to a total dose of 461 mg kg^{-1}) administered as above.

The curative method of scoring ulceration was used to evaluate the activity of fractions (8) and (9).

3.10 Activity of the chloroform fraction at pH 8 (f.10) and of the retained aqueous fraction (f.11) after the extraction of fraction (9) (curative model).

Fraction (9) was obtained from 49 g of banana sample (C) (7 g per rat), by the method mentioned in section (3.9).

Fraction (9) was solubilized in 30 ml of distilled water and the pH was adjusted to 8 by 1M , Na OH solution. This fraction was poured into a 100 ml separating funnel containing 15 ml of chloroform. The mixture was shaken vigorously and allowed to separate into two layers. The chloroform phase was separated from the aqueous one, and the aqueous fraction was re-extracted with the same volume of chloroform. The chloroform fractions were combined and evaporated to dryness at 35°C (f.10). The weight of the residue was determined and then solubilized in distilled water (87.6 mg ml^{-1}). The alkaline aqueous fraction was neutralized with HCl, freeze-dried (f.11) and a strong aqueous solution (152 mg ml^{-1}) was made with fraction (11).

Both of the fractions (10) and (11) were tested against the curative model of ulceration using 28 rats divided into 4 groups each of 7 animals as follows:

Treatment	Notes
Aspirin	16 g of normal diet during the treatment period 5 h after aspirin administration.
Aspirin with banana (C)	9 g of normal diet with 7 g of banana sample (C) during the treatment period as above.
Aspirin with fraction (10)	16 g of normal diet plus 3 oral doses of fraction (10). (equivalent to a total dose of 438 mg kg^{-1}) during the treatment period as above.
Aspirin with fraction (11)	16 g of normal diet plus 3 oral doses of fraction (11). (equivalent to a total dose of 760 mg kg^{-1}) during the treatment period administered as above.

The curative method of scoring ulcers and the mucosa weights were used to assess the activity of fractions (10) and (11).

a) Thin layer chromatography of fraction (11)

(retained aqueous fraction).

An aqueous solution of fraction (11) (10 mg ml^{-1}) was made up. Silica gel T.L.C. (type 60, 5 x 20 cm) plates were used and 5 µl of fraction (11) was spotted on to the plates. The chromatograms were developed in chloroform: Methanol: acetic acid: water: 45:15:2:3 v/v and the substances present were detected using the following reagents.

System 11 : Aniline hydrogen phthalate

System 12 : Ninhydrin reagent

System 13 : Iodine vapour

System 14 : Antimony trichloride reagent

System 15 : 15% phosphoric acid reagent

System 16 : Dragendorff's reagent

System 17 : U.V. light

System 18 : Anisaldehyde

System 19 : Phosphomolybdic acid reagent

3.11 Fractionation of the butanol extract (f.12) and the retained aqueous fraction (f.13).

50 g Of Sephadex G.10 was suspended in phosphate buffer pH (7.4) for 48 h with 100 mg of azide salt (bactericide). The Sephadex was packed into a column (30 cm length, 2.5 cm diameter), 49 g of banana sample (E) were extracted according to plan (2) (see section 3.3) to obtain fraction (11). Fraction (11) was solubilized in 20 ml of distilled water and added to a separating funnel with 40 ml of n-butanol. The mixture was shaken vigorously and on settling the aqueous phase was separated and re-extracted with butanol. The butanol fractions were combined and evaporated to dryness at 50°C under reduced pressure (f.12). The weight of the butanol fraction (12) was determined and the fraction was solubilized in 2 ml of distilled water. The residual aqueous fraction was evaporated to dryness at 45°C under reduce pressure (f.13) and was solubilized in 2 ml of distilled water.

The Sephadex column was washed with 500 ml of distilled water to remove all azide salts and then 2 ml of the butanol fraction (12) were added to the top of the gel, and the elution was carried out with sterile water. The eluant from the column was monitored at 280 nm and collected in 10 ml fractions, using L.K.B. equipment. The aqueous fraction (13) was also fractionated in a similar way to

the butanol extract; 30 tubes were obtained from each fractionation; 10 mg of dextran blue was solubilized in 2 ml of H_2O and eluted to determine the void volume of the column; 100 mg of phenylalanine was solubilized in 2 ml of H_2O and eluted through the column to compare the molecular weight of the constituents of the fractionation with that of phenylalanine. The eluted fractions were transfered to vials and reduced in volume to 1 ml by evaporation followed by freeze drying.

Thin layer chromatography was used to examine the residues in tube numbers: 5, 8, 9, 11, 12, 14, 15, 18, 21, 24 and 30 of the fractionated butanol and also the fractionated aqueous extract using silica gel type 60, 5 x 20 cm with the same solvent as before. The T.L.C. plates were treated with the following detecting agents.

- Aniline hydrogen phthalate (for redusing sugars).
- Ninhydrin reagent (for amines, amino acids and amino sugar).
- Phosphomolybdic acid (for lipides, steroids, hydroxy acids and phenols)
- Dragendorff's reagent (for alkaloids and terpenes).
- U.V. detection (for aromatics compounds and polyphenols)
- Antimony reagent (for vitamine A, steroids and glycosides).
- 15/ phosphoric acid (for charring agent for organics unsaturation)
- Iodine vapour(for all organic compounds).

By using the U.V. absorption values to locate the eluated molecular weight markers (phenylalanine and dextran blue), the fractions of the butanol

extract were classified as:

Tube 1 to 11: High molecular weight fraction

Tube 12 to 15: Medium molecular weight fraction

Tube 16 to 30: Low molecular weight fraction.

The fractions of the retained aqueous extract (f.13) were classified as follows:

Tube 11 to 16: high molecular weight fraction

Tube 17 to 30: medium molecular weight fraction.

3.12 Activity of the fractionated butanol extract (f.12-1, 2 and 3) and the retained aqueous fraction (f.13) compared with the activity of banana sample (E)(curative model).

The two aqueous fractions were combined together, freeze-dried and then solubilized in distilled water (29.9 mg ml^{-1}). An aqueous solution was made with the butanol high molecular weight fraction (12.1 mg ml^{-1}), the butanol medium molecular weight fraction (4.66 mg ml^{-1}) and the butanol low molecular weight fraction (8.4 mg ml^{-1}).

All the four fractions (e.g. 3 for the butanol fractions and one for the aqueous) were tested against the curative model of ulceration using 42 rats divided into 7 groups, each of 7, as follows:

Treatment	Notes
Aspirin	16 g of normal diet.
Aspirin with banana (E)	9 g of normal diet with 7 g of banana sample (E).
Aspirin with butanol high molecular weight fraction (12-1)	3 oral doses of butanol high molecular weight fraction (12-1). (equivalent to a total dose of 60.5 mg kg^{-1}) at 8 h intervals, plus 16 g of normal diet.
Aspirin with butanol medium molecular weight fraction (12-2)	Aspirin as above plus 3 oral doses of butanol medium molecular weight fraction (12-2). (equivalent to a total dose of 23.3 mg kg^{-1}) administered as above plus 16 g of normal diet.
Aspirin with butanol low molecular weight fraction (12-3)	Aspirin as above plus 3 oral doses of butanol, low molecular weight fraction (12-3). (equivalent to a total dose of 42 mg kg^{-1}) administered as above plus 16 g of normal diet.

Treatment (continued)	Notes (continued)
Aspirin with aqueous fraction (13)	3 oral doses of the aqueous fraction (13). (equivalent to a total dose of 149 mg kg^{-1}) administered as above. plus 16 g of normal diet.

The curative model of evaluating ulceration and the mucosa weights were used to assess healing from ulceration.

3.13 Anti-ulcerogenic test on the pigmented fraction (15) and the depigmented fraction (14) by charcoal treatment of fraction (13) (curative model)

Fraction (13) was obtained from 49 g of banana sample (E) following plan no. 2 (see section 3.3). Fraction (13) was solubilized in 20 ml of distilled water and 100 mg of activated charcoal was added. The mixture was shaken vigorously and then filtered through filter paper (Whatman no. 1). The filtrate was freeze-dried (f.14) and then solubilized in distilled water (13 mg ml^{-1}). The charcoal was washed with 50 ml of hot ethanol (50°C) and the filtrate was evaporated to dryness at 40°C under reduce pressure (f.15) and finally solubilized in distilled water, (17.7 mg ml^{-1}). Both the pigmented and depigmented fractions were tested against the curative model of ulceration by using 28 rats divided into four groups each of 7 rats as follows:

Treatment	Notes
Aspirin	3 oral doses of H_2O (equivalent to a total dose of 5 ml kg^{-1}) plus 16 g of normal diet.
Aspirin with fraction (13)	3 oral doses of the aqueous fraction (13). (equivalent to a total dose of 149 mg kg^{-1}) plus 16 g of normal diet.
Aspirin with fraction (14)	Aspirin as above plus 3 oral doses of the depigmented fraction (14). (equivalent to a total dose of 65.0 mg kg^{-1}) plus 16 g of normal diet.
Aspirin with fraction (15)	Aspirin as above plus 3 oral doses of the pigmented fraction (15). (equivalent to a total dose of 88.6 mg kg^{-1}).

The curative model of evaluating ulceration and mucosa weights were used to assess the activity of the previous fractions.

a) Thin layer chromatography of fraction (14)(depigmented fraction) and fraction (15) (pigmented fraction).

Aqueous solutions (15 mg ml^{-1}) of fraction (14) and fraction (15) were prepared and silica gel plates (5 x 20 cm) were spotted with 5 µl of the above solutions. The plates were developed in chloroform, ethanol, acetic acid, water.45:15:2:3 (v/v) and the T.L.C. plates were then detected with:

1 - Ninhydrin

2 - Iodine vapour

4. RESULTS

4.1 The anti-ulcerogenic activity of the aqueous fraction (0) and the retained residue of banana sample (A).

The percentage of the freeze-dried residue from the aqueous fraction of banana sample (A) was 13.2% . The ulcer index Table(9) showed that both of the banana sample (A) and the aqueous fraction of the same sample (f.0) were active in protecting animals against aspirin-induced ulceration. However not all the activity of the whole banana appeared in the aqueous extract as banana(A) had a significantly greater effect than the equivalent aqueous extract. Although the effect of the residue was not significant ($p>0.05$) it appears to have retained some of the activity . This may have been due to the retention of some water during the extraction process.

4.2 The effect of pepsin digestion on the active ingredient in banana banana sample (A).

The percentage of the aqueous banana fraction(1) resulting from pepsin digestion of banana sample (A) suspension was 14.3% The ulcer index Table (10) showed that the digestion of protein in the aqueous banana (A)suspension (f.1) did not abolish the anti-ulcerogenic activity of the aqueous fraction compared to the activity of banana sample (A). The active ingredient was therefore not a protein.

Table 9. The prophylactic anti-ulcerogenic activity of the aqueous fraction (O) and the retained residue of banana sample (A).

This Table(opposite) shows that the active factor was extracted by water. However compared with the control the activity was less in the extract. No significant activity was left in the residue although the residue would have retained some of the water used in the extraction (i.e. like a sponge) which might account for the extract value being less than for original banana sample (A).

Table 10. The prophylactic effect of pepsin digestion on the active ingredient in banana sample (A)

The results in Table 10 (opposite) show clearly that pepsin treatment did not abolish anti-ulcerogenic activity. This suggests that the factor is probably not a protein and is resistant to peptic-HCl activity in the stomach.

Table 9. The prophylactic anti-ulcerogenic activity of the aqueous fraction (O) and the retained residue of banana sample (A).

Treatment	ulcer index
Aspirin treatment	17.7 ± 2.16
Banana (A) treatment, plus aspirin	6.96 ± 1.66****
Aqueous extract (f.O) treatment, plus aspirin	11.18 ± 1.94***
Retained residue treatment, plus aspirin	12.24 ± 2.36

**** $p<0.001$; *** $p<0.01$ calculated by Wilcoxon rank sum test.

Table 10. The prophylactic effect of pepsin digestion on the active ingredient in banana sample (A).

Treatment	ulcer index
Aspirin treatment	17.18 ± 1.84
Banana (A) plus aspirin treatment	7.28 ± 1.84****
Digested banana aqueous extract (f.1) plus aspirin treatment	11.3 ± 2.1 *

**** $p<0.001$; * $p<0.05$ significances were calculated by Wilcoxon rank sum test.

4.3 The anti-ulcerogenic activity of successive extractions with petroleum ether, ethanol and water using soxhlet extraction.

The percentage of solids obtained by successive extraction with petroleum ether, ethanol and water of banana sample (A) using a soxhlet was respectively 0.7%, 5.4% and 15.4%. The ulcer index and the mucosa weights Table (11) showed that the petroleum ether fraction (2) which was extracted and evaporated at low temperatures (40°C) was negative in term of anti-ulcerogenic properties. The extraction with ethanol and water at 74°C and 100°C respectively for 15 h of the retained residue after petroleum ether extraction has destroyed the anti-ulcerogenic activity present in the cold aqueous extract of banana sample (A) (see Table 11).

4.4 Activity of the cold 95% ethanol extract (f.5), the aqueous extract (f.5́) and the retained residue of banana sample (A) after successive extraction.

The percentage of the 95% ethanol extract and the aqueous one of banana sample (A) were respectively 3.6% and 10%.

The ulcer index and the mucosa weights Table (12) showed that the anti-ulcerogenic activity of banana sample (A) was completely extracted by 95% ethanol. The subsequent aqueous extract (f.5́) of the residue retained after the 95% ethanol extraction and the residue

retained after the aqueous extraction were completely inactive in healing animals from ulceration. Therefore the anti-ulcerogenic activity was present in 3.6 g of the ethanol extract (f.5) out of 100 g of banana sample (A).

Table 11. The prophylactic anti-ulcerogenic activity of successive extraction with petroleum ether, ethanol and water using soxhlet extraction(sample A).

Treatment	ulcer index	mucosa weight in mg
Aspirin treatment	17.35 ± 1.42	56.48 ± 2.54
Aspirin treatment plus petroleum ether extract (f.2) of banana (A)	15.54 ± 1.59	58.78 ± 2.73
Aspirin treatment plus ethanol extract (f.3)	13.92 ± 1.78	61 ± 3.86
Aspirin treatment plus aqueous extract (f.4).	15.2 ± 1.49	58 ± 3.34

All values in this Table were not significant showing that the active factor was not extracted by pretroleum ether from banana, however in other experiments water extraction at room temperature did extract the active factor. Prolonged heating (e.g. by soxhlet extration) inactivated the factor.

Table 12. The curative activity of the cold 95% ethanol extract (f.5), the aqueous extract (f.5) and the retained residue of banana sample (A) after successive extraction.

Treatment	ulcer index "t"$_1$	mucosa weights "t"$_2$ in mg
Aspirin treatment	16.45 ± 1.3	68.1 ± 3.73
Aspirin treatment plus 95% ethanol extract (f.5)	10.34 ± 1.08***	78.38 ± 1.85**
Aspirin treatment plus aqueous extract (f.5)	18.38 ± 0.68	65.36 ± 3.05
Aspirin treatment plus retained residue of banana sample (A)	17.1 ± 1.65	68.4 ± 3.54

This table shows that at room temperature aqueous alcohol completely extracted the active factor. *** $p<0.01$; ** $p<0.02$;

t_1 calculated by Wilcoxon rank sum test; t_2 calculated by Student's 't' test.

a) T.L.C. Examination of the 95% ethanol extract (f.5)(sample A)

System 1: Aniline hydrogen phthalate (Reagent for sugars)

Spot	R_f value	Colour	Density
1	0.05	dark brown	strong
2	0.16	faint brown	strong
3	0.18	dark brown	strong
4 fructose	0.16	faint brown	strong
5 glucose	0.18	dark brown	strong

System 2: Dragendorff's reagent: the following substances were detected by Dragendorff's reagent with their appropriate R_f values: (alkaloids)

Spot	R_f value	Colour	Density
1	0.47	orange	strong
2	0.69	grey	medium
3	0.71	orange	strong
4	0.82	grey	medium

System 3: Phosphomolybdic acid reagent (for terpenes and steroids)

Spot	R_f value	Colour	Density
1	0.12	blue	weak
2	0.25	blue	weak
3	0.3	blue	weak
4	0.42	dark blue	strong
5	0.44	blue	medium
6	0.54	blue	medium
7	0.73	dark blue-black	strong
8	0.78	blue	medium
9	0.82	blue	medium
10	0.86	blue	medium
11	0.94	dark blue	strong
12	0.96	dark blue	strong
13	0.98	dark blue	strong

System 4: Ninhydrin reagent: the following substances were detected by ninhydrin: (peptides and amino-acids)

Spot	R_f value	Colour	Density
1	0.02	dark red	strong
2	0.07	dark red	strong
3	0.14	red-orange	weak
4	0.15	pink	medium
5	0.22	dark red	medium
6	0.27	dark red	weak

System 5: Anisaldehyde detection: (for sterols and steroids)

Spot	R_f value	Colour	Density
1	0.13	brown	weak
2	0.24	dark grey	medium
3	0.31	grey	medium
4	0.57	grey	medium
5	0.76	grey	strong
6	0.90	brown	strong

System 6: Antimony trichloride reagent: (for terpenes)

Spot	R_f value	Colour	Density
1	0.79	red	medium
2	0.92	brown	weak
3	0.94	brown	medium
4	0.98	brown-violet	strong
5	0.99	brown	weak

System 7: 15% phosphoric acid and heat: (for steroids, sterols and terpenes)

Spot	R_f value	Colour	Density
1	0	brown	weak
2	0.24	brown	weak
3	0.5	brown	medium
4	0.72	red	medium
5	0.87	blue	strong
6	0.9	violet	strong
7	0.93	blue	medium
8	0.96	violet	medium
9	0.98	brown	medium

System 8: Diazotized sulphanilic acid reagent: (for phenolic compounds)

Diazotized sulphanilic acid reagent gave negative results on the T.L.C. plates.

System 9: Iodine vapour: (for unsaturated compounds)

Spot	R_f value	Colour	Density
1	0.29	orange	medium
2	0.46	orange	strong
3	0.64	orange	weak
4	0.76	orange	medium
5	0.83	orange	strong
6	0.85	orange	strong
7	0.92	orange	strong
8	0.96	orange	strong
9	0.98	orange	strong

System 10: Ultra-violet detection at 283 nm.: (for aromatic compound)

Spot	R_f value	Colour	Density
1	0	yellow	medium
2	0.49	violet	strong
3	0.57	white	strong
4	0.73	yellow-white	strong
5	0.90	yellow	medium
6	0.96	yellow	strong
7	0.98	yellow	medium

b) 5-Hydroxytryptamine (5-HT) content of fraction (5) of banana sample (A) and (F).

The 5-HT content of fraction (5) was the same in both banana samples (A) and (F) and was at a level 1 $\mu g\ g^{-1}$ of dried banana powder.

4.5 Activity of the chloroform extract (f.6) and the residue retained (f.7).

The percentages of the chloroform extract (f.6) and the residue retained (f.7) of the 95% ethanol extract (f.5) were 1% and 2.55% respectively.

The ulcer index and the mucosa weights Table (13) showed that the anti-ulcerogenic activity present in fraction (5) (95% ethanol fraction) cannot be extracted by chloroform. Therefore only the residue retained (f.7) after chloroform extraction possessed anti-ulcerogenic activity.

4.6 The anti-ulcerogenic activity of the acetone extract (f.8) and the residue retained (f.9) of banana sample (B).

The percentage of the acetone extract (f.8) and the residue retained (f.9) were respectively 0.67% and 0.99%. The ulcer index Table (14) showed that acetone extraction of the dried residue (f.7) did not extract the anti-ulcerogenic activity and the activity was retained in the residue (f.9). The residue (f.9) was significantly active in healing ulceration when compared to the activity of banana sample (B) where fraction (9) was obtained .

Table 13. The curative activity of the chloroform (f.6) extract and the residue retained (f.7)(sample A).

Treatment	ulcer index "t"$_1$	mucosa weights "t"$_2$ in mg
Aspirin treatment	15.5 $\pm$ 0.83	56.38 $\pm$ 2
Aspirin treatment plus banana (A)	6.96 $\pm$ 0.58****	68.00 $\pm$ 1.79 ****
Aspirin treatment plus the residue retained (f.7)	8.03 $\pm$ 1.38***	65.05 $\pm$ 1.21 ****
Aspirin treatment plus chloroform extract (f.6)	14.47 $\pm$ 0.7	59.31 $\pm$ 1.77

This table shows that chloroform did not extract the active factors

**** $p<0.001$; *** $p<0.01$; t_1 : significance calculated by Wilcoxon rank sum test; t_2 : calculated by Student's t test.

Table 14. The curative anti-ulcerogenic activity of the acetone extract (f.8) and the residue retained (f.9)(sample B).

Treatment	ulcer index
Aspirin treatment	15.86 $\pm$ 2.12
Aspirin treatment plus 7g of banana (B)	9.46 $\pm$ 1.36***
Aspirin treatment plus the residue retained (f.9)	9.28 $\pm$ 1.37***
Aspirin treatment plus acetone extract (f.8)	13.65 $\pm$ 2

This table shows that acetone did not extract the active factors.

*** $p<0.01$ calculated by Wilcoxon rank sum test.

4.7 Activity of the chloroform extract at pH 8 (f.10) and the retained aqueous fraction (11)(sample c)

The percentage of the chloroform extract at pH 8 (f.10) and the retained aqueous fraction (11) were 0.94% and 1.63% respectively when these extracts were obtained from banana sample (C).

Extraction with chloroform of fraction (8) at pH 8 extracted the weak basic salts. The ulcer index and the mucosa weights Table (15) showed that the basic salts (f.10) did not have any effect in healing ulceration. However the retained aqueous fraction (11) healed ulceration induced by aspirin compared to the activity of banana sample (C) which also healed ulceration.

a) T.L.C. Examination of fraction (11):

System 11: Aniline hydrogen phthalate: The substances obtained with this system were identical to those obtained with system 1 (p.137)

System 12: Ninhydrin reagent: The same substances obtained in system 4 (p.139)

System 13: Iodine vapour: only one orange spot was found with a Rf value of 0.75.

System 14: Antimony trichloride: the same spot which was detected with iodine vapour gave a positive result with antimony trichloride. This spot was red.

Table 15. The curative activity of the chloroform extract at pH (f.10) and the retained aqueous fraction (11) (sample c)

Treatment	ulcer index "t"$_1$	mucosa weights "t"$_2$ in mg
Aspirin treatment	15.83 ± 0.77	61.73 ± 2.63
Aspirin treatment plus 7 g of banana sample (C)	8.13 ± 1.7****	72.28 ± 1.99****
Aspirin treatment plus chloroform extract (f.10)	13.16 ± 1.82	64.81 ± 1.79
Aspirin treatment plus retained aqueous fraction (11)	9.16 ± 1.74***	69.7 ± 1.64**

In this table the insolubility of the factor in chloroform was confimed. However the activity was retained in the aqueous fraction. Therefore chloroform was useful in removing basic, inactive, unwanted material from the aqueous extract.

**** $p<0.001$; *** $p<0.01$; ** $p<0.02$;

t_1 : significance calculated by Wilcoxon rank sum test.

t_2 : significance calculated by Student's t test.

System 15: 15% Phosphoric acid: the same spot which was detected with iodine in system 13, and antimony trichloride in system 14 gave a red colour with 15% phosphoric acid.

Fraction (11) gave a negative result with the following reagents:

System 16: Dragendorff's reagent

System 17: U.V. detection

System 18: Anisaldehyde reagent

System 19: Phosphomolybdic acid reagent.

4.8 T.L.C. Examination of the butanol extract (f.12-1, 2 and 3) fractionation and the retained aqueous fraction (13) (sample E).

The use of U.V. detection with the L.K.B. fractionating system showed that the void volume of the column (30 cm length and 2.5 cm diameter) was between 55 and 75 ml of the eluant. Phenylalanine which absorbs in U.V. (see figure 9, p.149)appeared between 195 and 207 ml of the eluant.

The weights of the fractionated butanol fraction and the aqueous ones are given in Table (16).

a) Butanol extract fractionation:

The butanol extract fractionation was classified to:

i) - High molecular weight fraction

ii) - Medium molecular weight fraction

iii) - Low molecular weight fraction.

Table 16. The amount of material extracted by solvants from banana powder and expressed as: $\frac{\text{dry weight of fraction}}{\text{dry weight of banana powder}} \times 100$

The extraction was carried out according to the extraction scheme(p.109)

Fraction	sample (A)	sample (B)	sample (C)	sample (E)
-95% ethanol extract (f.5)	3.6	2.9	3.6	2.08
-chloroform extract (f.6)	1	1.1	0.95	0.56
-insoluble residue in chloroform (f.7)	2.55	1.66	2.58	1.58
-acetone extract (f.8)	0.45	0.67	0.48	0.23
-insoluble residue in acetone (f.9)	2.13	0.99	2.09	1.19
-chloroform extract at pH 8. (f.10)			0.94	0.39
-retained aqueous fraction (11)			1.63	0.78
-butanol high molecular weight fraction (12-1)				0.13
-butanol medium molecular weight fraction (12-2)				0.05
-butanol low molecular weight fraction (12-3)				0.09
-aqueous high molecular weight fraction (13-1)				0.17
-aqueous medium molecular weight fraction (13-2)				0.19
-depigmented aqueous fraction (14)				0.14
-charcoal adsorbed fraction (15)				0.19

i) Butanol high molecular weight fraction

The fractionation of the butanol extract is represented in Figure 9. This fraction correspond to eluant from 50 ml to 110 ml which have a brown colour and absorb in U.V. The detection of the volumes between 51-70, 81-90 and 101-110 gave negative results with the reagents below:

- Ninhydrin
- 15% phosphoric acid reagent
- Antimony trichloride reagent
- Aniline hydrogen phthalate

However, the detection of the above fractions of the high molecular weight with iodine vapour and U.V. gave positive results for streaks present on the T.L.C. plate. Presumably this fraction corresponds to the pigmented components of banana.

ii) Butanol medium molecular weight fraction.

This corresponds to eluant volumes between 111 and 150 ml absorbing in U.V. region and they were brown in colouration. The fractions consisting of the volumes between 111-130 ml, 131-140 ml and 141-150 ml with the reagents below gave only one spot with R_f value = 0.74

- 15% phosphoric acid	Red
- Antimony trichloride	Red
- Iodine	Orange
- U.V. light	White

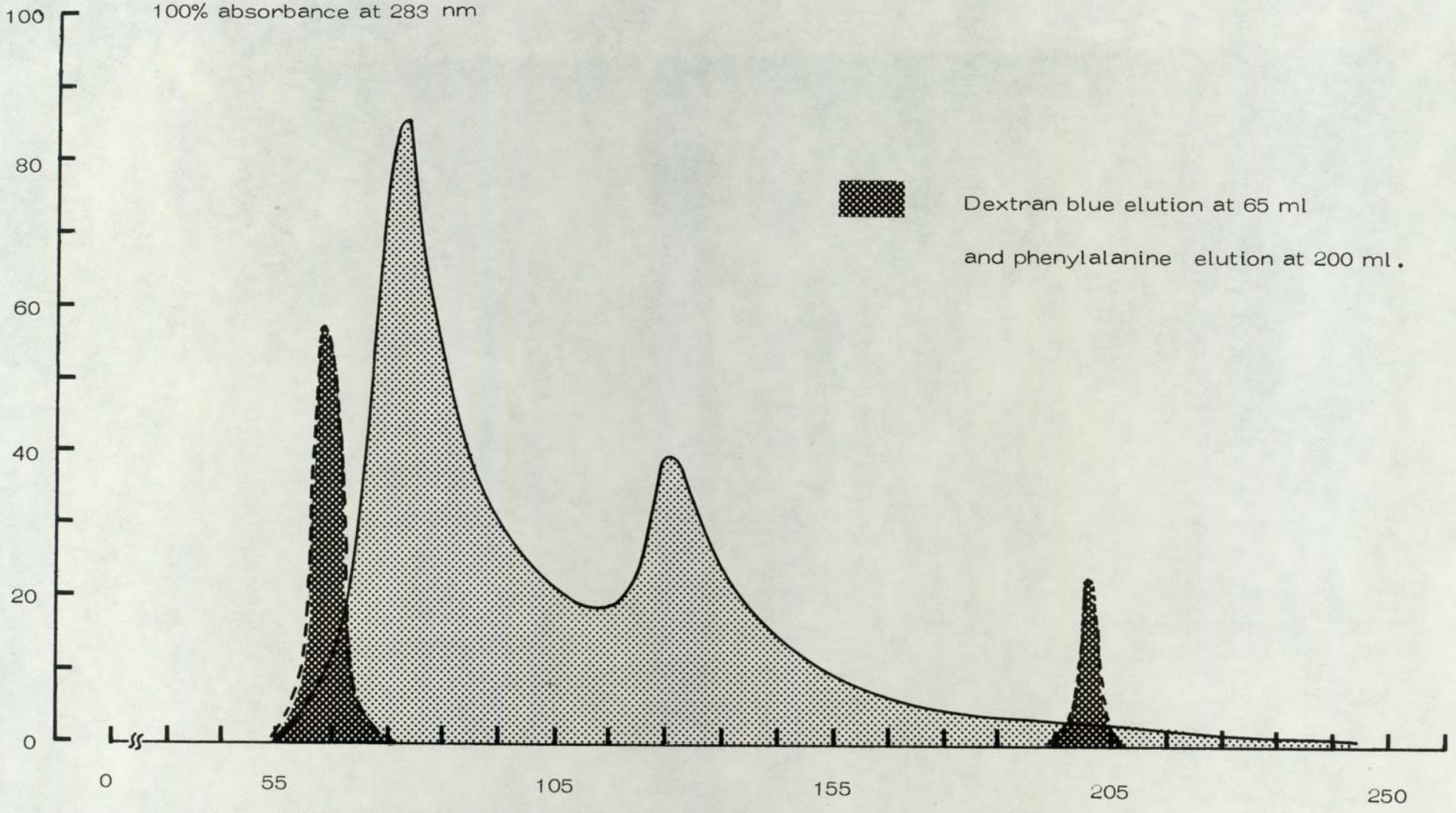


Figure 9. Column chromatography of a butanol extract of banana sample (E).

The diagram above shows and elution profile of the butanol extract (f. 12) of banana on sephadex G-10

The detection with ninhydrin and aniline hydrogen phthalate reagents in the above fractions (111-150mls) gave negative results. Detection with iodine and U.V. in the same fraction gave a single positive result for a substance migrating with the solvent front. This substance was identical to the one present in the front solvent in (see section 4.8.a.i) which corresponded to pigments.

iii) Butanol low molecular weight fraction

The low molecular weight fraction corresponded to the eluate volume between 151 and 300 ml. All the individual 10 ml fractions were a pale yellow colour. Substances eluted in the solvent front between 151-160 and 161-170 mls gave negative results with the following reagents:

- Antimony trichloride reagent
- 15% phosphoric acid
- Aniline hydrogen phthalate
- Ninhydrin reagent

However iodine and U.V. revealed a substance(s) migrating with the solvent front which corresponded to fragmented pigments.

The volume eluted between 171-180 mls gave negative results with the following reagents:-

- Antimony trichloride
- 15% phosphoric acid
- Ninhydrin

Positive reactions were obtained with iodine and U.V. of substances migrating with the solvent front and this showed that the same pigment present in volumes eluted between 150-170 ml was also present in this fraction. The volume between 171-180 ml also contained a substance reactive with aniline hydrogen phthalate with an R_f value = 0.06; which was brown in colour. The addition of a few drops of acetone to volumes eluted between 171-180 ml followed by incubation at $4^{o}C$ for one hour gave a white precipitate of crystals. The R_f value of the crystals was identical to sucrose (R_f = 0.06) when examined by T.L.C. and the infra-red spectra of the white crystals (KBr disc) was identical to that of sucrose. The detection of the same volumes (171-180 ml) with ninhydrin reagent gave a trace of the following compounds.

R_f	Colour	Density
0.09	red	trace
0.15	orange	trace
0.16	pink	trace

Substances in the eluate between 181-190 mls showed weak activity with aniline hydrogen phthalate with an identical R_f value to sucrose.

The detection of substances in the eluant coming off between 191-200 and 201-210 ml with aniline hydrogen phthalate showed that two streaks on the T.L.C. plate were sugars identical to the sugars obtained in system 1 with R_f values 0.17 and 0.14. These sugars corresponded to fructose and glucose which have identical R_f values (see system 1, p. 137)

b) Aqueous fraction:

The aqueous fraction was divided into a high molecular weight and a medium molecular weight one using dextran blue (G.200) and phenylalanine as molecular weight markers.

i) High molecular weight fraction:

This fraction (U.V. positive) eluted at volumes between 101 and 160 ml. Substances in this fraction gave negative results with the reagents below:

- 15% phosphoric acid
- Antimony trichloride
- Ninhydrin
- Aniline hydrogen phthalate

U.V. and iodine vapour as T.L.C. detecting agents showed similar results for substances present in the butanol high molecular weight fraction. However the substances in the

fraction have a lower molecular weight than the ones present in the butanol high molecular weight fraction. These substances are very likely to be chemically similar to the ones obtained in the high molecular weight fraction (pigments) but with lower degree of polymerisation or molecular weight.

ii) Medium molecular weight fraction

This fraction corresponded to the eluate between 165 and 190 ml. T.L.C. plates sprayed with the reagents below gave negative results:

- Antimony trichloride
- 15% phosphoric acid
- Aniline hydrogen phthalate

U.V. and iodine vapour gave positive results with substances migrating at the solvent front, similar to the result obtained with the medium molecular weight of butanol fraction.

Detection with ninhydrin gave positive results as follows:

Volume in ml	R_f value	Colour	Density
161-170	0.09	red	weak
	0.15	orange	weak
	0.29	red	weak
171-180	0.03	red	medium
	0.09	red	strong
	0.15	orange	strong
	0.16	pink	strong
	0.29	red	strong
181-190	0.03	red	medium
	0.09	red	weak
	0.15	orange	medium
	0.16	pink	weak
	0.22	red	medium
	0.29	red	weak

Since the high molecular weight fraction of the aqueous extract was similar in composition to the butanol high molecular weight fraction, the only difference between them was in the degree of polymerisation. Therefore the two fractions of the aqueous extract (high and medium molecular weight fraction) were combined together and the three fractions of the butanol extract were tested individually.

4.9 Activity of the three butanol fractions (12-1, 2 and 3) and the aqueous extract (f.13) compared with the activity of banana sample (E).

The ulcer index and the mucosa weights Table (17) showed that the aqueous extract was active in healing ulceration when compared to the activity of sample (E) at a dose of 7 g of banana per rat over 48 h All the butanol fractions (high, medium and low molecular weight fractions) were negative for anti-ulcerogenic activity.

4.10 The anti-ulcerogenic activity of the depigmented aqueous fraction (14) and the substances adsorbed by charcoal (f.15).

The percentage of the depigmented aqueous fraction (14) and the adsorbed charcoal fraction (15) are given in Table (16).
The ulcer index and the mucosa weights Table (18, p 157) showed that the use of charcoal reduced the anti-ulcerogenic activity present in the aqueous fraction (13).

a) T.L.C. Identification of the depigmented aqueous fraction (14) and the adsorbed charcoal substances (f.15).

The detection of the T.L.C. plates with iodine vapour and ninhydrin showed that the depigmented fraction (14) gave positive results with all ninhydrin reactive substances (amino group) present in fraction (13), while

Table 17. The curative activity of the three butanol fractions (12-1, 2 and 3) and the aqueous extract (f.13) compared with the activity of banana sample (E).

Treatment	ulcer index "t"$_1$	mucosa weights "t"$_2$ in mg
Aspirin treatment	15 ± 1.17	73 ± 2.57
Aspirin treatment plus 7 g of banana (E)	10.81 ± 1.15**	79.21 ± 1.77 *
Aspirin treatment plus aqueous fraction (13)	10 ± 1.01***	81.7 ± 1.85 ***
Aspirin treatment plus butanol high molecular weight fraction (12-1)	14 ± 2.51	74.2 ± 1.5
Aspirin treatment plus butanol medium molecular weight fraction (12-2)	14.57 ± 1.19	69.5 ± 3.12
Aspirin treatment plus butanol low molecular weight fraction (12-3)	14.8 ± 1.53	72.81 ± 2

** $p<0.02$; * $p<0.05$; *** $p<0.01$; t_1 : significance calculated by Wilcoxon rank sum test; t_2 : significance calculated by Student's t-test.

Table 18. The curative anti-ulcerogenic activity of the depigmented aqueous fraction (14) and the substances adsorbed by charcoal (f.15) compared with the aqueous fraction (13).

Treatment	ulcer index	mucosa weights (mg)
Aspirin treatment	15.93 ± 0.41	65.77 ± 2.32
Aspirin treatment plus aqueous fraction (13)	9.07 ± 0.78***	74.02 ± 2**
Aspirin treatment plus aqueous depigmented fraction (14)	12.00 ± 1.15	70.00 ± 2.2
Aspirin treatment plus adsorbed charcoal substances (f.15)	13.94 ± 0.85	68.61 ± 2.35

The results above show that charcoal treatment not only removed the pigment but removed the active substance as well. Therefore the active factor can adhere firmly to charcoal. The active substance remained adsorbed to the charcoal.

*** $p<0.01$ significance calculated by Wilcoxon rank sum test;

** $p<0.02$ significance calculated by Student's t test.

the charcoal adsorbed substances included the pigment present in fraction (13) (high molecular weight of the aqueous fraction). However a positive reaction with ninhydrin reactive substances was also obtained in the pigmented fraction (15).

5. DISCUSSION

It has been reported that the anti-ulcerogenic activity of banana is due to its buffering effect (Hanszen, 1934) and it is probable that green banana remains in the stomach for a much longer time than an average diet, which might prolong its buffering action. The present findings do not support that hypothesis since the anti-ulcerogenic activity of banana was found to be due to extractable substances and these substances both protected and healed rats from ulceration induced by aspirin.

Rider et al., (1967) reported that the anti-ulcerogenic activity of banana was due to its low protein content compared to the amount of protein present in a normal diet. The basis for his suggestion was that protein promotes gastric acid secretion. This investigation has shown that the pepsin digestion of a banana suspension failed to abolish the anti-ulcerogenic activity in rats given an equal amount of normal diet. It would appear that the anti-ulcerogenic action of banana is due to substances other than proteins. A treatment for the management of peptic ulcer is to use sulphated polysaccharide which forms a gum with pepsin in the gastric juice. This reduces the ulcerogenic effects of the gastric juice (Cook et al., 1963). However the active 95% ethanol extract did not extract polysaccharide, pectin, starch, etc.) and that solvent is only suitable for extracting oligosaccharides

and monosaccharides. Therefore the anti-ulcerogenic activity of banana was unlikely to be due to sulphated polysaccharides forming gums with pepsin to decrease the digestive activity of the gastric juice.

Prostaglandins have a cytoprotective effect in protecting rats against ulceration (Robert et al., 1979). The fatty acids arachidonic acid and linolenic acid are the precursors of prostaglandin synthesis (Vane, 1973).

However, prostaglandin or fatty acids were unlikely to be the active anti-ulcerogenic substances in banana since the acetone fraction (6) and the chloroform one (f.7) were inactive in protecting animals against ulceration and these fractions would be expected to contain fatty acids and prostaglandins on solubility grounds. In addition the retained residue (f.9) was completely free of fatty acid components and was very active in healing animals from ulceration. The anti-ulcerogenic ingredient in banana powder is highly polar and it is very unlikely that activity is due to compounds having non-polar radicals since the extraction of banana powder or its active fractions with petroleum ether, chloroform or acetone failed to extract the anti-ulcerogenic activity. That activity was consistently extractable by polar solvents such as water or 95% ethanol. The successive extraction of banana with ethanol and water by soxhlet extraction which involved heating during the extraction procedure abolished the

anti-ulcerogenic properties of banana. Therefore successful extraction was achieved without heating, since the active factors appear to be heat labile. The use of the 95% ethanol as solvent to extract the anti-ulcerogenic activity has advantages over water for several reasons. The percentage of the active fraction obtained with water extraction of banana (sample A) was 13.2% which was four times greater in mass than the active fraction extracted with 95%ethnol . However the biological activity was the same in both . Also aqueous extraction after a 95% ethanol extraction of banana confirmed that all the active ingredients in banana were extracted by the 95% ethanol.

The extraction of banana by water requires a large volume of water relative to banana (4 volumes at least) to avoid sludge formation with water. However the 95% ethanol solvent did not form a sludge with banana and was easy to remove by evaporation under a reduced pressure. At 40^{o}C water was more difficult to remove and required a higher temperature or a long period of freeze drying.

Carbenoxolone was reported to be active in protecting rats against ulceration induced by stress (Perkins and Vars, 1973). However the anti-ulcerogenic activity of banana is due to substances different in structure from carbenoxolone since carbenoxolone would be expected to be extracted by chloroform at alkaline pH. This is due to basic salt formation with the -COOH group. However

fraction (9) which was extracted at pH 8 by chloroform was negative in terms of anti-ulcerogenic properties. In addition, carbenoxolone has a molecular weight of 614and absorbs in U.V. at 256 nm. However the active fraction (13) (medium molecular weight) was slightly higher in molecular weight than the molecular weight of phenylalanine (165) and that fraction did not adsorb in U.V. Therefore the anti-ulcerogenic properties of fraction (13) was not due to carbenoxolone. The extraction of the active fraction (5) (95% ethanol fraction) with chloroform and acetone eliminated all lipids, fatty acids, sterols (reactive substances with phosphomolybdic acid, anisaldhyde, dragendroff's reagent) from fraction (9) without affecting the anti-ulcerogenic activity.

The extraction of fraction (9) with chloroform at pH 8 removed most of the weak acids which form a basic salt at pH 8 without affecting the anti-ulcerogenic activity of fraction (11). It was important to eliminate all chloroform and acetone extractable substances from fraction (9) before extracting with chloroform at alkaline pH because a milky colloidal suspension was formed if fraction (5) was extracted directly with chloroform at alkaline pH. The positive result obtained with 15% phosphoric acid , antimony trichloride, iodine and U.V. light on the T.L.C. plate in examination of the butanol medium molecular weight fraction suggests that the compound present may be a terpene-like compound. However,

the negative anti-ulcerogenic properties of that fraction did not encourage further investigation or purification of that compound. The extraction of fraction (11) with butanol removed a sticky gum from fraction (13) which was due to high sugar content in fraction (11). However fructose and glucose were detected by T.L.C. in fraction (12) (butanol low molecular weight fraction) and that finding is in agreement with Barnell and Barnell (1947) who reported that banana contains glucose and fructose and that these two sugars increase gradually as the banana ripens. The isolation of white crystals of sucrose from fraction (13) (butanol, low molecular weight fraction) was also in agreement with the above author's findings. It should be possible to correlate between the free sugar or starch content of banana as an index of ripening since the unripe banana contains the anti-ulcerogenic substances. However the lack of detailed information about the date of collection, source and names of the banana used in this project made such correlations impossible.

The active fraction (13) contained only ninhydrin positive substances and some pigment. The pigments were abundant in the high molecular weight of butanol fraction (12-1) which did not show anti-ulcerogenic activity. Therefore the anti-ulcerogenic activity of banana is very likely to be due to one or more of the six ninhydrin positive substances or to substances present in micro concentration in that fraction undetectable with the T.L.C.

If the activity was due to ninhydrin reactive substances, it was highly polar and possessed a molecular weight higher than phenylalanine (mw = 165) and similar to the molecular weight of sucrose (mw = 342). Since the sucrose in the butanol low molecular weight fraction (12-3) eluted at a similar volume to the ninhydrin reactive substances (170-180 ml). The percentage of the active fraction (13-2) was 0.19% when banana sample (E) was used. This percentage represents a 500 fold of purification by using plan number 2. for the extraction of banana. The loss of the anti-ulcerogenic activity of banana fractions (14) and (15) was probably due to the adsorption of ninhydrin reactive substances by charcoal; since both of fraction (14) and (15) contained in part the ninhydrin reactive substances.

CHAPTER FOUR

MODE OF ACTION OF BANANA AS COMPARED TO STANDARD AGENTS

1. INTRODUCTION

1.1 Function, structure, biochemical estimations and histochemistry of gastric mucus

a) Function:

The two types of mucus discussed earlier and this project is concerned with the gelatinous mucus.

Mucus is a viscous gelatinous substance which coats the gastro-intestinal mucosa. It has several main physiological roles. It protects the mucosal cells from the lumen content of enzymes, acids, bacteria, chemicals and from osmotic gradients. It lubricates the surface membrane during the passage of food (Florey, 1962; Heatley, 1959; Spiro, 1963). It also provides a barrier for the containment of secreted HCO_3^- and restricts the access of H^+ to the mucosa surface.

b) Structure:

Much of our present knowledge of the chemical composition of gastric mucus has been derived over the past decade from the development of gas-liquid chromatography and high pressure liquid chromatography. The principal constituents of mucus are glycoproteins where one or more chains of sugar residues are attached to serine, threonine or asparagine residues in proteins. The glycoproteins of many animal species have been extensively studied. Allen and Garner, 1980 have studied the gastric glycoproteins of the pig

and they suggested the general structure in Figure 10. Glycoproteins consist of four sub-units, each of molecular weight 5×10^{-5} daltons linked to each other by disulphide bridges. About three quarters of the protein in each subunit is covered by a sheath of closely packed polysaccharide chains. Each chain has an average of 15 sugar residues and there are 160 chains to each protein sub-unit. The remainder of the protein is non-glycosylated but contains cysteine residues which cross-link to bind the sub-units together. The carbohydrate content of glycoproteins consist of: D-xylose, L-arabinose, D-galactose, D-mannose, D-glucose, L-fructose, N-acetyl-D-glucosamine and N- and O-acetyl and N-glycolyl-derivatives of neuraminic acid residues (Slomiany and Meyer, 1972). The terminal carbohydrates in each side chain are N-acetyl-galactosamines, N-acetylglucosamine, galactose and fucose in the ratio 1:3:4:2 (Schrager, 1970; Allen and Starkey, 1974). The juxtaposition of these four sugars is highly specific for the blood group antigens A, B, AB and O. Group O has fucose as the terminal residue in its side chain: Group A has fucose and N-acetylgalactosamine and group B has fucose and galactose. Group AB has both the A and B modalities.

These blood group related structures are of interest since a high incidence of both gastric and duodenal ulcers has been associated with blood group O. Amino acids make up about 13% of the total

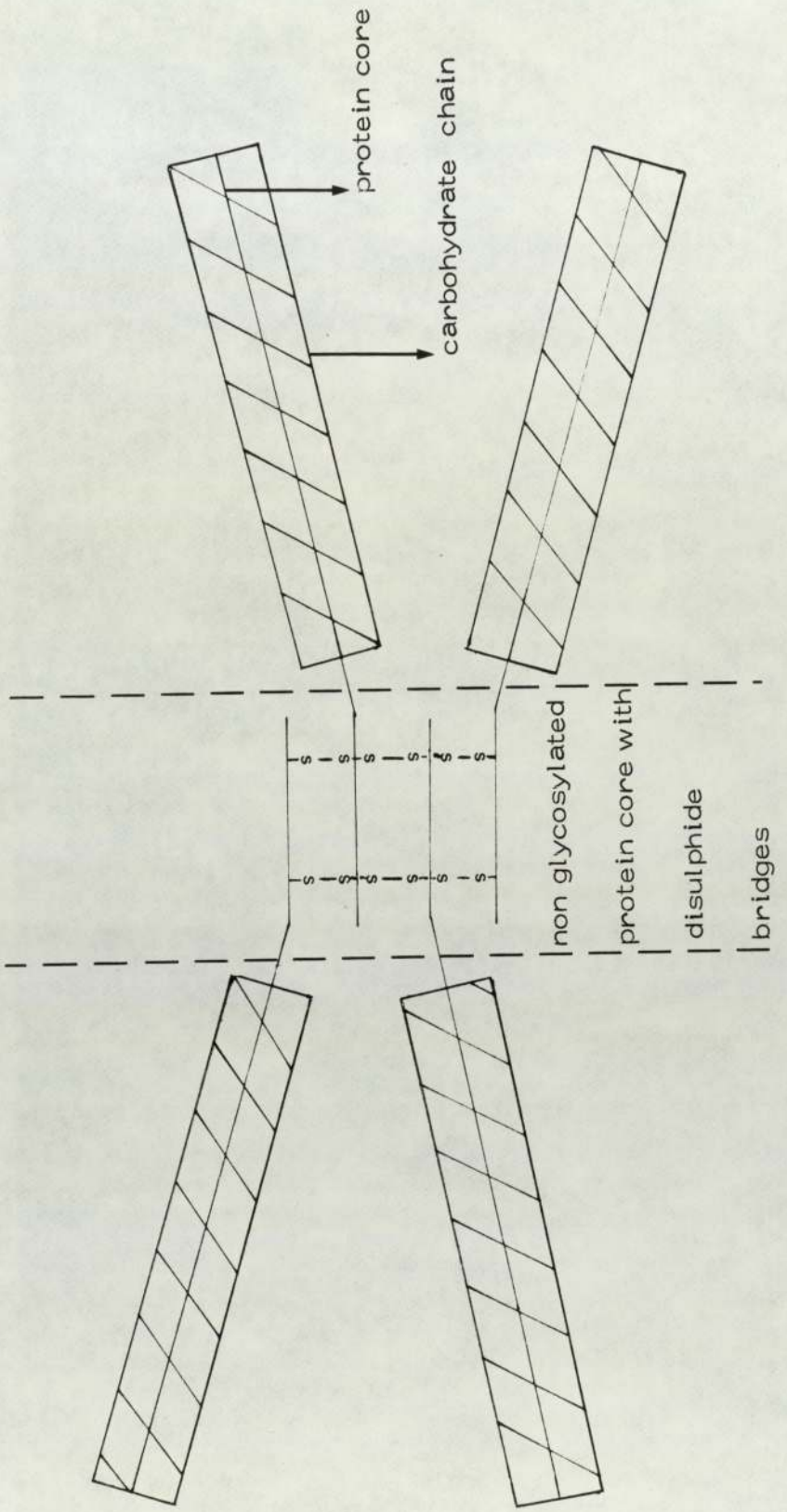


Figure 10. A diagramatic representation of the four glycoprotein sub-units joined to form the gastric glyco protein (after Allen and Garner, 1980).

glycoprotein in many species. Threonine, serine, proline, alanine and glycine form about 70% of the total amino acids present. Threonine is present to 18.1%, alanine to 15.8% and proline to 15.03% of the total (Allen and Garner, 1980). The high concentration of these amino-acids is obvious since the attachment of sugars are made with these residues.

Studies on gastric mucus secretion show that mucus glycoprotein secretion in the rat is stimulated by pentagastrin, cholecystokinin and cholinergic stimulants present in the fundus (Vagne and Perret, 1974).

Non-steroidal anti-inflammatory drugs e.g. phenylbutazone and aspirin, inhibit mucus secretion (Croft, 1977; Menguy and Master , 1965) and corticosteroids have been reported to have a similar action (Robert and Nezamis, 1963).

c) Biochemical estimation of mucus:

The measurement of individual carbohydrates in mucus was carried out by Grossberg et al., (1950) when they measured hexosamine and neuraminic acid in canine gastric mucus. In the present study the carbohydrate constituents of the gastric mucus (hexoses, hexosamines, fucose and neuraminic acid) were estimated from the gastric mucosal layers removed from the stomach by scraping with a microscope slide.

d) Histochemistry of mucus:

Mucins can be classified into acidic and neutral mucins on the basis of histochemical staining.

i) Acid mucins:

The hexosamine units of mucus are attached to either sialic acid, glucuronic acid or iduronic acid residues which contribute to the acidity of the mucus. Sulphate groups can also be present and contribute to the acidity. Therefore the acidic mucins may be either strongly acidic as sulphated mucins or weakly acidic as non-sulphated mucins.

ii) Neutral mucins:

They consist of hexosamines and hexoses and are not associated with sialic acids or sulphate groups. Periodic acid Schiff (for neutral mucins) and Alcian blue stains (for acidic and sulphated mucins) are suitable stains both for neutral mucins and for acidic and sulphated mucins examined in the present study.

iii) Periodic acid - Schiff's reagents and Alcian blue stain:

Periodic acid is a strong oxidising agent which oxidises the vicinal diol (cis or trans 1, 2-glycols) to form the dialdehyde. The dialdehyde groups stain with fuschin which is present in Schiff's reagent. Alcian blue is a water soluble copper phthalocyanin dye which is very reactive to acidic groups. The use of Alcian blue at pH1 and 2.5 to distinguish between acidic and sulphated mucins is

described by Lev and Spicer (1964). At pH1, strongly acid groups (e.g. sulphated mucins) are ionized and stained, whereas at pH 2.5 the weakly acidic groups (e.g.COOH) are ionized and stain but at this pH the strongly acidic sulphated mucins do not stain (Cook, 1964).

Staining by the combined Alcian blue and Periodic acid Schiff's reagent was also used by Spicer and Mayer (1960) to differentiate between the acidic and neutral groups of mucopolysaccharides.

1.2 Cimetidine and the gut

Cimetidine is the major histamine H_2-receptor antagonist in clinical use and is undoubtedly a striking therapeutic advance in the treatment of ulceration. The discovery of this drug was credited to Black et al., (1972) who discovered the H_1 and H_2-histamine receptors. Black et al., (1974) reported the discovery of cimetidine as a new H_2-histamine antagonist. In both rats and dogs cimetidine is rapidly and extensively absorbed. It is excreted largely unchanged in the urine. The plasma half-life is about one hour (Taylor and Cresswell, 1975). Similar results have been obtained in man (Griffiths et al., 1977). Cimetidine inhibits gastric acid secretion (Volume and acid outputs) in the dog, rat (Brimblecombe et al., 1978) and in man after stimulation by pentagastrin, histamine or by food (Burland et al., 1975, Fielding et al., 1976, Pounder et al., 1977). The fact that

Cimetidine is an H_2-receptor antagonist and that it inhibits acid output in response to various stimulants has been cited as evidence that histamine is the common mediator influencing the parietal cells and that other agonists act by releasing histamine (Debas , 1977). Cimetidine at a dose of 25 mg kg^{-1} administered orally to rats almost completely abolishes basal acid secretion (Brimblecombe et al., 1978). Some evidence suggests that cAMP is involved inthe regulation of gastric acid secretion. Dousa and Code (1974) found that cimetidine decreases adenylate cyclase levels in the gastric mucosa of guinea pigs. Similar results have been obtained in man (Simon and Kather, 1977, 1978) and cimetidine counteracts stimulation of cAMP formation by histamine or 4-methylhistamine. Cimetidine has only mild side effects compared to those in some other anti-ulcer drugs. Gynecomastia, nausea and vomiting have been reported in patients treated with cimetidine over a long period (Hall, 1976; Sharpe and Hawkins, 1977). The main problem with cimetidine is the recurrence of severe ulceration which may be asymptomatic (Wallace et al., 1977).

1.3 Prostaglandins and the gut:

Prostaglandins are hydroxy fatty acids and are derived from the natural poly-unsaturated acid arachidonic acid. Prostaglandins are present in the gastric intestinal tract and have been implicated in physiological and pathological processes in the gut.

Prostaglandins are not released from stores but are formed during periods of stimulation (Coceani et al., 1967, 1968). Species differences are found. PGE-1 is the most predominant prostaglandin in rat gastric mucosa (Bennett et al., 1967; Shaw and Ramwell, 1968) whilst human gastric mucosa contains mainly PGE-2 at a concentration of 400 $\mu g\ g^{-1}$ of mucosa (Bennett et al., 1968). PGE-1 is also present in basal gastric secretion in the human and is secreted at a rate of 2.4 $\mu g\ min^{-1}$. Prostaglandins have a wide range of important physiological roles in the gastro-intestinal tract but this study is mainly concerned with the anti-ulcerogenic effect of prostaglandins on gastric secretion and ulcer healing as a comparison to the anti-ulcerogenic effect of banana.

It has been known for several years that prostaglandins particularly PGE-1, PGE-2 and PGF-2α do not exert identical actions on gastric secretion (Bass, 1974). PGE-1 and PGE-2 at 1 $\mu g\ kg^{-1}\ min^{-1}$ totally inhibit acid and pepsin secretion in the dog after stimulation by food, pentagastrin, carbachol, reserpine, 2-deoxyglucose or histamine (Robert et al., 1967, 1968b). PGE-1, PGE-2, PGA but not PGF-2α were shown to inhibit acid secretion both in vitro and in vivo in the dog and rat (Robert et al., 1967, 1968b; Way and Durbin, 1969; Waller, 1973). The secretion of other components in gastric secretions (e g. pepsin and mucus) were also inhibited by PGE-1, PGE-2 and PGA (Robert et al., 1968a).

Prostaglandins have a direct effect on the physiology of the vascular system, PGE-1 and PGE-2, PGA and PGI were described as vasodilators but during the inhibition of acid secretion the gastric blood flow was reduced but this was probably as the result, rather than the cause, of the anti-secretory effect (Robert 1973, Waller, 1973). These findings together with increased release of mucosal PG'S during the stimulation of secretion (Shaw and Ramwell, 1968) have led to the hypothesis that prostaglandins have a physiological role as negative feedback inhibitors of acid secretion and functional vasodilators in the gastric mucosa.

PGF-2α is a poor inhibitor of acid and pepsin secretion (Usardi et al., 1974) and has a vasoconstrictor action. Prostaglandins of the series E, I and F-2α were shown to be protective against disruption of the gastric mucosal barrier in animals. These agents prevent gastric ulceration (increased flux of H^+, Na^+, K^+ and pepsin secretion) produced by the intragastric administration of either aspirin, indomethacin or taurocholate in both the dog and rat (Whittle, 1977; Konturek et al., 1979; Dajani et al., 1978).

Robert (1979) has reported that prostaglandins have cytoprotective properties against ulcer induction by alcohol, HCl, or thermal injury in the rat stomach. He also reported that the cytoprotective effect of prostaglandins is unrelated to the inhibition of gastric secretion since the cytoprotection is maximal at a dose

which has no effect on gastric secretion. The cytoprotective action of prostaglandins is difficult to explain. A common feature of prostaglandins is that they maintain the mucosa barrier against the back diffusion of ions. However the precise mode of action of prostaglandins in protecting animals from gastric ulceration is ambiguous. Ghaudhury and Jacobson (1978); Wollin et al., (1974) and Thompson et al., (1977) found that PGE-2 stimulated adenylate cyclase activity in the gastric mucous cells of the guinea-pig and rat. Soll (1978) confirmed these findings and he localized the activity to being in the non-parietal cells of isolated gastric mucosa cells from the surface epithelium which was the site of cytoprotection. If that is the case then prostaglandins increase the cAMP levels in non-parietal cells and should therefore decrease histamine-stimulated accumulation of cAMP in parietal cells (Soll, 1978). Prostaglandins synthesis is catalysed by 'prostaglandin synthetase' (a membrane bound enzyme system) which is stimulated by adrenaline, noradrenaline and dopamine (Pace-Asciak, 1972; Lippman, 1974) and inhibited by non-steroidal anti-inflammatory drugs e.g. aspirin, indomethacin, phenylbutazone (Vane, 1971; Tarun et al., 1978; Lippman, 1974).

In man prostaglandins both of the E and A series have been found to reduce basal and stimulated acid secretion. However the prostaglandins were not

effective by oral administration (Bass, 1974). This may be due to hydrolysis by prostaglandin 15-OH dehydrogenase in the gut. Attempts have been made to develop synthetic prostaglandins resistant to hydrolysis. These are 15($\frac{S}{R}$)-15-methyl PGE-2, methyl esters which are resistant to hydrolysis at the 15-OH group (Karim et al., 1973a).

1.4 Cyclic adenosine 3', 5'-monophosphate (cAMP) and the gut

Cyclic adenosine 3', 5'-monophosphate (cAMP) is a nucleotide which functions as a intracellular 'second messenger' in which it transmits and amplifies the chemical signals delivered to the cellular membrane by hormones. It therefore has a key role in controlling cellular activity. In recent years there has been increasing evidence to indicate that cAMP may be involved in gastrointestinal function and in controlling gastric secretion. Recently it was confirmed both in vivo and in vitro that cAMP levels increased in response to histamine or gastrin administration (Kimberg, 1974; Simon and Kather, 1977, 1978). Carbenoxolone also increases cAMP levels by inhibiting both phosphodiesterase and the enzymes deactivating prostaglandins (Parke,1978). At present there is insufficient evidence to suggest that cAMP mediates the secretion of pepsinogen from the chief cells or mucin from the gastric mucus cells.

The mechanism of action of cAMP is not clear but it may be involved in the activation of carbonic anhydrase via a protein kinase (Bersimbaev et al., 1971; Salganick et al., 1972).

1.5 5-Hydroxytryptamine and the gut:

The gastrointestinal tract is known to be a rich source of 5-hydroxytryptamine (5-HT), and 5-HT in the blood is derived primarily from the gastrointestinal store of 5-HT (Stacy and Young, 1964) located in the entero-chromaffin cells (Benditt and Wong, 1957). 5-HT is continuously released into the gastric lumen at an approximate rate of 10 ng g^{-1} min^{-1} (Gross and Sturkie, 1975).

Our knowledge of the physiology and the pharmacology of 5-HT secretion is scanty. Early observation linked 5-HT with gastric mucus secretion in dogs (Black et al., 1958) and cats (Decorral, 1956). A similar finding in the rat colon was found by Bradbury et al., (1980) who estimated the hexose and fucose content in the colonic perfusate following intravenous infusion of 5-HT at a dose of 2 n mol kg^{-1} min^{-1}.

5HT was also reported to be involved in the pathogenesis of peptic ulceration (Furuhashi, 1968; Hedinger and Veraguth, 1957; Wilhelmi, 1957). Ulceration was induced in mice 4 h after the s.c. injection of 5-HT at a dose of 10 mg kg^{-1} (Watanabe, 1980).

They found that ulceration was associated with a decrease of volume secretion and pepsin output with slight increase in acidity. The results were confirmed in rats at a dose 15-30 mg kg^{-1}, s.c. of 5-HT where ulceration was associated with vasocontriction and ischaemia. There was also an increase in the motility of the stomach, a decrease in mucosal blood flow, an inhibition of protein synthesis in the mucosa, thrombosis and reflux of the duodenal contents back into the stomach (Milne and Cohn, 1957, White and Magee, 1958, Hori, 1962, Yano et al., 1977; Hashizume et al., 1978; Watanabe, 1980). The mechanism by which 5-HT induces ulceration is not clear but some evidence suggests that 5-HT decreases the stability of lysosomes in the mucosal cells and that the lysosomal hydrolases are responsible for necrosis in the mucosal layer (Ferguson et al., 1973).

Ferguson et al., (1973) also found that PGE-1 decreased the severity of 5-HT-induced ulceration by stabilizing the lysosomal membranes. A similar suggestion that PGE-1 inhibits 5-HT induced ulceration in the rat by the same mechanism was made by Debnath et al., (1978).

2. MATERIALS

2.1 Materials - histology and histochemical stains and reagents

a) Bouin's solution:

This fixing solution consisted of 75 ml of a saturated aqueous solution of picric acid mixed with 25 ml of 40% (v/v) formaldehyde and 5 ml of glacial acetic acid.

b) Ehrlich's Haematoxylin

This solution was prepared by adding 100 ml of absolute alcohol to 2 g of haematoxylin and the solution was left for several weeks with occasional shaking. Then the following reagents were added:

Glycerol	100 ml
Distilled water	100 ml
Glacial acetic acid	10 ml

The mixture was shaken and then filtered.

c) Eosin solution:

1% w/v eosin in water.

d) Acid alcohol:

1 ml of concentrated HCl in 99 ml of 70% alcohol.

e) Schiff's reagent:

Fuschin (0.9 g) was dissolved in 200 ml of distilled water and 0.5 ml of thionyl chloride added. After standing overnight the solution was decolourised by 1 g of activated charcoal. After shaking the solution was filtered and kept in a dark bottle at $4^{o}C$.

f) Periodic acid solution:

1% ($w.v.^{-1}$) of periodic acid in water.

g) Alcian blue (AB) pH 2.5:

1% ($w.v.^{-1}$) Alcian blue solution in 3% ($v.v.^{-1}$) acetic acid was filtered before use.

h) Alcian blue (AB) pH 1:

1% ($w.v.^{-1}$) Alcian blue in 0.2 M. HCl.

i) Sulphite rinsing solution:

This was prepared immediately before use by adding 36 ml of 1% ($w.v.^{-1}$) potassium metabisulphite to 30 ml of 1M.HCl and making up to 600 ml with distilled water.

3. METHODS

Both the prophylactic and curative methods of evaluating anti-ulcerogenic activity were used and have been described in detail in Chapter 2, in summary they are:-

3.1 Prophylactic method:

The rats are maintained on a normal diet for two days under restful conditions. The treatment is then administered over two days followed by a further two days fasting period. Then aspirin is administered orally at 150 mg kg^{-1} and the rats killed 5 h later and the stomachs removed and assessed for evidence of ulceration.

3.2 Curative method:

Again the animals were allowed to stablize on a normal diet for two days before the 48 h of fasting. Aspirin was then administered orally at 150 mg kg^{-1} and treatment commenced 5 h later, treatment was maintained for a further two days after which the animals were killed and the stomach removed and evaluated for ulceration. In both methods the animals were allowed free access to water over the experimental period.

3.3 Evaluation of severity of ulceration:

The effect of treatment on the induction of ulcers by aspirin in both the prophylactic and curative methods described above was evaluated by either:

- Determination of the ulcer index (Chapter 2, section 3. a, b)
- Determination of mucosa weights (Chapter 2, section 3.c)
- Determination of the thickness of mucosa layer (section 3.4 a)
- Determination of gastric acid secretion (section 3.5.).

3.4 A comparison of the anti-ulcerogenic action of banana with that of aluminium hydroxide

The "prophylactic" method for evaluation of the anti-ulcerogenic activity was used in this experiment.

Rats were divided into 6 groups of 10 rats in each group as follows:

Treatment	Notes
None	-
Aspirin	Aspirin administration on the 7th day 5 h before killing the rats.

Treatment	Notes
Banana	5 g of banana (sample A) was mixed with 9 g of food and was consumed over 2 days prior to fasting.
Banana treatment plus aspirin	As above except that aspirin was administered 5 h before killing the rats on the 7th day.
$Al(OH)_3$ treatment	After climatization for two days on a normal diet, rats were given 14 g of normal diet each over the 3rd and the 4th day of the experiment. An aqueous $Al(OH)_3$ suspension was administered orally (8 mg kg^{-1}) at the start of the 2 days fasting period (0 hr) followed by subsequent doses at 24 and 47 h during the fasting period. Rats were killed 6 h after the last dose of $Al(OH)_3$
$Al(OH)_3$ treatment plus aspirin	As above except that aspirin was administered 1 h after the final dose of $Al(OH)_3$.

After killing the rats, the stomachs were examined and evaluated by the ulcer index. After evaluation one cm square specimens were removed from the body of the stomach and fixed in Bouin's fluid for 24 h . The tissues were dehydrated in alcohol and cleared for 8 h in chloroform before embedding in paraffin wax blocks.

Sections (5 μ) were cut with a Cambridge rotatory microtome. The sections were transferred to microscope slides by floatation on a warm 1% albumin solution. After mopping off the excess fluid with adsorbent tissue paper the slides were left to dry for 12 hours at room temperature prior to staining.

a) Haematoxylin and eosin stain:

The wax was removed from sections by immersing them in xylene and rehydration in water was carried out prior to staining. The slides were then immersed in Ehrlich's haematoxylin for 15 minutes. Excess of haematoxylin was drained off the slides and the sections blued in running tap water. The blued sections were dipped in acid - alcohol for one second then washed again in water. After drying with cellulose tissue the sections were counter stained with 1% eosin for 2-4 min . After drying again the sections were dehydrated by immersion in alcohol and cleared in xylene followed by mounting with D.P.X. mounting medium. The cover slips were sealed with nail varnish to prevent drying. The sections were examined under the microscope and the minimum and maximum thickness of the mucosal membrane was measured and the mean taken to give the average thickness of the gastric mucosa. Ulcerated areas represented the minimum thickness and the protected areas the maximum thickness.

b) Periodic acid Schiffs (P.A.S.) stain.

After removal of wax and rehydration the sections were oxidised for 10 minutes in 1% aqueous periodic acid followed by washing in running water for 5 minutes. The sections were then immersed in Schiff's reagent for 30 minutes followed by rinsing in sulphite solution for 1 minute followed by 3 minutes immersion in a second fresh sulphite rinse. The sections were washed for ten minutes in water and then dehydrated with alcohol. The slides were mounted in D.P.X. as before.

c) Combined Alcian blue and P.A.S. staining (AB-P.A.S.):

After rehydration of the sections they were stained with the appropriate Alcian blue solution (pH 1 or 2.5) for 20 minutes. At pH 1 only the strongly acidic sulphated compounds reacted with the dye. The water rinse was omitted to avoid neutralization of the sections and the rapid blueing of non-sulphated mucins (carboxyl group of acid mucopolysaccharide). However the staining of carboxyl groups was prevented by drying the slide completely with filter paper after staining with Alcian blue. (Lev and Spicer, 1964). The sections were then transferred to periodic acid solution. The P.A.S. staining was carried out as previously described and finally the stained sections were mounted in D.P.X.

3.5 Evaluation of gastric acid secretion in rats treated with banana aqueous extract (f.O).

The aqueous banana extract (sample A) was obtained as described in Chapter 3, section 3.4 and a saturated solution (0.88 g ml^{-1}) was prepared in distilled water. The prophylactic model for evaluating ulceration was used in this experiment to evaluate both ulceration and gastric acid secretion. Two groups of 7 rats were set up as follows:

Treatment	Notes
Aspirin	3 oral doses of distilled water (total doses 5 ml kg^{1}) at (0) h , 8 h of the treatment period and the last dose of H_2O was given one h before aspirin administration.
Aqueous banana (f.O) treatment with aspirin	3 oral doses of the aqueous extract administered (equivalent to a total dose of 4.4 g kg^{-1}) as above for the distilled water.

On the seventh day, all animals were dosed with aspirin one hour after the last dose of the treatment. One h later the rats were anaesthetized by an intraperitoneal injection of 300 µl kg^{-1}, of sodium pentobarbitone. The abdominal cavity was opened and the pyloric end of the stomach was ligated for 3 h . At the end of the

experiment the rats were killed and the gastric juices were collected and centrifuged for 2 min using a clinical centrifuge. The volumes were noted and the acidity of the gastric juices were determined by titration with 0.1 N sodium hydroxide with phenolphthalein as the indicator.

The ulcer index for each stomach was evaluated and 1 cm^2 specimens were dissected from the body of the stomach to evaluate the thickness of the gastric membrane as previously described.

3.6 Evaluation of banana (sample E) fraction (13) activity by the intra-peritoneal route.

A solution (30 mg ml^{-1}) of fraction (13) was prepared in distilled water and sterilized by filtration through a membrane (G.S., 0.22 µm) diameter. The curative model of ulceration was used to investigate the activity of fraction (13) when administered by the intra-peritoneal route (i.p.). Twenty rats were divided into two groups of ten as follows:

Treatment	Notes
Aspirin	Three i.p. doses of sterile H_2O (equivalent to a total dose of 5 ml kg^{-1}) at (0) h , 8 h and 16 h 5 h after aspirin treatment.
Aspirin with fraction (13)	Three i.p. doses of fraction (13) (equivalent to a total dose of 150 mg* kg^{-1}) administered as above.

* equivalent to 7 g of banana powder

At the end of the experiments the ulcer index and the mucosa weights were evaluated.

3.7 Estimation of the carbohydrate content of the scraped gastric mucosa after treatment with 2, 3 and 4 g of banana sample (A).

Twenty-eight rats were divided into four groups of seven as follows:

Treatment	Notes
Aspirin	14 g of normal diet during the treatment period.
Aspirin plus 2 g of banana	12 g of normal diet with 2 g of banana during the treatment period.
Aspirin plus 3 g of banana	11 g of normal diet plus 3 g of banana.
Aspirin plus 4 g of banana	10 g of normal diet plus 4 g of banana.

At the end of the experiment the ulcer index was evaluated and specimens were taken from the body of the stomach of rats treated with aspirin or aspirin with banana (4 g only) for histology and estimation of carbohydrate content.

a) Histology:

The preparation of the sections, staining with H.E. and periodic acid Schiff-Alcian blue were carried out as described in section 3.4. a, b and c.

b) Preparation of the samples for hexosamine , fucose and sialic acid determinations

30-40 mg Of each mucosal homogenate sample was hydrolysed for 150 minutes with 5 ml of 2M HCl in stoppered tubes placed in a boiling water bath.

After hydrolysis the samples were filtered and made up to 6 ml with distilled water. All spectrophotometric determinations were carried out on a Pye-Unicam SP 600 spectrophotometer.

i) Hexosamine determination:

A 0.2 ml portion of each hydrolysate was diluted to 0.5 ml with distilled water and assayed by the method of Dische and Borenfreund (1950), Glucosamine hydrochloride (BDH Ltd) was used as a standard.

ii) Fucose determination:

50 µl Of the hydrolysate was diluted to 1 ml with water and assayed by the method of Gibbons (1955) using L(-) fucose (BDH Ltd) as standard.

iii) Sialic acid determination:

20-30 mg Of each homogenized mucosal sample was hydrolysed with 2 ml of 0.1 N $H_2 SO_4$ at 90°C for one h (this procedure splits off the bound sialic acid without dehydration).

The hydrolysate was made up to 4 ml after filtration. 0.2 ml Of the filtrate was assayed according to the Warren (1959) method. N-acetyl neuraminic acid (BDH Ltd) was used as standard. The maximal absorption of sialic acid is at 549 nm and it was found that there was a second absorption maximum peak at 533 nm due to 2-deoxyribose for which a correction was made as the absorption of this material at 549 nm was large. The correction factor was obtained by evaluating the molecular extinction coefficient of N-acetyl neuraminic acid (NANA) and 2-deoxyribose (2 DR) (BDH Ltd) and substituting them into the following equation:

$$\mu \text{mol NANA} = \left[\frac{E_3}{(E_2E_3) - (E_1E_4)} \times OD1 - \frac{E_4}{(E_2E_3) - (E_1E_4)} \times OD_2 \right] \times 4.3$$

where $O.D._1$ = optical density at 549 nm

$O.D._2$ = optical density at 532 nm

where E_1 = molecular extinction coefficient of NANA at 532 nm 10^{-3}

E_2 = molecular extinction coefficient of NANA at 549 nm 10^{-3}

E_3 = molecular extinction coefficient of 2 DR at 532 nm 10^{-3}

E_4 = molecular extinction of 2 DR at 549 nm 10^{-3}

The molecular extinction coefficients were obtained and they were slightly different to the ones quoted by Warren (1959).

$E_1 = 19.5$, $E_2 = 46$, $E_3 = 139$, $E_4 = 39$

This gave μ mol NANA $= \left[0.106 \times O.D._{549} - 0.03 \times O.D._{532} \right] 10^{-3}$

3.8 Determination of Deoxyribonucleic acid (DNA) in the gastric mucosa of rats treated with banana sample (A).

Gastric mucosal DNA was estimated in the mucosal scrapings from stomachs after treatment with banana sample (A). Highly polymerized DNA from calf thymus (Sigma Ltd) was used as controls. Banana sample (A) was given mixed with food at 3 g over 48 h per rat The "curative" model of ulceration was used in this experiment. Three groups each of ten rats were used as follows:

Treatment	Notes
None	14 g of normal diet during the treatment period.
Aspirin	diet as above plus aspirin (150 mg kg^{-1}, p.o.)
Aspirin plus banana (A)	aspirin as above plus 3 g of banana sample (A) plus 11 g of normal diet during the treatment period.

At the end of the experiment the ulcer index and the mucosa weights were evaluated as previously described.

a) Extraction and determination of DNA in the gastric mucosa

The scraped mucosa samples were suspended in 10 ml of distilled water. Since the specimens are rich in free DNA 0.3 ml of 30% bovine serum albumin solution were added to the specimens. The specimens were well shaken to precipitate DNA and 3 ml of 50% (w.v.$^{-1}$) trichloroacetic acid (T.C.A.) was added to each sample.

The samples were frozen at -30°C for three days and then thawed in a shaking water bath at 37°C and centrifuged for 30 min at 1800 (r.p.m.) at 4°C. The supernatants were removed and 10 ml of 5% (w.v.$^{-1}$) of T.C.A. was added to each and the centrifugation repeated at 2500 (r.p.m.) at 4°C for 30 min . The supernatants were discarded and the pellets were hydrolysed for 15 min at 90 $\pm$ 1°C with 6 ml of 5% T.C.A. After cooling to room temperature they were again centrifuged at 2500 (r.p.m.) for 30 min. The supernatants were retained and hydrolysed as described above The supernatants were combined, frozen and thawed, and centrifuged for 30 min at 2500 (r.p.m.). The final supernatants were lyophylizied and finally dissolved in 4 ml of distilled water. As standards 3, 6, 9, 12, 15, 18 and 21 µg of highly polymerized calf-thymus DNA samples were extracted and hydrolysed in the same way. The same range of concentrations of DNA were chosen for hydrolysis and assayed in order to determine the efficiency of the extraction procedure for the samples. The samples were duplicated and 0.1 ml of each sample was assayed (Croft, et al., 1966) where the colorimetric reaction was allowed to develop at 6°C over 50 hours. The results were estimated in µg of DNA mg^{-1} of tissue and the total DNA in the complete mass of scraped mucosa from each stomach.

3.9 Effect of cimetidine, prostaglandin E-2, 5-hydroxytryptamine and dibutyryl 3', 5' adenosine cyclic monophospate on ulceration induced by aspirin and their anti-secretory activity.

Cimetidine was obtained from Smith Kline and French Limited and a solution (48 mg ml^{-1}) was prepared in sterile water. Prostaglandin E-2 (PGE-2) was a generous gift from Dr Brooks (UpJohn Limited, Sussex). A stock solution in ethanol (10 mg ml^{-1}) was prepared and 120 µl of the stock was diluted to 10 ml with sterile water (120 µg ml^{-1}) and stored at 4°C. 5-Hydroxytryptamine creatine sulphate (5-HT) (Sigma) was dissolved in sterile water. A stock solution (300 µg ml^{-1}) was prepared and stored at 4°C.

A stock solution of sodium salt of N^6, O^2-dibutyryl adenosine 3', 5' cyclic monophosphate acid sodium (dib-cAMP) (Sigma Chemical Company) (120 µg ml^{-1}) in sterile water was prepared and stored at 4°C.

a) Assessment of cimetidine, PGE-2, 5-HT and dib-cAMP activity on ulceration induced by aspirin.

The curative method was used to evaluate the effect of these agents on ulceration induced by aspirin. The effect of each of the above agents on ulceration was investigated in a similar way to 'banana extract' treatment (3 oral doses at 8 h intervals, 5 h

after aspirin treatment) which is described as "post-treatment" compared to "pre-treatment" where the agent was administered 45 min before administration of aspirin. All the agents were administered orally except PGE-2 which was injected subcutaneously. Seventy rats were used in this experiment, divided into 10 groups of 7 rats as follows:

Treatment	Notes
None	Oral dose of H_2O (5 ml kg^{-1})
Aspirin	Oral dose of aspirin (150 mg kg^{-1})
Pre-cimetidine plus aspirin	Oral dose of cimetidine (240 mg kg^{-1}) plus aspirin as above.
Aspirin plus post-cimetidine.	Aspirin as above plus 3 oral doses of cimetidine (total doses 240 mg kg^{-1})
Pre-PGE-2 plus aspirin.	PGE-2 (600 μg kg^{-1}, s.c.) plus aspirin as above.
Aspirin plus post-PGE-2	Aspirin as above plus 3 injections of PGE-2 (total dose 600 μg kg^{-1}, s.c.).
Pre-5-HT plus aspirin.	Oral dose of 5-HT (1.5 mg kg^{-1}) plus aspirin as above.
Aspirin plus post-5-HT	Aspirin as above plus 3 oral doses of 5-HT (total doses 1.5 mg kg^{-1}).
Pre-dib-cAMP plus aspirin	Oral dose of dib-cAMP (600 μg kg^{-1}) plus aspirin as above.
Aspirin plus post-dib-cAMP.	Aspirin as above plus 3 oral doses of dib-cAMP (total doses 600 μg kg^{-1}).

At the end of the experiment the ulcer index was evaluated and the mucosa was removed by scraping, freeze-dried and weighed.

i) Estimation of carbohydrate in the scraped gastric mucosa

The preparation of the samples for the estimation of carbohydrate was described in section 3.7. The methods for estimating sialic acid and hexosamines were also described in the same section.

ii) Fucose and hexose determinations:

Fucose and D-galactose were purchased from BDH Ltd and were used as standards. Fucose and hexose determinations were carried out according to the method of Dische and Shettles (1948). The extinction molecular coefficient (E) of fucose was determined at two wavelengths (390 nm and 430 nm) and maximum absorbance for fucose was found to be at 390 nm. At 430 nm hexose may contribute a small change to the absorbance hence the difference between the extinction at 390 nm and 430 nm provides a method specifically for determining the fucose content of the sample. Glucose and nucleic acid give very poor absorbance spectra for this reaction. The following concentrations were used as standards for fucose determination: 5, 10, 15, 20, 25 and 30 $\mu g\ ml^{-1}$.

Over this concentration range a linear relationship exists between concentration and extinction values.

A small number of samples were analysed in each batch by the above procedure to ensure the exact time for boiling and adding the cysteine hydrochloride (Sigma) solution. D-galactose was used for a standard curve at the following concentrations 1, 3, 5,7 and 9 $\mu g\ ml^{-1}$. A linear relationship exists between the galactose concentrations and their extinction over this range.

The concentration of the galactose in the samples was calculated by calculating the regression correlation coefficient.

iii) Protein determination:

10–15 mg Of each of the freeze dried mucosa samples were suspended in 4 ml of distilled water and homogenized with a Potter homogenizer to disintegrate the tissue. The samples were duplicated and 50 µl of the homogenized samples were assayed for proteins by the method of Lowry et al., (1951). 50, 100, 200, 300, 400 and 500 $\mu g\ ml^{-1}$ solutions of bovine serum albumin were used to establish a standard curve. A linear relationship was observed between the chosen protein content and the extinctions. The calculation of the protein content in the samples was carried out by the regression correlation coefficient.

$$Y = ax + C$$

where $a = 2.5.10^{-3}$ and $c = 6.49.10^{-2}$

b) An evaluation of the anti-secretory activity of cimetidine, PGE-2, 5-HT and dib-cAMP in the 5 h modified Shay rat.

This was investigated at doses 240 mg kg^{-1} for cimetidine, 600 µg kg for PGE-2 and dib-cAMP and 1.5 mg kg^{-1} for 5-HT (see Table 19 for experimental procedure). Forty-five minutes after aspirin treatment, the rats were anaesthetized by sodium pentobarbitone (300 µl kg^{-1}, s.c.), ligated for 5 hours after which the pyloric end of the stomachs were carefully removed and the gastric juice collected. The volume and the acidity of each juice was determined as described in section 3.5.

Table 19: Experimental procedure for evaluating the anti-secretory activity of cimetidine, PGE-2, 5-HT and dib-cAMP in the 5 h modified Shay rat.

Treatment	0 - 48 h	Day 3			
		48 h	48.75 h	49.50 h	54.50 h
Aspirin	fasting period	oral dose of H_2O (5 ml kg^{-1})	oral dose of aspirin 150 mg kg^{-1}	pylore ligated	killed
Cimetidine plus aspirin	fasting period	oral dose of cimetidine (240 mg kg^{-1})	aspirin as above.	as above.	as above
PGE-2 plus aspirin	fasting period	PGE-2 (600 µg kg^{-1}, s.c.)	aspirin as above	as above	as above
5-HT plus aspirin	fasting period	oral dose of 5-HT (1.5 mg kg^{-1})	as above	as above.	as above
Dib-cAMP plus aspirin	fasting period	oral dose of Dib-cAMP (600 µg kg^{-1})	as above	as above.	as above

4. RESULTS

4.1 The protective roles of banana and aluminium hydroxide:

The ulcer index values (Figure 11), showed that Al $(OH)_3$ at a dose 3 x 8 mg kg^{-1}, 48 h had a significant protective effect against aspirin induced ulceration. The banana (A) diet at a dose 5 g over 48 h was more effective ($p<0.005$) than Al$(OH)_3$ ($p<0.05$) in preventing ulceration. On examination of animals treated with Al-$(OH)_3$ it was found that the gastric mucosa was covered with a white mucin membrane in four out of ten animals treated with the combined aspirin and Al $(OH)_3$. The severity of the ulceration decreased when animals were pre-treated with Al $(OH)_3$ and only small pin point haemorrhages were found with shallow ulcers. Measurements of the thickness (Figure 12) of the mucosa membrane showed that banana treatment without aspirin increased the thickness of the mucosal membrane by 14% compared to untreated rats and by 28% compared to aspirin treatment. Aspirin administration at a dose of 150 mg kg^{-1} in 48 h fasted rats decreased the thickness of the gastric mucosa by 15% compared to untreated rats. The combined banana plus aspirin treatment redused this decrease in the mucosa thickness by 3% compared to untreated rats and by 20% when compared to rats treated with aspirin. Pre-treatment with Al $(OH)_3$ did not increase the thickness of gastric mucosa membrane compared to untreated rats. The combined Al $(OH)_3$ treatment with aspirin had a protective effect by increasing

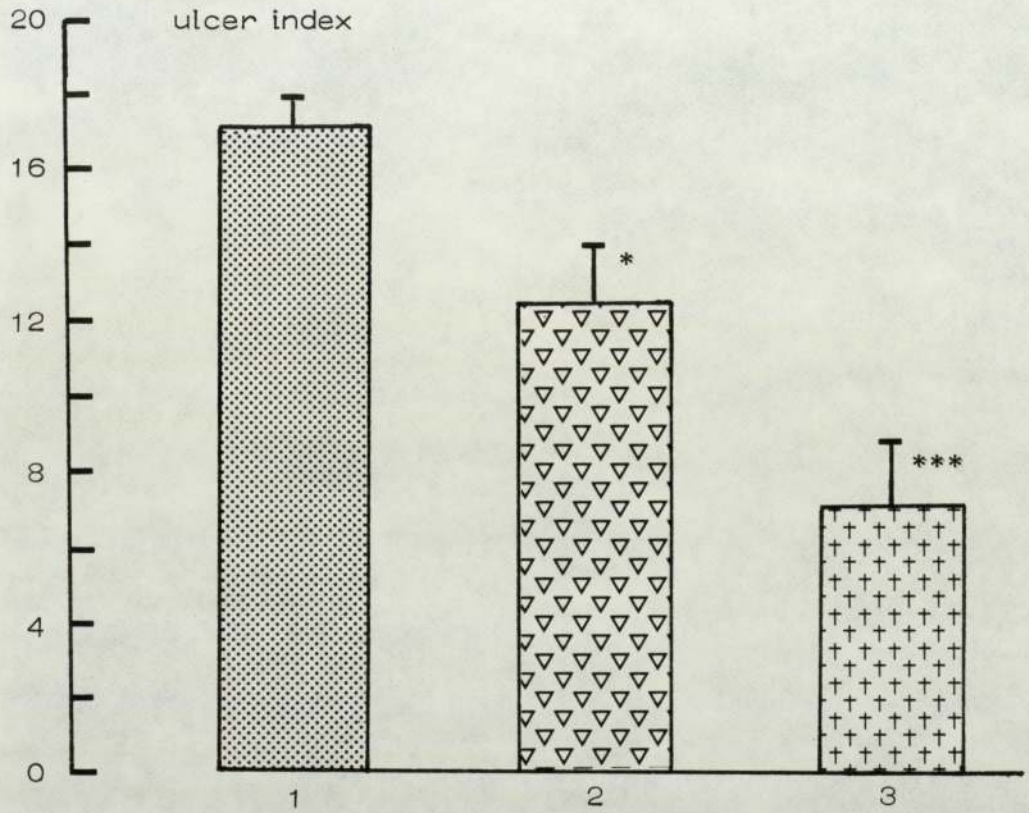


Figure 11. Ulcer indices of stomach treated with $Al(OH)_3$ and banana sample (A).

The figure shows histagrams representing the values obtained from $Al(OH)_3$ treated stomachs (2) compared with banana treated stomachs (3) and non-treated aspirin controls (1).

* $p<0.05$, *** $p<0.01$, significance of calculated by Wilcoxon rank sum test.

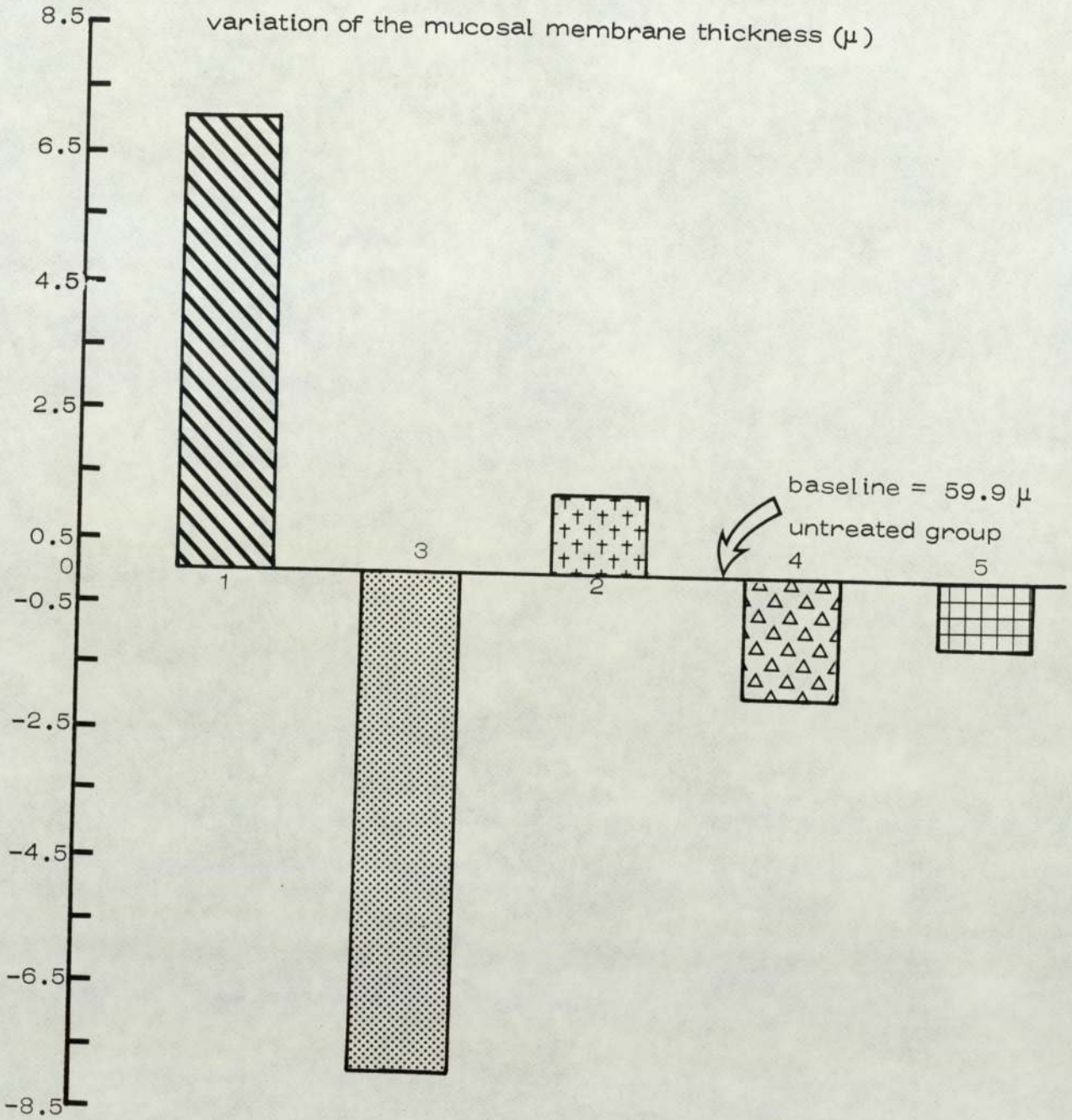


Figure 12. The effect of banana and $Al(OH)_3$ treatment on the thickness of the mucosa layer.

The Figure shows histograms representing the variation of thickness (in μ) of the mucosa after either aspirin (3), banana (1), $Al(OH)_3$ with aspirin (4) or banana with aspirin (2). The base line adjusted to value represents the normal untreated rats (59.9 μ). Values above the basal line show an increase in thickness and values below a thinning of the mucosa.

the gastric mucosa thickness by 13% compared to animals treated with aspirin alone.

a) Histopathology:

i) Non fasted rat:

Sections stained with P.A.S. - Alcian blue at pH 1 and 2.5 of unfasted rats indicate that the superficial cells of the gastric mucosa body secrete a mixture of neutral and acidic substances. The secretion from the cells lining the gastric faveolae appeared to be predominantly neutral mucin while at the base of the faveolae the cells secreted mainly acidic mucins as shown by P.A.S. - AB at pH 2.5 and traces of sulphated mucins as detected by P.A.S. - AB at pH 1.

The neck mucus cells showed a high affinity for P.A.S. The gastric pits were tightly closed and were full of mucus.

ii) Effect of fasting:

Fasting greatly decreased the thickness of the superficial film of mucopolysaccharide. The secretion of the mucus neck cells did not appear to be as much affected. H and E staining revealed irregularities at the mucosal surface (Figure 13). These probably correspond to the pin point erosions found in the macro-examination of fasted stomachs. The same area was examined with AB - P.A.S. stain at pH 2.5 and a slight widening of the gastric pits

was observed with fissures appearing between the glands. There was a complete absence of superficial mucin film.

iii) Effect of aspirin

The effect of the oral administration of aspirin at a dose of 150 mg kg^{-1} to fasted rats showed damage ranging from superficial erosions to complete deep ulcers which penetrated the whole glandular mucosa but not the muscularis mucosa. Exfoliation of the gastric mucosal surface was present in many sections. The gastric pits were widened and fissures, erosions and necrosis were found in the mucosa. The combined P.A.S. - AB at pH 1 and 2.5 showed that the mucin film coating the mucosa was thin. In one animal the mucin surface was completely absent and the mucous neck cells were rarely functioning, (Figure 14), vasodilation was observed in the capillaries. It was noted that vasodilation was prevalent in the sections where the upper part of the mucosa was exfoliated and the mucosa in this region was more eosinophylic than in other parts of the mucosa. In the ulcerated area the mucous neck cells only stained lightly for neutral mucins which may indicate low secretory activity.

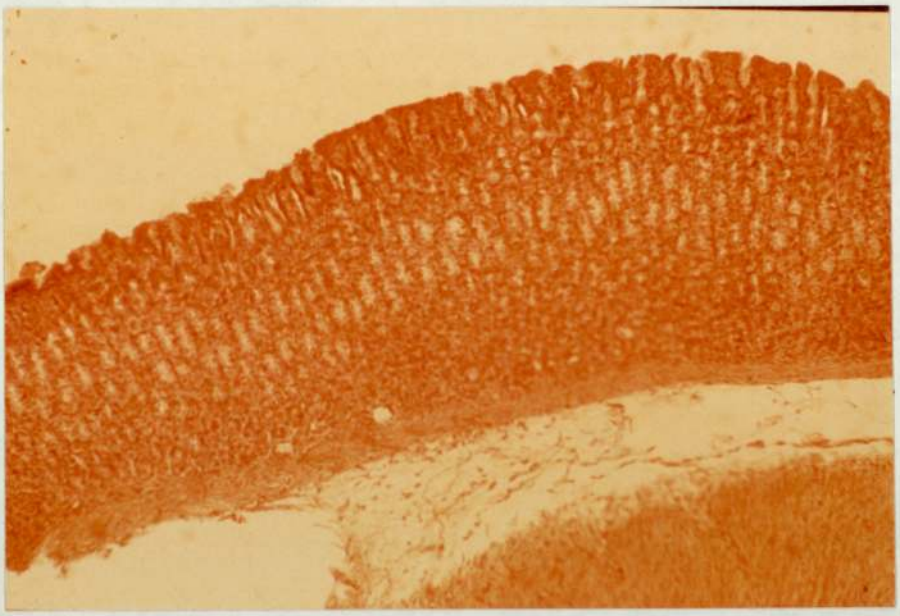


Figure 13. Section of the body of rats stomach starved for 48 h and stained with H.E (× 100 magnification)

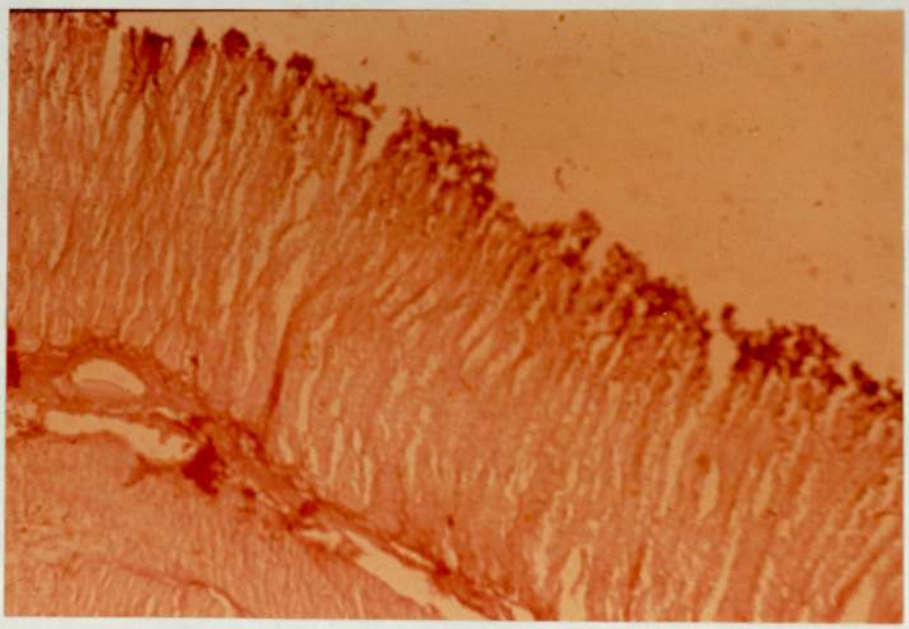


Figure 14. Effect of aspirin in 48 h starved rats. Section obtained from the stomach (body) stained with P.A.S. - AB, pH 2.5. (x 160 magnification).

iv) Effect of banana:

Banana treatment had a considerable protective effect against aspirin induced ulceration. The observed ulceration pattern in rats treated with combined banana with aspirin were found to vary from superficial erosions to medium ulcers. Deep ulceration involving the whole glandular mucosa was rarely found when the rats were previously treated with banana. The necrotic effect of aspirin in rats treated with banana was localized compared with a general necrosis in rat stomachs treated with aspirin only.

The H and E stain showed that sections from rats treated with banana combined with aspirin were less eosinophylic than aspirin alone and the eosinophylic staining was localized rather than diffuse as found with aspirin. Although banana treatment without aspirin was effective in increasing the thickness of the gastric mucosa membrane the P.A.S. - AB at pH 1 and 2.5 showed that banana also increased mucin secretion when compared to untreated rats (fasted only). In the banana treated rats a thick film of mucins (neutral and acidic mucins) was present on the mucosal surface. The mucous neck cells were active in secreting neutral mucins (Figure 15). Superficial erosions were rarely found in rats treated with banana only. The combined banana with aspirin treatment decreased the thickness of the surface mucin and it was occasionally

discontinuous particularly in the eroded and ulcerated areas of the stomach (Figure 16). At one ulcerated site P.A.S. - AB reactive substances below the ulcer stained with less intensity than in undamaged areas (Figure 17). However residual activity persisted in contrast with aspirin where secretory activity was totally lost. No vasodilation was observed in aspirin treated rats after previous treatment with banana. This may be due to the retention of an effective mucosal cell barrier with an increased secretion of mucin stimulated by banana. This would inhibit the back diffusion of H^+ to the basal layers of the mucosa.

v) Effect of aluminium hydroxide

Staining with P.A.S. - AB, pH 1 and 2.5 in rats treated with $Al(OH)_3$ at a dose 3 x 8 mg kg^{-1} during the 48 h fasting period showed that there was no variation in mucus content in the neck of the glands compared to untreated fasted rats only. A surface film of mucins persisted although it was thin. This was probably due to the $Al(OH)_3$ since it was absent in the fasted controls. No widening was observed in the gastric pits compared to untreated fasted rats. When $Al(OH)_3$ treatment was combined with aspirin the thin film of neutral mucin persisted on the surface except where erosions and ulceration occurred when the film was absent. No vasodilation was observed in the capillaries.

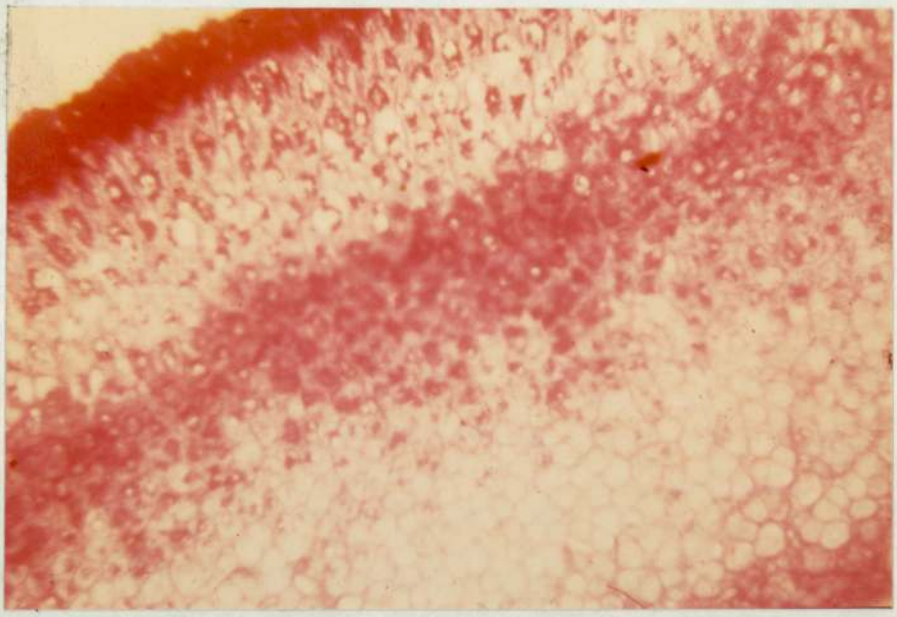


Figure 15. Effect of banana sample (A) treatment on rat stomach. P.A.S. - AB, pH 1. (x 400 magnification).

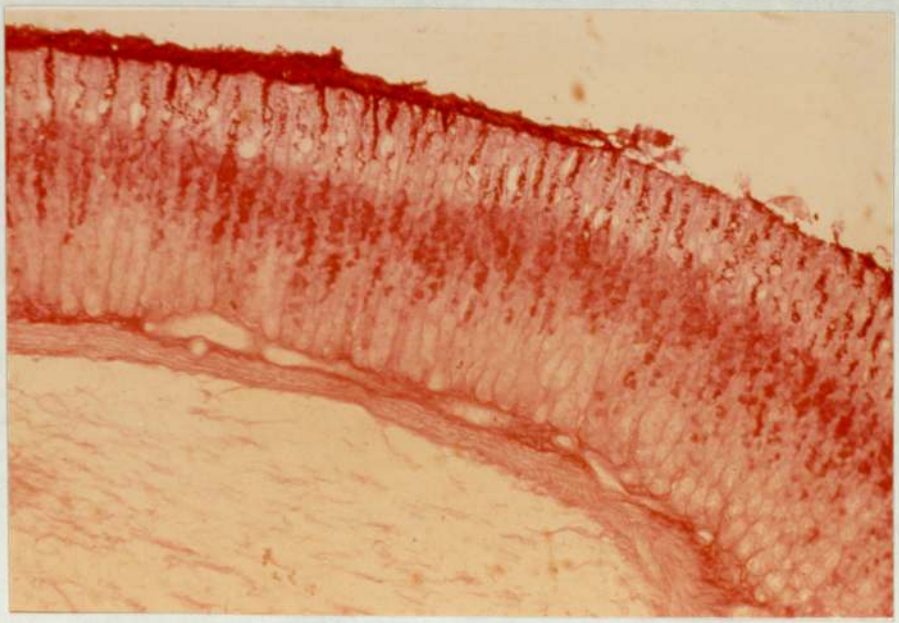


Figure 16. Effect of aspirin in rat stomach (body)treated with banana sample (A). P.A.S. - AB, pH 2.5, (x 100 magnification).

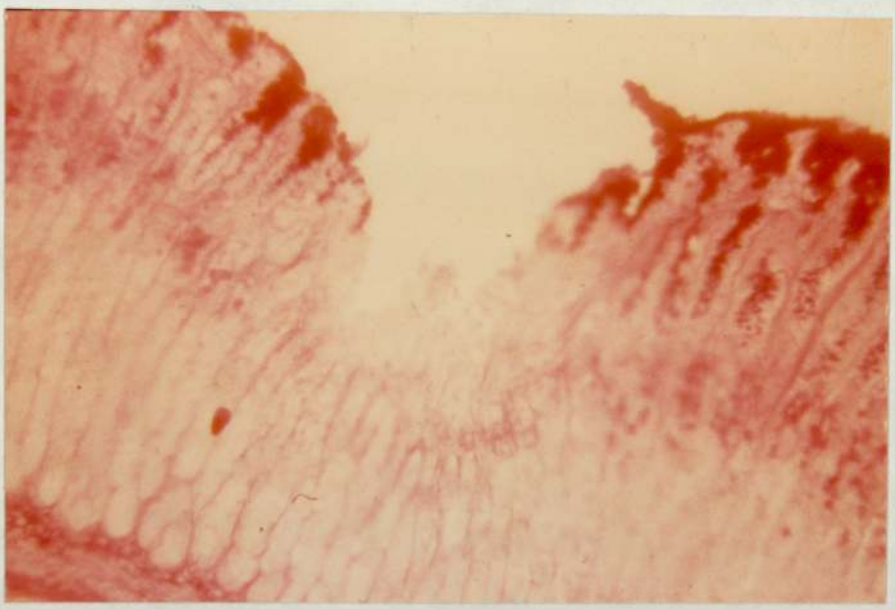


Figure 17. Effect of aspirin in rat stomach (body) treated with banana sample (A). P.A.S. - AB, pH 2.5 (x 400 magnification).

4.2 Gastric acid secretion in rats treated with the aqueous extract (f.O) of banana sample (A).

The aqueous banana extract was effective in protecting rats against ulceration induced by aspirin. The anti-ulcerogenic activity of the aqueous extract was accompanied by increases in gastric mucosal thickness when compared to aspirin treated rats (Table 20).

The aqueous banana extract had no effect on gastric acid secretion in ligated pyloric rats when compared to aspirin treated controls (Table 21).

4.3 The anti-ulcerogenic activity of fraction (13) administered by the intra-peritoneal route.

The ulcer index Table (22) showed that banana fraction (13) administered by the i.p. route was effective in healing rats from ulcers induced by aspirin. This finding was supported by the mucosal weights which show a significant increase in banana treated rats against aspirin treated controls (Table 22). The macroscopic examination of stomachs treated with fraction (13) (i.p.) indicate that the healing features were identical to those found by oral treatment with the same fraction.

4.4 The dose response for banana Sample (A) in healing ulceration.

The ulcer index determination of animals treated with banana at a dose of 2, 3 and 4 g showed that banana at all three doses was effective in healing ulcers. A dose of 3 g of banana was a significant improvement over 2 g but the 4 g dose was the same as a 3 g dose.

The mucosal weights support the results obtained with ulcer index values at 3 and 4 g doses of banana but significance was not found with mucosal weights after treatment with 2 g of banana Table (23).

a) Carbohydrate estimation in scraped mucosa of rats treated with 2, 3 and 4 g of banana Sample (A).

The quantitative estimation of sialic acids, hexosamines and fucose in the stomachs of rats treated with 2, 3 and 4 g of banana per 48 h did not show any significant variation when the values were calculated as carbohydrate per mg of tissue (Table 24). The total content of carbohydrate in total mucosa from stomachs is given in Table (25). The total sialic acid content of the scraped gastric mucosa was increased significantly in rats treated with 3 and 4 g of banana but not at 2 g when compared to controls. The total hexosamine also increased in the mucosa at 3 and 4 g of banana but not at the 2 g level. No variation was found in total fucose content at all dose levels. A comparison between the banana treated groups and

Table 20. Ulcer index and the gastric mucosal thickness of rats' stomachs treated with the aqueous extract (f.O) of banana sample (A).

Treatment	ulcer index	gastric mucosal thickness (μ)
Aspirin treatment	17.7 ± 2.1	46.7 ± 3.1
Aspirin treatment with aqueous banana extract (f.O)	11.5 ± 1.8 ***	56.9 ± 2.3 **

The results show that the active factor was water soluble.
*** $p<0.01$, (Wilcoxon- rank sum test) **$p<0.02$ (Student t test).

Table 21. Volume, acidity and acidity output of the gastric secretions of rats treated with aqueous banana extract (f.O)

Treatment	volume (ml) $100\ g^{-1}$ body weight	acidity mEQ of $H^+.L^{-1}$	acidity output mEQ of H^+
Aspirin treatment	1.08 ± 0.13	80.59 ± 8.65	88.53 ± 7.46
Aspirin treatment with aqueous banana extract (f.O)	1.11 ± 0.12	83.55 ± 8.8	89.17 ± 7.97

The results show that the factor was not anti-secretory.

Table 22. The curative anti-ulcerogenic activity of fraction (13),(sample E) by intra-peritoneal route

Group	ulcer index	mucosa weights (mg)
Aspirin treatment	15.85 ± 0.74	61.46 ± 1.9
Aspirin treatment plus i.p. treatment with fraction (13)	9.8 ± 1.21 ***	70.54 ± 2.42 **

The table above shows that the active factor acts systemically.
*** $p<0.01$ (Wilcoxon-rank sum test); ** $p<0.02$ (Students t test)

Table 23. The dose response of banana (A) in healing ulceration.

Treatment	ulcer index	mucosa weights (t_2) (mg)
Aspirin treatment	16.6 ± 1.1	56.72 ± 1.41
Aspirin treatment with 2 g of banana (A)	11 ± 3.5 **	59 ± 2.87
Aspirin treatment with 3 g of banana (A)	4.65 ± 0.55 ****	69.34 ± 1.66 ***
Aspirin treatment with 4 g of banana (A)	4.68 ± 0.62 ****	68.95 ± 1.97 ***

**** $p<0.001$; *** $p<0.01$; ** $p<0.02$

t_1 calculated by Wilcoxon rank sum test;

t_2 calculated by Students t test.

Table 24. Carbohydrate determination in scraped mucosa of rats treated with 2, 3 and 4 g of banana Sample (A)

Treatment	sialic acid $\mu g\ mg^{-1}$ of tissue	hexosamine $\mu g\ mg^{-1}$ of tissue	fucose $\mu g\ mg^{-1}$ of tissue	total sugar $\mu g\ mg^{-1}$ of tissue
Aspirin treatment	0.21 ± 0.02	11.35 ± 1.23	3.49 ± 0.36	15.46 ± 1.39
Aspirin plus 2 g banana over 48 h	0.22 ± 0.02	12.39 ± 1.69	2.91 ± 0.64	15.51 ± 2.33
Aspirin plus 3 g banana over 48 h	0.24 ± 0.01	14.11 ± 1.38	2.65 ± 0.4	17 ± 1.78
Aspirin plus 4 g banana over 48 h	0.26 ± 0.02	13.91 ± 1.36	2.84 ± 0.41	17.01 ± 1.77

This table shows that banana treatment did not increase the mucus content in ulcerated stomachs when the carbohydrate was estimated per unit mass of tissue.

Table 25. Total carbohydrate determination in scraped mucosa of rats treated with 2, 3 and 4 g of banana sample (A).

In Table 24 it was found that banana did not increase the carbohydrate content per unit mass of tissue. In this table (opposite) the sialic and hexosamine values increase with increasing doses of banana. This strongly suggests that the increase in mass is much higher than the increase in mucus constituents. This finding is discussed in the text. Possible reasons for the lack of increase in fucose levels in response to banana are also discussed in the text. Total carbohydrate also increased in response to banana treatment.

*** $p<0.01$; ** $p<0.02$; * $p<0.05$ (Students t test).

Table 25. Total carbohydrate determination in scraped mucosa of rats treated with 2, 3 and 4 g of banana Sample(A).

Treatment	total sialic acid (µg)	total hexosamine (µg)	total fucose (µg)	total carbohydrate (µg)	% increase of total carbohydrate
Aspirin treatment	12.23 ± 1.75	651 ± 55	201 ± 17.8	864 ± 74	
Aspirin plus 2 g banana over 48 h	13.85 ± 1.76	725 ± 78	191 ± 30.8	929 ± 110	7
Aspirin plus 3 g banana over 48 h	16.91 ± 1.08 *	927 ± 96 **	186 ± 33.1	1129 ± 130	31
Aspirin plus 4 g banana over 48 h	18.23 ± 1.63 **	1031 ± 118 ***	198 ± 34.4	1247 ± 154 *	34

*p<0.05 ; **p<0.02; ***p<0.01 (Student's t-test)

the aspirin controls showed that there was a considerable increase in the total carbohydrate levels in mucosa when the rats were treated with banana.

b) Histopathology:

i) Recovery from fasting:

The recovery from 48 hours fasting by feeding the rats was characterized by epithelization of the surface mucosa by the mucin surface film and the gastric pits were tight and full of mucin.

ii) Recovery from aspirin treatment

The pathological aspect of aspirin treatment were described in section (4.1.a, iii). Healing from aspirin-induced ulceration with no subsequent treatment was slow compared to the healing observed after subsequent banana treatment.

Five hours after aspirin administration the gastric pits were loose but 48 h later histological examination showed them to be much tighter at the surface of the mucosa. At the base of the glands some widening of the spaces between the glands persisted as did some necrosis in the tissue which stained actively with eosin. After 48 h some granule cells appeared in that area (Figure 18). The vasodilation due to aspirin administration tended to persist in most of aspirin treated sections. In some sections vasodilation of the capillaries was accompanied by complete

breakdown of the blood vessels with haemorrhage. In eroded areas the mucous neck cells were heavily stained with P.A.S. positive substances (Figure 19) compared to fasted rats not treated with aspirin. The stain for P.A.S. - AB at pH 2.5 showed that acidic mucus substances were absent at the base of the gastric pits in eroded areas. However in eroded areas the surface was also totally devoid of acidic mucin. Healing was also characterized by the formation of fibrotic tissue in the damaged area. The tissue was also infiltrated with granule cells. Negative reactions with P.A.S. - AB at pH 1 in the base of the faveole was found in all rats treated only with aspirin. It appears that healing from aspirin induced ulceration is characterized by hypersecretion of neutral mucins from the cells present in the neck of the glands and that these cells are also responsible for the regeneration of the surface mucous cells lost by exfoliation by the ulcerogenic agent. The sulphated mucous cells at the base of the faveolae were the last to function and that may have been due to their environment.

iii) Aspirin with banana:

The healing effect of banana on aspirin-induced ulceration was rapid. The ulcers were much less severe than in animals treated only with aspirin and the deep ulcers extending over the whole glandular mucosa frequently found with aspirin treatment

Figure 18. Effect of 48 h feeding on aspirin (150 mg kg^{-1}) -induced gastric ulceration in the rat . H.E. (x 160 magnification).

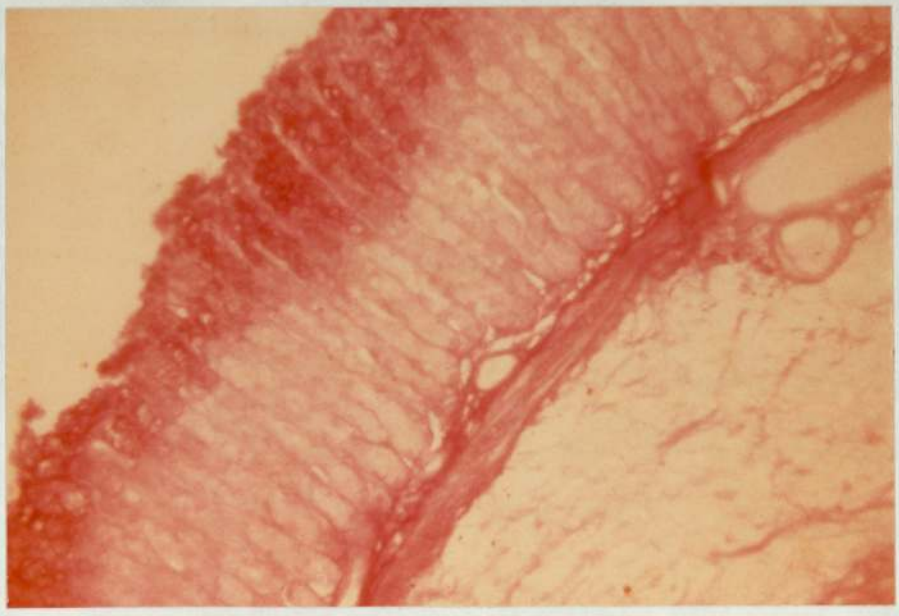


Figure 19. Effect of 48 h feeding on aspirin (150 mg kg^{-1}) -induced gastric ulceration in the rat P.A.S. - AB, pH 2.5. (x 160 magnification).

were rarely found. The gastric pits were tight and no widening was observed at the base of the glands. Eosinophylic tissue was absent at the base of the glands (Figure 20). A characteristic of the sections from animals treated with banana after aspirin was the regeneration of the mucin surface film (neutral and acidic mucins) and the surface mucous cells were heavily stained by P.A.S. - AB at pH 2.5 (neutral and acidic mucins). The mucous neck cells were apparently very active in secreting neutral mucins. This activity extended to the basal cells located below the neck cells in rats treated with banana but was absent in rats treated with aspirin only. This may indicate a rapid proliferation of cells in the neck region due to banana and extension of that activity into upper and basal tissues. The cells in the base of the faveolae were also active in secreting acidic mucins (P.A.S. - AB at pH 2.5) while the activity was relatively weak with aspirin treatment only. Another characteristic found in sections treated with banana was that at the base of the foveoles P.A.S. - AB at pH 1 positive substances (i.e. sulphated mucins) were found. Sulphated mucins were rarely observed at the base of the faveolae when the rats were treated with aspirin. It is possible that the absence of sulphated mucins in aspirin treated rats may contribute in part to the necrosis noted at the base of the faveolae in stomachs treated with aspirin only.

Examination by a binocular microscope showed that there was an increase in the thickness of the gastric mucosa at the ulcer edge. Ulcers were accompanied by hypersecretion of mucus in rats treated with banana. Microscopical studies with H and E staining of the same area showed that the process of healing was characterized by a proliferation of regenerating cells both at the base and the edges of the ulcer and a high concentration of mucous cells was observed in the ulcerated area (Figure 21). The P.A.S. - AB staining at pH 2.5 of the same areas showed that cells were very active in secreting neutral and acidic mucins.

4.5 Deoxyribonucleic acid (DNA) content in stomachs from rats treated with banana Sample(A).

The ulcer index, the mucosal weights, the DNA ($\mu g\ mg^{-1}$) of tissue and the total DNA content of gastric mucosal scrapings are given in Table (26).

It was previously reported that banana sample (A) was effective in healing animals of ulceration induced by aspirin and the healing was associated with increases in mucosal thickness and mass. The increase in the DNA content of the scraped mucosa after banana treatment (banana with aspirin) compared with aspirin treatment only supports these findings and the increase in DNA reflects an increase in cell mass.

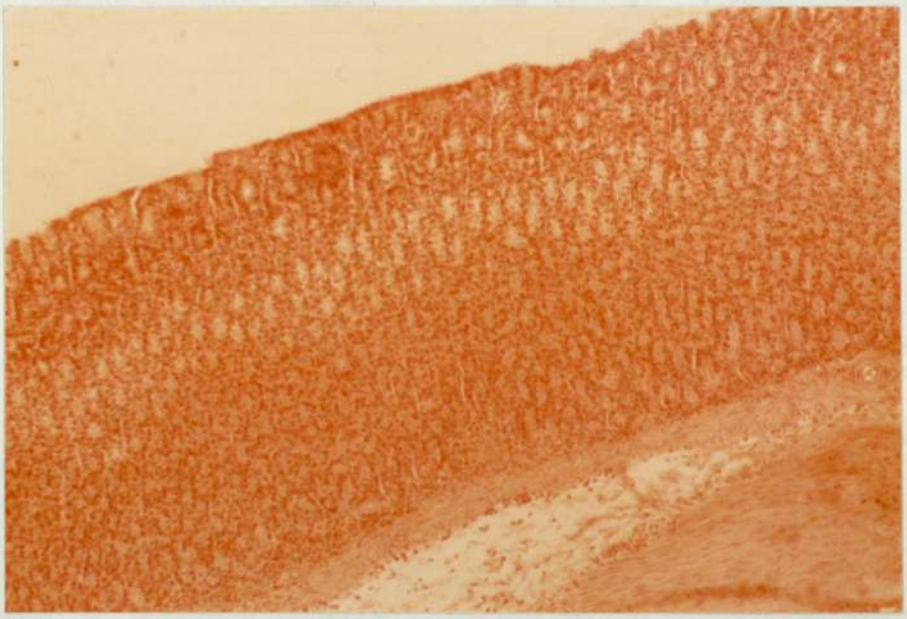


Figure 20. Effect of banana sample (A) treatment on aspirin-induced gastric ulceration in the rat H.E. (x 160 magnification).

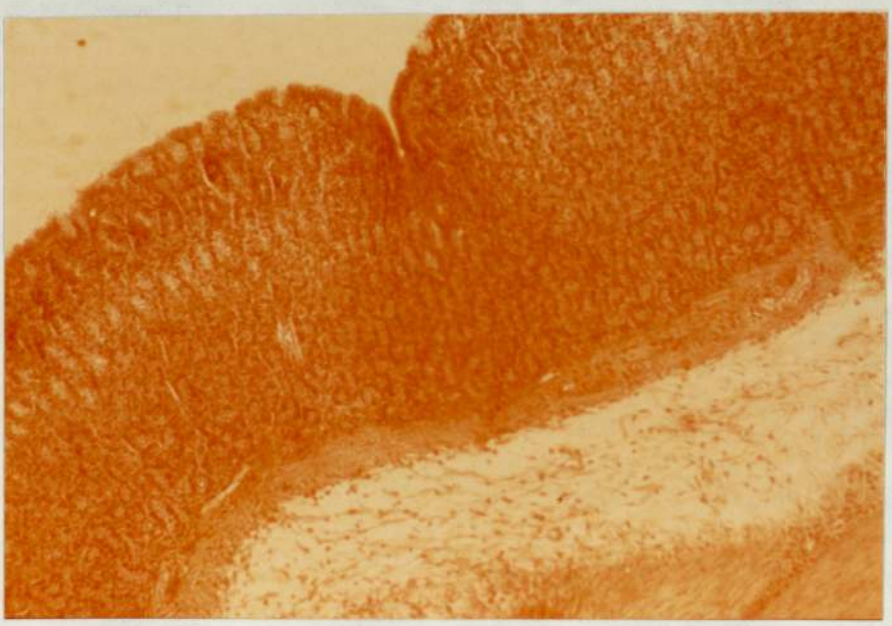


Figure 21. Effect of banana sample (A) treatment on aspirin-induced gastric ulceration in the rat. H.E. (x 160 magnification).

Table 26. The curative effect of banana on the DNA content of rat gastric mucosal scrapings:

Treatment	ulcer index (t_1)	mucosal weights (mg) (t_2)	DNA µg mg^{-1} of tissue (t_2)	total DNA (µg) per freeze dried mucosa
No treatment	1.80 ± 0.2 ****	73.2 ± 2.1	6.97 ± 1.11	511 ± 78
Aspirin treatment	14.42 ± 0.61	71.2 ± 2.1	7.12 ± 0.72	507 ± 53
Aspirin with banana (A)	7.02 ± 0.45 ****	84.9 ± 1.58 ****	9.55 ± 0.68 **	811 ± 59 ***

The Table(above) gives the DNA content (u g, mg^{-1} of tissue and total DNA) ofmucosa after banana treatment. Banana increased both the DNA content per unit mass and also the total DNA per stomach. This result supports other evidence in this thesis that banana promotes cellular proliferation.

**$p<0.02$, ***$p<0.01$, ****$p<0.001$, t_1 :significance calculated by Wilcoxon rank sum test compared to aspirin treated animals. t_2: significance calculated by Students t test compared to aspirin treated animals

The increase in DNA content of banana treated rats was significant both when the DNA was estimated per mg of tissue and when the total DNA content of the scraped mucosa was estimated. The mucosal weights of the untreated animals were not significantly different from the mucosal weights of the aspirin treated animals and no variation was found between either the total DNA content or the DNA per mg of tissue. The efficiency of the DNA extraction was calculated and was found to be 92%. Therefore a correction was made in Table (27) for this factor and the % increase of DNA in banana treated rats compared to aspirin treated ones.

4.6 Anti-secretory activity of cimetidine, prostaglandin E-2 (PGE-2), 5-hydroxytryptamine (5-HT) and dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP) using the "Shay rat" model.

Cimetidine and PGE-2 possessed anti-secretory activity in decreasing both volume and acid output in rat while 5-HT decreased only the gastric volume (Table 28).

Cimetidine at a dose 240 mg kg^{-1} given orally was highly significant in inhibiting gastric acid secretion (volume and acid output). PGE-2 at a dose 600 $\mu g\ kg^{-1}$ (s.c.) was equally effective in inhibiting gastric secretion in both volume and acidity. 5-HT at a dose 1.5 mg kg^{-1} administered orally showed a significant

Table 27. The effect of banana treatment on gastric mucosal DNA after correcting for 92% extraction.

Treatment	DNA $\mu g\ mg^{-1}$ of tissue	total DNA per freeze-dried mucosa	% of total increase on DNA
No treatment	7.52 ± 1.19	551 ± 84	-
Aspirin treatment	7.68 ± 0.77	547 ± 57	1
Aspirin treatment plus banana	10.31 ± 0.73 **	875 ± 63 ***	60

The Tableabove is the correction of the raw data in Table 26. The correction is based on the experimental controls in the analytical procedure where 92% of standard DNA added was found. The Table therefore gives the theoretical content of DNA in the tissues compared to the actual amount found recorded in Table 26.

**$p < 0.02$; ***$p < 0.01$, (Student's t-test)

Table 28. The anti-secretory activity of cimetidine, PGE-2, 5-HT and dib-cAMP using the modified "Shay rat" model.

Treatment	volume in ml $100g^{-1}$ body weights	% of inhibition	H^+ mEQ l^{-1}	output H^+ mEQ	% of inhibition (acidity)
Control	1.45 ± 0.17	-	35.2 ± 6.31	46.51 ± 6.2	-
Cimetidine	0.26 ± 1.11 ****	82	46 ± 7.84	12.8 ± 5.24 ****	72
PGE-2	0.3 ± 0.11 ***	79	41.1 ± 7.99	11 ± 5.22 ****	76
5-HT	0.7 ± 0.1 ***	51	93.3 ± 12.6 ****	52.5 ± 7.66	-
Dib-cAMP	1.36 ± 0.21 ***	6	42.2 ± 6.8	49.3 ± 7.63	-

TheTable (above) shows that cimetidine and PGE-2 possessed anti-secretory properties (volume and acid output). However a very high dose was used. This was an attempt to prolong their response to be used for a comparative purpose when examining the mode of action of banana. 5-HT was anti-secretory in terms of volume only but the acid output was not different from the control. Dib-cAMP was not an anti-secretory agent(volume and acid output)

***$p < 0.01$, ****$p < 0.001$, (Student's t-test)

decrease in gastric volume only and no variation was observed in the output of gastric acidity. dib-cAMP at a dose of 600 mg kg^{-1} was not effective in altering gastric acid secretion in terms either of volume or acidity.

4.7 The assessment of cimetidine, prostaglandin E-2 (PGE-2), 5-hydroxytryptamine (5-HT), and dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP) as protective and healing agents in rat gastric ulceration studies.

The assessment of cimetidine, PGE-2, 5-HT and dib-cAMP as agents in healing and protecting rats from ulceration is summarized in Table (29) as ulcer indices and mucosal weights.

All the pre-treatments with cimetidine, PGE-2 and dib-cAMP at doses of 240 mg kg^{-1} for cimetidine, 600 µg kg^{-1} for PGE-2 and dib-cAMP, had a significant effect in protecting rats from aspirin-induced ulceration. Post-treatment with these agents at the same dose levels given in three oral administrations (except for PGE-2 s.c.) spaced at 8 h intervals, 5 h after aspirin treatment did not heal ulceration. Pre-treatment with PGE-2 on examination the stomachs appeared healthy and showed no evidence of deep ulceration or haemorrhage. Dib-cAMP was much less potent than PGE-2 in protecting the animals against ulceration. However the examination of rats stomach pre-treated with dib-cAMP showed

Table 29. The assessment of cimetidine, PGE-2, 5-HT and dib-cAMP as agents in protecting (pre-treatment) and healing (post-treatment) rats of ulceration.

The results (opposite) show that although cimetidine, PGE-2 and dib-cAMP inhibited ulceration (pre-treatment) as shown by the ulcer indices but they did not heal (post-treatment) ulcers. The protective effect of cimetidine, PGE-2 and dib-cAMP was not associated with an increase in mucosa weights which is different to banana mode of action. 5-HT did not prevent or heal ulcers.

Table 29. The assessment of cimetidine, PGE-2, 5-HT and dib-cAMP as agents in protecting and healing rats of ulceration.

Treatment	ulcer index	% inhibition of ulcer index	mucosal weights in mg
Aspirin	16.05 ± 1.17		81.45 ± 3.05
None	1.83 ± 0.64 ****		82.27 ± 3.13
Pre-cimetidine	7.14 ± 1.16 ****	56	80.21 ± 3.71
Post-cimetidine	14.17 ± 0.84		80.64 ± 1.52
Pre-PGE-2	4.42 ± 0.9 ****	73	83.04 ± 4.31
Post-PGE-2	14.25 ± 1.28		85.15 ± 2.43
Pre-5-HT	14.00 ± 1.43		81.28 ± 3.21
Post-5-HT	13.42 ± 1.71		83.48 ± 3.84
Pre-dib-cAMP	9.42 ± 1.71 ***	41	84.77 ± 3
Post-dib-cAMP	13.02 ± 0.64		85.22 ± 1.9

Pre and post indicate that animals were treated with the appropriate agents either before or after aspirin administration.

****$p<0.001$; ***$p<0.01$ (Wilcoxon rank sum test).

that the degree of ulceration was less severe than in aspirin treated controls.

The examination of the stomach after pre and post-treatments with cimetidine showed that a thin film of white mucus covered most of the mucosal surface. Its presence is an indication of an inhibition or absence of the digestive activity of the gastric juice. The stomachs of animals pre-treated with cimetidine showed only superficial erosions and no signs of deep ulceration or haemorrhage. Cimetidine was not effective in healing ulceration after only two days treatment.

Pre and post-treatment with 5-HT had no effect in decreasing the ulcer index. Examination of the stomachs showed that there was a hypersecretion of mucin but the mucin was stained yellow (bile pigment) and less viscous than normal. The mucus was similar to gastritis secretion as reported by Rhodes (1972). It was noticed in rats treated with 5-HT that a reflux of the duodenal secretions back into the stomach had occurred. Bile was evident in the stomachs of rats that had been both pre and post-treated with 5-HT and the mucosa was stained with pigment. In one animal the yellow-green colouration was so intense that it could be seen through the stomach wall using back lighting prior to opening the stomach. The severity of the ulceration in animals both pre and post-treated with 5-HT was of the same order as with animals treated with aspirin. Cimetidine,

PGE-2, 5-HT and dib-cAMP did not induce any significant increases in the gastric mucosal weights (Table 29).

a) Mucopolysaccharide and protein estimations

i) Protein estimation

The protein content of the stomach of rats treated with cimetidine, PGE-2, 5-HT and dib-cAMP are given in Table (30). It was found that only PGE-2 pre-aspirin treatment induced just a significant decrease in protein content when compared with aspirin controls. While a significant decrease was obtained with PGE-2 in pre- and post-aspirin treatments when the results were compared with normal controls.

Forty-eight hours after the administration of aspirin no variation in protein levels was found in aspirin treated rats when compared to untreated controls. The same results were found for 5-HT, dib-cAMP and cimetidine treatment.

ii) Hexosamine content:

The hexosamine content of mucosa of all treatments is shown in Table (30). PGE-2 was found to be only effective in reducing the hexosamine content at a dose of 600 $\mu g\ kg^{-1}$ in pre-aspirin treated rats when the results were compared to untreated control rats.

Table 30. Protein and hexosamine content of rats gastric mucosal scraping after pre and post-treatment with cimetidine, PGE-2, 5-HT and dib-cAMP.

Treatment	protein $\mu g\ mg^{-1}$ of tissue	hexosamine $\mu g\ mg^{-1}$ of tissue
None	153 ± 8.3	117 ± 10.9
Aspirin	147 ± 10.15	100 ± 10.6
Pre-cimetidine	149 ± 18.5	102 ± 11.06
Post-cimetidine	134 ± 11	104 ± 11.18
Pre-PGE-2	109 ± 14.3 ** or t	88 ± 7.74 *
Post-PGE-2	118 ± 12.1 *	90 ± 8.22
Pre-5-HT	131 ± 14.7	127 ± 12.14
Post-5-HT	150 ± 15.8	109 ± 12.3
Pre-dib-cAMP	142 ± 19	94 ± 6.56
Post-dib-cAMP	145 ± 17	109 ± 11.17

No agent increased the protein or hexosamine content per unit mass. PGE-2 even depressed the values slightly in the pre-treatment procedure.

** $p<0.02$; * $p<0.05$ (significance compared to untreated rats using the Students t test; t $p<0.05$ significance compared to aspirin treated groups.

Aspirin at a dose 150 mg kg^{-1} had no effect in reducing the hexosamine content of mucosa scraped from stomachs, 48h after its administration. No variation in the hexosamine content of mucosa was found in both cimetidine, dib-cAMP and 5-HT treated animals.

iii) Hexose content of rat mucosa:

The hexose content of rat mucosa is given in Table (31). No significant variation in hexoses levels was found in either pre- or post-treated rats with cimetidine, PGE-2 and dib-cAMP 49 h and 24 h after the administration of the agents compared to untreated controls. No variation was found between normal and aspirin treated rats 48 h after aspirin administration. An increase in hexose content was found in rats post-treated with 5-HT at a dose 1.5 mg kg^{-1} (in three oral doses at 8 h interval) when compared to aspirin treated rats. Rats pre-treated with 5-HT showed no significant increases in mucosal hexose content compared to aspirin treated rats.

iv) Fucose and sialic acid content of rat mucosa:

The fucose and sialic acid content of the mucosa is given in Table (31). No variation was found in either sialic acid or fucose levels in rats treated with aspirin, 48 h after the aspirin was administered. None of the agents e.g. cimetidine, 5-HT, dib-cAMP or PGE-2 induced a significant variation in fucose or sialic acid levels, 24 h and 49 h after the last doses of those agents.

Table 31. Hexose fucose and sialic acid content of rats gastric mucosal scraping after pre- and post-treatment with cimetidine, PGE-2, 5-HT and dib-cAMP.

Treatment	hexose $\mu g\ mg^{-1}$ of tissue	fucose $\mu g\ mg^{-1}$ of tissue	sialic acid $\mu g\ mg^{-1}$ of tissue
None	150 + 11.92	32.4 + 3.2	14.4 + 0.68
Aspirin	137 + 14.6	28.65 + 3.53	13.8 + 1.75
Pre-cimetidine	153 + 18	31.64 + 2.23	14.3 + 0.88
Post-cimetidine	129 + 12	30.82 + 2.47	13.2 + 0.96
Pre-PGE-2	153 + 21.4	26.52 + 2.31	16.06 + 1.87
Post-PGE-2	123 + 11.4	23.14 + 3.79	13.7 + 1.37
Pre-5-HT	165 + 11	30.72 + 3.05	14.2 + 1.32
Post-5-HT	185 + 16.2 *	28.07 + 2.68	13.5 + 1.35
Pre-dib-cAMP	144 + 11.7	36.62 + 3.4	16.7 + 2.16
Post-dib-cAMP	153 + 7	29.64 + 3.86	14.1 + 2

All values were not significant apart from a small increase in hexose values when 5-HT was used in the curative procedure.

* $p<0.05$; significance was calculated by Students t test compared to aspirin untreated animals.

b) Histopathology and histochemistry:

The histochemistry of untreated animals was discussed in section 4.1.a and the histochemistry and histopathology of rats treated with aspirin was discussed in section (4.1.a.iii).

i) Pre and post-treatment with cimetidine:

H and E staining of stomach sections from rats subjected to pre- and post-treatment with cimetidine showed that there was a uniform regularity in the surface of the mucosa in undamaged areas. The gastric pits were tight in sections of stomachs from animals both pre- and post-treatment with cimetidine. No necrosis was found in sections pre-treated with cimetidine. Only slight vasodilation was present after post-treatment with cimetidine. The P.A.S. - AB in pre-treated and in healthy areas of the post-treated rats with cimetidine showed that the gastric pits were packed with neutral mucins. (Figure 22). The cells in the neck of the glands stained heavily with P.A.S. reagent.

The distribution of sulphated and acidic mucins in the base of the pits in rats pre-treated with cimetidine were similar to untreated control rats but in rats post-treated with cimetidine the stain for acidic and sulphated mucins was weak at the base of the pits. In the eroded and ulcerated areas of rats post-treated with cimetidine there was an increase in P.A.S. activity (neutral mucins) in cells in the neck of the glands. In both cimetidine pre- and post-treated rats

the parietal cells in the base of the glands were slightly swollen compared to untreated controls and that may be due to the hypoactivity of the parietal cells in secreting gastric acid secretion.

ii) Pre- and post-treatment with prostaglandin E-2 (PGE-2).

The H and E stain in sections from stomachs pre-treated with PGE-2 showed that the gastric pits were tight and no eosinophylic staining was present in the sections. Slight eosinophylic staining was evident only in damaged areas. Post-treatment with PGE-2 gave a similar result to pre-treatment except that eosinophylic staining of the stomach was evident. Vasodilation was just evident after post-treatment with PGE-2 but was absent in rats pre-treated with PGE-2. The P.A.S. - AB at pH 2.5 and pH 1 in pre- and post-treated rats with PGE-2 showed that the mucus secretion in healthy areas were slightly decreased (Figure 23) compared to healthy areas in sections from rats treated with aspirin only. The P.A.S. staining was less intense compared to those from cimetidine treated rats. However both cimetidine and PGE-2 inhibited gastric acid secretion. The decrease in P.A.S. activity in pre- and post-treated rats with PGE-2 is in agreement with the decrease in protein and the hexosamine found with the biochemical determination of carbohydrates. In pre- and post-treated rats with PGE-2, the cells at the base of the glands were swollen compared to untreated rats or those treated with aspirin only.

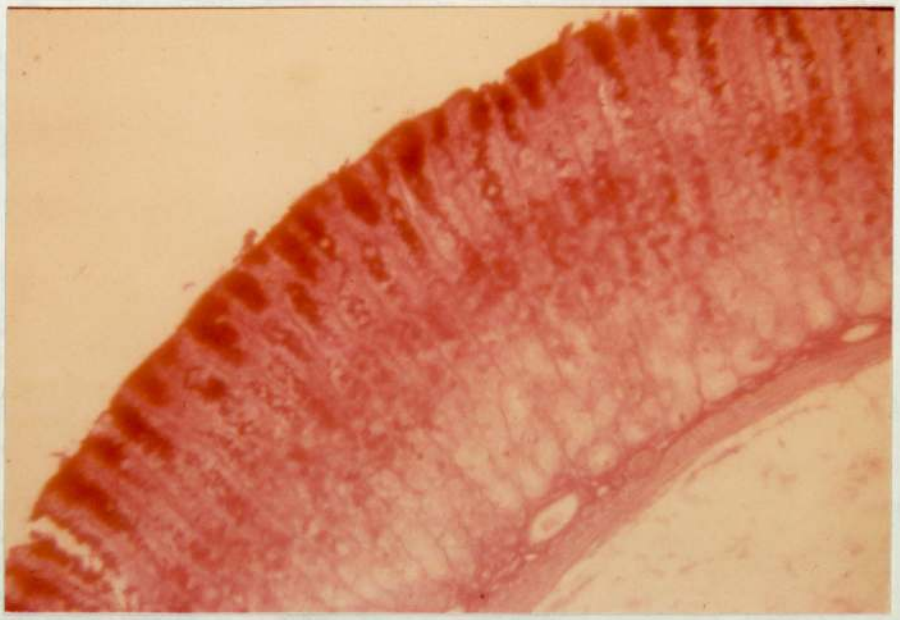


Figure 22. Effect of post-cimetidine (240 mg kg^{-1}) treatment on aspirin-induced gastric ulceration in the rat P.A.S. - AB, pH 2.5. (x 160 magnification).

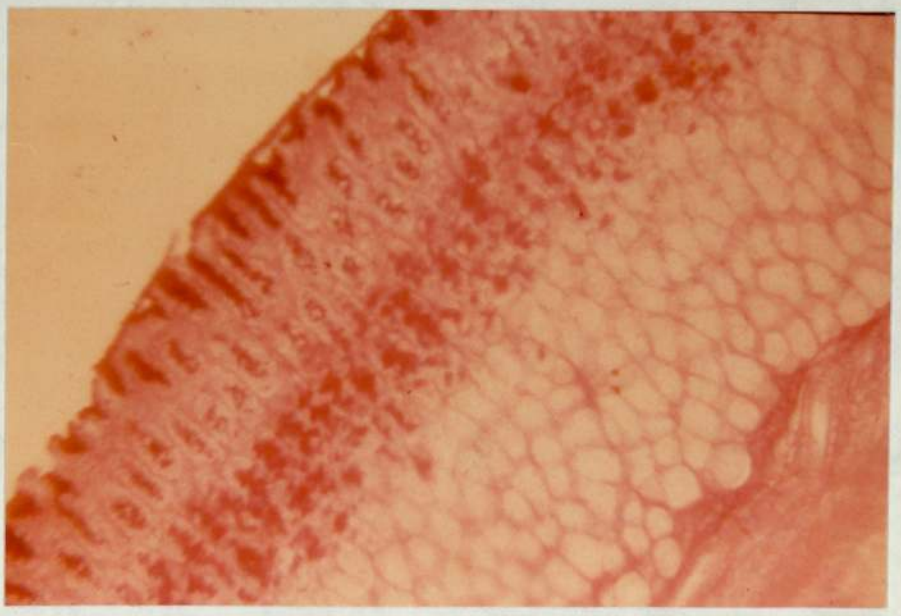


Figure 23. Effect of pre-PGE-2 treatment (600 µg kg^{-1}) on aspirin-induced gastric ulceration in the rat P.A.S. - AB pH 2.5. (x 200 magnification).

iii) Pre- and post-treatment with 5-hydroxytryptamine (5-HT):

The examiniation of stained sections with H and E from animals pre- and post-treated with 5-HT at a dose 1.5 mg kg^{-1} showed that the mucosa was eosinophylic, vasoconstriction was also found in the capillaries which is associated with thrombosis. The P.A.S. - AB stain at pH 2.5 showed that the mucosa was necrotic and this necrosis penetrated into areas where the surface of the mucosa appeared to be normal and protected by a film of mucin (Figure 24). The necrosis in some sections extended to the muscularis mucosa although the surface mucosa was covered with neutral mucin. Neighbouring cells to the eroded areas of the muscularis mucosa showed evidence of hypertrophy. The mucosa was oedematous and it was observed in these sections that the mucus secretion was fluid when the stomachs were originally opened for examination. The P.A.S. stain in oedematous tissue diffused throughout the body and the base of the glands and small patches of diffused neutral mucin could be identified at the base of the glands. In one section major necrosis was also found in the mucosa at a region distant from adjacent ulceration. However the pattern of necrosis was different to rats treated with aspirin only. A section from mucosa stained with bile pigments showed that in the stained regions the mucin film was less organized on the surface mucosa than normal. 5-HT had a considerable effect in stimulating neutral

mucin secretion from the mucous cells at the surface. The increase in neutral mucin was not accompanied by an increase in acidic mucin on the surface, or at the base of the faveolae.

iv) Pre and post-treatment with dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP).

The H and E stain in sections of stomachs from rats subjected to pre-dib-cAMP treatment showed that no necrosis in the base of the glands had occurred compared to rats treated with aspirin only. Only slight eosinophylic staining was localized at the base of the glands. No vasodilation was observed. The combined P.A.S.-AB stain at pH 2.5 of sections pre-treated with dib-cAMP showed that the surface mucosa was actively covered with neutral and acidic mucins (Figure 25) and the mucous neck cells were active in secreting neutral mucin. Traces of sulphated mucins were observed at the base of the faveolae (P.A.S. - AB at pH 1). The H and E staining of sections post-treated with dib-cAMP showed that the surface mucosa was eroded but no widening was present at the base of the glands (Figure 26). Some vasodilation was observed but it was less severe than in sections from rats treated with aspirin only. The combined P.A.S. - AB stain at pH 2.5 and pH 1 in non-eroded areas showed that the surface mucosa and the gastric pits were packed with neutral mucin and acidic mucin secretory activity was also evident (Figure 27). In eroded and ulcerated areas the section was similar to sections from rats treated with aspirin only. A surface mucin film was rarely evident on the mucosa surface.

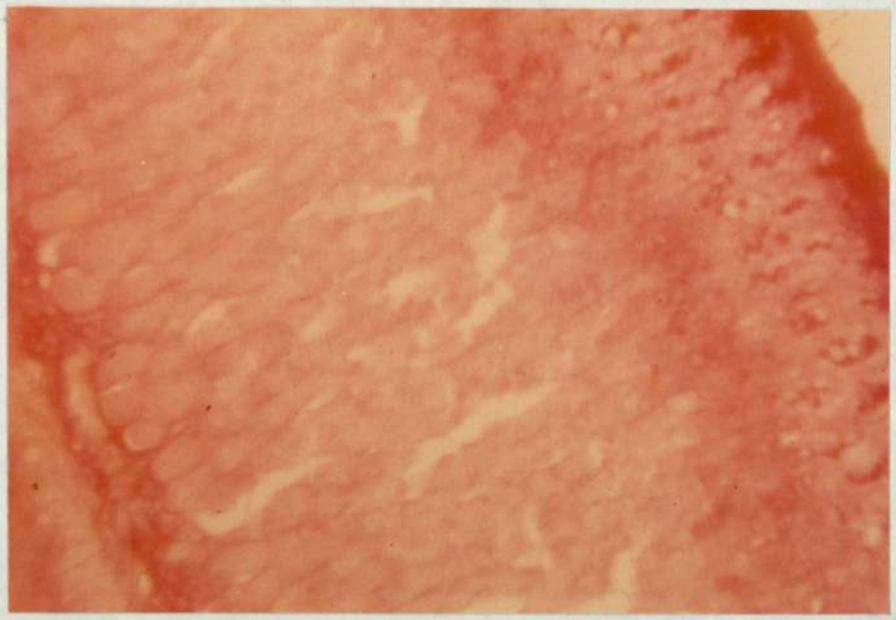


Figure 24. Effect of pre-5-HT treatment (1.5 mg kg^{-1}) on aspirin-induced gastric ulceration in the rat P.A.S. - AB, pH 1. (x 400 magnification).

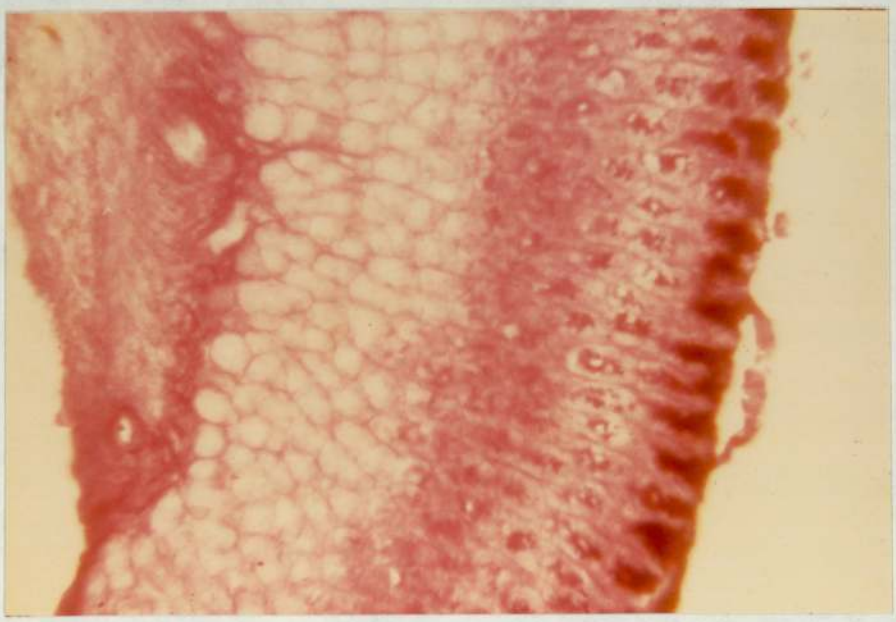


Figure 25. Effect of pre-dib-cAMP treatment (600 $\mu g\ kg^{-1}$) on aspirin-induced gastric ulceration in the rat P.A.S. - AB, pH 2.5. (x 200 magnification).

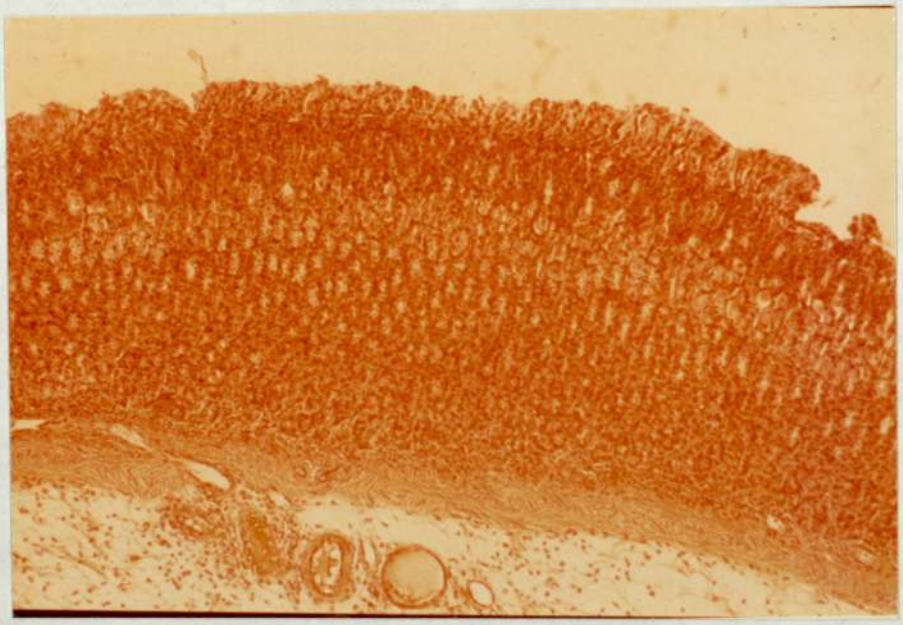


Figure 26. Effect of post-dib-cAMP (600 $\mu g\ kg^{-1}$) treatment on aspirin-induced gastric ulceration in the rat. H.E. (x 160 magnification).

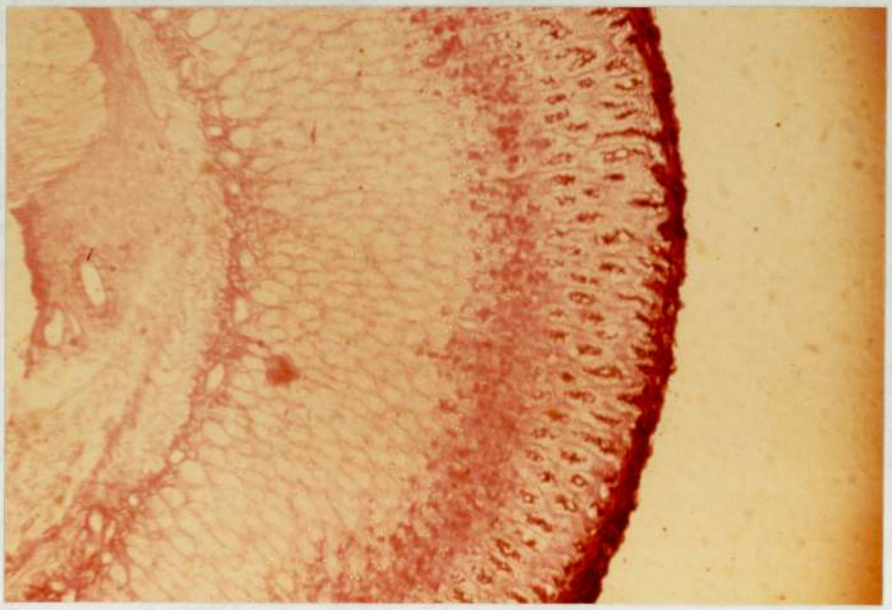


Figure 27. Effect of pre-dib-cAMP (600 $\mu g\ kg^{-1}$) treatment on aspirin-induced gastric ulceration in the rat P.A.S. - AB, pH 2.5. (x 160 magnification).

5. DISCUSSION

5.1 The protective effect of banana and Al $(OH)_3$ in preventing ulceration.

Both the ulcer index and the mucosal thickness results show that banana powder sample (A) at a dose 5 g per rat over 48 h protects rats against aspirin induced ulceration and that its effect was greater than Al $(OH)_3$ at a dose of 3×8 mg kg^{-1} over 48 h. The results also show that protection by banana is different to the neutralizing effect of Al $(OH)_3$ since only banana induced an increase in mucosal thickness. Al $(OH)_3$ had no effect on mucosal thickness.

This increase in mucosal thickness in rats treated with banana is probably the major protective factor against ulceration induced by aspirin in the rat.

a) Histopathology and histochemistry:

i) Effect of fasting on the gastric mucosa:

It is now generally accepted that the mucus barrier protects the underlying mucosa from ulceration and a diminution in mucus secretion may at least in part contribute to the development of erosions and ulceration (Piper et al., 1965, Glass et al., 1967).

The results suggested that fasting decreased the rate of secretion of mucus which would provide suitable conditions for

aspirin to induce ulcers. These findings are in agreement with Dekanski et al., 1975 who found that fasting decreased the rate of uptake of (C^{14}) N-acetyl hexosamine by mucosal cells.

The histology indicated that at the base of the gastric pits and in cells situated in the neck of the glands, the staining for acid, neutral and sulphated mucins was similar to that in fed controls. However the surface epithelial cells in fasted rats gave less intense staining patterns for neutral and acidic mucins than in fed rats suggesting that surface secretion was diminished. In fasted rats the gastric pits were slightly enlarged with a decrease in mucus staining in that region. This would allow access of pepsin and other gastric acid secretions which would presumably have erosive effects on the surface cells lining the gastric pits. These enlarged, damaged gastric pits may correspond to the pin point erosions observed by binocular microscopic examination of the stomachs.

ii) Effect of aspirin treatment on the gastric mucosa.

The mucosal thickness was found to be a useful index for the quantitative estimations of the effects of various treatments on the stomach. It was conveniently measured as mean values of several measurements in sections taken from stomachs after various treatments in vivo. The use of such sections also offered the advantages that the same section could be used for subsequent histology and histopathology studies. The histology showed that

aspirin treatment, after fasting, had reduced the mucosal thickness. when banana was combined with aspirin treatment , the banana redused the thinning induced by aspirin alone.

The decrease in mucosal thickness coupled with the observed ulceration and exfoliation of the surface mucosal cells are in agreement with the results of Max and Menguy (1969, 1970) who reported that aspirin induces exfoliation in the mucosa surface cells.

The deeply ulcerated areas of the mucosa in rats treated with aspirin probably developed in the less resistant parts of the mucosa where the open gastric pits were evident and mucus secretion was diminished due to previous fasting. These sites would act as foci for ulceration since the gastric juice would be able to penetrate into deeper tissues. Exfoliation of surface cells was often associated with vasodilation. This observation was also reported by Bruggeman et al., (1979). The reason for the vasodilation observed is not clear but it may well be due to the back diffusion of H^+ from the lumen to the serosa, following damage to the mucosal barrier by aspirin. Previous reports have stated that carbohydrate constituents decreased after aspirin administration (Robert et al., 1963, Menguy and Desbaillets, 1967). The histological studies were in agreement with these observations since it was noticed that the staining for mucin in rats treated with aspirin was much less intense than in sections from untreated controls.

iii) Effect of banana in preventing ulceration:

The main protective effect of banana was to increase the thickness of the gastric membrane by increasing the number of cells and therefore the amount of mucus secreted. The increase in thickness of the mucosal membrane was not due to a morphological modification of the mucosa as the cells appeared normal when stained with H. and E. In the combined treatment of banana with aspirin, eosinophylic stained tissue was localized in the superficial areas where erosions and medium ulcers occurred. The general histopathological picture was mild compared to the deep ulceration and general necrosis present when aspirin was used alone. This may explain why vasodilation was not found in animals treated with aspirin combined with banana. The increase in mucosal thickness, following banana treatment was associated with increase on mucin secretion (neutral, acidic and sulphated). This is of interest because Garner et al. (1979) reported that HCO_3^- is secreted by the surface mucosal cells and that HCO_3^- passes into and through the mucus barrier and therefore sets up a protective neutralizing cover against a small amount of H^+ which normally diffuse back from the lumen of the stomach to the serosa.

The surface mucus film was much thicker in animals treated with banana than in fasted animals. The gastric pits were

tight and filled with mucin which would block the entry and penetration of gastric juice through the mucosal tissue to a far greater extent than in fasted rats with enlarged pits and decreased thickness of the surface film of mucus. Banana treatment also increased the sulphated mucin content compared to fasted rats. Since it has been reported that sulphated mucins inhibit pepsin activity (Balikin, 1950), this increase in sulphated mucin content may reduce the erosive effect of pepsin on the mucosa.

iv) Effect of aluminium hydroxide treatment in preventing ulceration.

$Al(OH)_3$ treatment neutralized gastric acid secretions and decreased the erosive power of the gastric juice. The retention of the surface mucus film and the absence of widening at the gastric pits were due to the decreased erosive action of HCl and pepsin. The absence of vasodilation in the capillaries was probably due to the neutralization of H^+ and the retention of an effective mucosal barrier against the back diffusion of H^+.

5.2 Effect of banana aqueous extract on gastric acid secretion

The results showed that the anti-ulcerogenic action of banana was not due to any modification of gastric acid secretion. It has been reported that the presence of food in the gut has a

stimulating effect on mucus secretion and cellular turnover in the gastro-intestinal tract of the rat (Heird et al., 1974).

Since the anti-ulcerogenic action is in unripe banana, which is less digestible than ripe banana, it is possible that unripe banana may persist in the stomach much longer than food in a normal diet. This retention of banana may contribute to its protective effect as shown by the increase in mucosal thickness and stimulation of mucus secretion. However some of the present findings do not support this since:

i) An aqueous extract of banana administered orally provided protection against ulceration by increasing significantly the thickness of the mucosa and reducing the severity of aspirin induced ulceration.

ii) Banana fraction (13) injected i.p. promoted the healing of aspirin-induced ulcers and erosions.

iii) The anti-ulcerogenic activity differed widely between various samples of unripe banana.

Therefore the protection and healing of ulceration by banana is probably due to extractable substances from banana and they have a direct action in increasing mucosal membrane thickness and mucus secretion.

The present finding that banana was effective in increasing mucosal thickness was confirmed in September, 1981 (Dr M. Day - personal communication) in independent work sponsored by Reckitt and Colman PLC. The lack of variation on gastric acid secretion and pepsin activity was also confirmed by Sanyal (1981, unpublished work).

The increase in mucosal weights and mucosal thickness and the lack of variation on gastric acid secretion may indicate that the cell proliferation of the mucosa does not concern the parietal cells. This hypothesis is of a great importance in that the treatment with banana stimulates the cells which contribute to the healing effect only in the mucosa rather than the parietal cells which contribute a pathological factor in the ulcerogenesis. Further work is needed to confirm this suggestion by using autoradiographic methods.

5.3 Intra-peritoneal treatment with banana fraction (13).

One of the oldest treatments for peptic ulceration was to prepare a coating agent consisting of a colloidal solution of gum and proteins which protected the gastric mucosa from endogenous ulcerogenic factors such as HCl and pepsin. Banana was found to have a powerful buffering effect on gastric acid secretion (Hanszen, 1934). It was suggested that the anti-ulcerogenic activity of banana was due to its high content of basic salts (Killian, 1959). However the present findings suggest that the active substance(s) in banana are not acting

topically, but systemically, where the active ingredient(s) must be absorbed first to contribute its anti-ulcerogenic activity. The results are therefore not in agreement with the suggestions of Hanszen(1934) and Killian (1959). The increase in mucosal weights and thickness in response to banana treatment correlated with the effectiveness of healing

5.4 Effective dose of banana:

The mucosal weights and the ulcer index evaluation observed after sample(A) treatment, indicate that banana was active in healing ulcers at a dose 3 g, over 48 h per animal. Under our experimental coditions, the procedure of the work was planned after discussions with Dr M.D.Day. Doses below this level e.g. 2 g, over 48 h per animal did not significantly affect the increase in mucosal weights but did improve healing in animals at a significance level $p<0.02$. However 2 g of banana administered over 48 h was effective in increasing the total carbohydrate by 7% compared to aspirin treated controls whilst 3 g of banana increased the total carbohydrate by 30 %. Obviously 2 g of banana administered in 48 h represents the lowest dose for the sample under test to be effective After considering the significance values for the ulcer indices at doses 2 g and 3 g of banana and the gains of 7 % and 30 % of carbohydrate at the respective doses, it is obvious that 3 g of banana in 48 h was the better dose .Doses above 3 g of the same sample (e.g. 5- 7 g)maintained but did not increase the anti-ulcerogenic effect. It was also noted that the effect of aspirin on mucosal weights varied between experiments.It was noted in preliminary work that different batches of aspirin varied in their

ulcerogenic activity in different experiments. This may have accounted for the variation between some experiments in the effect of aspirin on mucosal weights. In subsequent experiments care was taken to use aspirin that had been kept in a dessicator to avoid hydrolysis to salicylic acid by atmospheric moisture.

a) Carbohydrate estimations:

The estimations of hexosamines, sialic acids and fucose did not show any increases in their values when the appropriate sugars were estimated as µg per mg of tissue after banana treatment. However the histological studies between animals treated with and without banana indicated that animals treated with banana showed higher activity with P.A.S. - AB stain than in untreated animals. The estimation of total sugars in stomachs indicated that there was a increase in hexosamines and sialic acids in animals treated with 3 g and 4 g of banana in 48 h but the fucose value did not vary. With banana treatment the increase in mass (mucosal weights) was much higher than the increase in carbohydrate which may be due to the fact that not all the new cells secrete carbohydrate directly after cell division. The lack of a rise in fucose levels is difficult to explain. Fucose was found to be increased after the oral administration of aspirin which may be due to alterations in the mucus composition (Dekanski et al., 1975). Some authors suggest that hexosamines and sialic acids are the most sensitive of the individual sugars for measuring mucus secretion and relating secretion rates to ulceration (Lukie and Forstner, 1972, Shillingford et al., 1974). Mucus composition varies, and it possible that the increased mucus secretion observed may not have involved increases in mucoproteins rich in fucose. However further work is needed to establish the truth of this suggestion.

b) Histological observations of aspirin induced-ulceration.

i) Aspirin treatment plus two days recovery:

After two days recovery from aspirin induced ulceration the secretory activity of the mucous neck cells and cells lining the foveoles were much greater than in fasted rats that had not been treated with aspirin. Consequently the gastric pits were packed with neutral mucins. Other workers (St.John et al., 1973, Hietanen, 1975) have observed that after a short recovery period from aspirin, followed by fasting, rats showed some resistance to further aspirin-induced ulceration. The present finding may explain this as a hyper-secretion of mucus in the recovery phase leading to the plugging of gastric pits with mucin which block the further access of ulcerogenic factors to mucosal tissue. This would reduce the severity of ulceration since the hypersecretion was located in areas damaged in the first aspirin treatment and these areas will be particularly resistant to further aspirin attack. Areas which resisted attack by the first aspirin treatment where mucus secretion was similar to that in untreated fasted rats were likely to be vulnerable areas in the second aspirin attack. However since these areas resisted the first aspirin treatment they may have shown a residual resistance to the second aspirin treatment. The carbohydrate analysis of gastric mucosa from rats treated with aspirin only failed to show differences in total mucin levels when compared to untreated fasted rats (see Table 30 and 31). This may reflect differences in mucin distribution rather

than in total mucin content which would be consistent with the histological findings. It is possible that an absence of the mucin surface film may be compensated for in the analysis by an increase in the carbohydrate content in damaged tissues in the recovery process.

It appears that the healing from aspirin induced ulceration was more rapid in the upper part of the glands where no widening was observed, in contrast to the widening and the eosinophylic picture which persisted in the lower part of the glands. This may be due to:

1. The cells present in the neck of the glands regenerated more rapidly than those in the lower regions (Lipkin, 1973; Bell et al., 1967; Winawer and Lipkin, 1969).
2. The back diffusion of H^+ from the lumen to the lower part of the glands may damage the cells which contain the endogenous ulcerogenic factors. In particular H^+ release would induce vasodilation which was observed in the sections from aspirin treated animals. This could be potentiated by histamine release in the pyloric region and would have an additive effect on the capillary network.

The absence of acidic mucins in the surface mucosa particularly in the eroded areas is due to the removal by exfoliation

of the surface cells which secrete neutral and acidic mucins. These cells are the last to regenerate in the surface mucosa. These cells originate from cells present in the neck of the gland (Lipkin, 1973).

In general the healing after aspirin treatment is localized in eroded areas. However banana treatment as discussed below induced a much faster and more complete healing process.

ii) Aspirin treatment followed by banana:

The healing from aspirin-induced ulceration induced by banana treatment was a consequence of rapid cell proliferation. That activity was found particularly in the neck of the glands where the cells were actively migrating towards the surface of the mucosa to heal ulcerated and eroded areas of the mucosa. Lipkin (1973) reported that the cells in the neck of the glands were the most active cells in the mucosa to proliferate . The presence of a surface film of mucin is a consequence of a rapid cellular regeneration to replace cells exfoliated from the surface by aspirin treatment. The mucus neck cells were very active in secreting P.A.S. staining substances and the activity extended to the upper and lower regions of the gland. Such activity may be due to cellular regeneration extending to both the lower and upper parts of the glands. This extension of secretory activity was only found in animals treated both with aspirin and

banana and was absent when the banana treatment was omitted. The absence of vasodilation, eosinophylic stained mucosa and the disappearance of spaces between the glands may be due to a diminution in the back diffusion of H^+. Another factor may well be the effect of banana in increasing the mitotic activity of the mucosal cells which led to an increase in cells and therefore an increase in the total of mucus secreted.

Mucus packed the gastric pits and formed a cover over the surface mucosa which would constitute an effective barrier against erosive factors. The surface mucous cells also play an important role in secreting HCO_3^- (Garner et al., 1979) which neutralises the small amount of H^+; which normally diffuses back through the surface mucosa from the lumen.

5.5 Deoxyribonucleic acid (DNA) content in banana treated stomachs

It was found that the mucosal weights were decreased 5 h after the administration of aspirin in previously fasted rats (Macdonald, 1976). In the present studies the mucosal weights recovered to normal values after two days on a normal diet in rats treated with aspirin only compared to untreated ones. Banana treatment, after aspirin, significantly increased the mucosal weights compared to both untreated and rats treated only with aspirin. The increase in mucosal weights found in rats treated with banana compared to untreated controls and aspirin treated animals showed

that banana not only protects against ulceration but can also heal ulcerated mucosa. If cells do proliferate after banana treatment then the DNA content should rise. All rats treated with banana showed significant increases in DNA as $\mu g\ mg^{-1}$ of tissue and total DNA. This increase correlates with the increase found in mucosal weights, and thickness (Figure 12)and is also in agreement with the histological findings (see section 4.4.b,iii) where cellular migration was evident in ulcerated areas of the mucosa after banana treatment. The DNA increased by 60% and the total carbohydrate increased by 30% in rats treated with 3 g of banana compared to rats treated with aspirin only. However no variation in carbohydrate content was found when the carbohydrate was calculated as $\mu g\ mg^{-1}$ of tissue and that may be due to the increase in mass which is much higher than the increase in carbohydrate.

The DNA content of cells is about 1% of the total weight (Legay et al., 1964) and the values obtained for DNA were in this region. Therefore Crofts et al (1966) method of DNA estimation was sensitive enough to detect the increase in DNA due to banana treatment. The increase in DNA content and mucosal weights of the scraped gastric mucosa was not due to a decrease in endogenous ulcerogenic factors such as HCl or pepsin since the acid and pepsin secretion values (personal communication with Professor Sanyal, 1981) with and without banana treatment were the same.

5.6 Effects of cimetidine, prostaglandin E-2 (PGE-2), 5-hydroxytryptamine (5-HT) and dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP) as both protective and healing agents for ulcers.

a) Cimetidine

The inhibition of gastric acid secretion (volume and acid output) after administration of cimetidine is in agreement with the findings of Durant et al., 1977. The ulcer index (Table 30) of stomachs treated previously with cimetidine at anti-secretory doses indicated that acid is needed for the production of aspirin-induced ulceration. This agrees with the findings of Brodie and Chase 1967, and Guth et al., 1979). Post-treatment with cimetidine at oral doses of 240 mg.Kg^{-1}, over 48 h were effective in inhibiting gastric acid secretion for 24 hours at least since 0.34 mg kg^{-1} min^{-1} of cimetidine was effective in inhibiting gastric acid secretion in the perfused stomach and the half life of cimetidine is 90 min. (Durant et al., 1977). However 3 doses at 80 mg kg^{-1}, over 24 h did not heal rats of ulceration (Table 30). In contrast banana administered 5 h after aspirin administration healed ulcers. Cimetidine administered before aspirin had a protective effect since it reduced the ulcer index compared with untreated aspirin controls. However neither the pre- or post-treatment with cimetidine increased the mucosa weights as found with banana. Therefore it is unlikely

that Cimetidine has an action similar to that of banana.

The effect of cimetidine was due only to its anti-secretory action. The accumulated 'dead' mucin on the mucosa surface after cimetidine treatment may be due to the inhibition of the digestive power of the gastric juice. Normally the mechanical effect of food aided by acid and pepsin would remove this layer.

b) Prostaglandin E-2 (PGE-2)

The decrease in gastric acid secretion (volume and acid output) after PGE-2 administration is in agreement with the findings of Robert (1973) and Bolton et al., (1978). The inhibition of acid secretion after PGE-2 administration is similar to the inhibition induced by cimetidine. The decrease in ulcer index in response to PGE-2 pre-treatment was 17% greater than the ulcer index observed with cimetidine pre-treatment. However the difference in values may be due to the additive effects of PGE-2 giving cytoprotection (Robert et al., 1976., 1979.) in addition to its anti-secretory properties.

It is known that non-steroidal anti-inflammatory drugs e.g. aspirin inhibit prostaglandin synthesis by inhibiting cycloxygenase (Vane , 1971). Therefore the administration of PGE-2 may make up for the endogenous loss and contributes a protective role against ulceration.

The mechanism of the cytoprotective effect of prostaglandins is poorly understood and most of the proposed mechanisms are contradictory. The cytoprotective effect of PGE-2 is similar but not identical to banana. PGE-2 did not increase the mucosal weights as was found with banana. PGE-2 is protective against ethanol-induced ulceration when PGE-2 is administered even one minute before ethanol administration (Robert et al., 1979) and therefore the mechanism of cell proliferation does not explain the cytoprotective effect of prostaglandins and the failure of PGE-2 to increase mucosal weight indicates that its cytoprotective effect was not due to cell proliferation.

The vasoactive changes induced by prostaglandins cannot offer a conventional cytoprotective role since PGE-1, PGE-2, PGA, and PGI are described as vasodilators (Tarun et al., 1978, Robert et al.,1979, Cheung., 1980) and PGF-2 α is vasoconstrictor (Usardi et al., 1974).

The cytoprotective effect of prostaglandins may be due to their effect in increasing the HCO_3^- secretion from the surface mucous cells (Kauffman et al., 1980).

PGE-2 may act on the permeability of the cell membrane and inhibit the back diffusion of H^+ in ulcerated stomachs after aspirin treatment in the rat. This hypothesis is not convincing since PGE-2 did

not inhibit the back diffusion of H^+ in ulcerated stomachs after ethanol treatment although PGE-2 did show cytoprotective effects against ulceration (Robert et al., 1979).

c) 5-Hydroxytryptamine (5-HT):

The observed decrease in the volume of the gastric acid secretion in response to oral administration of 5-HT at a dose 1.5 mg kg^{-1} is in agreement with Yano et al., (1977) findings in which 15 mg kg^{-1} of 5-HT orally administered, decreased the volume of gastric acid secretion in rats. The lack of variation of ulcer index values between rats treated orally with 5-HT at a dose 1.5 mg kg^{-1} compared to untreated aspirin controls confirms that there is no relationship between the 5-HT content of banana and its mode of action as a protective agent. This contradicts Sanyal's et al.,(1961) suggestion that the anti-ulcerogenic effect of banana is due to its 5-HT content. Although there was no variation in the gastric acidity and a decrease in gastric volume secretion in rats treated with 5-HT, the oedematous nature of the surface mucosa may be a consequence of several factors. 5-HT increases and disturbs the motility of the stomach (Hori ., 1962, Yano et al., 1977) and such disturbances in motility can be contributed to, in part, by the reflux of duodenal contents back into the stomach, carrying with it the detergent effect of bile salts. This could seriously disturb the gastric mucus and cause cytolysis of epithelial cells which would deplete the mucus content (Rhodes, 1972).

It was noticed that green-yellow bile stained the mucosa after 5-HT treatment in several animals. Bile salts can also break down the mucosal barrier and alter the permeability of the mucosa and permit the back diffusion of H^+ (Geall et al.,1970). The oedema aspects of the mucosal surface is likely to be a consequence of vascular impairment observed in the histology and is in agreement with Milne and Cohn (1957).

d) Dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP).

Dib-cAMP was found to protect animals against ulceration induced by aspirin. The lack of variation on mucosal weights and the absence of the curative activity of dib-cAMP would exclude any relationship between banana mode of action and that of dib-cAMP.

The histochemical studies of sections pre- and post-treated with dib-cAMP showed that a significant barrier of mucus was present on the surface mucosa. The increase in mucus on the surface mucosa may be a consequence of the effect of dib-cAMP on HCO_3^- production in the surface mucosa as reported by Kauffman et al., (1980).

CHAPTER FIVE

FINAL DISCUSSION AND SUGGESTIONS FOR FURTHER WORK

FINAL DISCUSSION AND SUGGESTIONS FOR FURTHER WORK

1. The treatment of gastric ulcer with banana

Previous work has shown that banana has anti-ulcerogenic action against ulcers induced by stress (Sanyal et al., 1965) histamine (Elliot and Heward, 1976) and phenylbutazone (Sanyal et al., 1961). The present work has shown that banana protects rats from aspirin-induced ulceration. It was also found that in addition to protecting rats against ulceration, banana also has curative properties in that it assisted in the healing of ulcers already induced by aspirin. This finding parallels the finding of Sanyal, (1981,personal communication) when he reported the curative effect of banana in man.

2. Variation in banana activity

The anti-ulcerogenic activity of banana varies from one sample to another but never within the same sample. That activity was stable when banana was dried and stored at room temperature but was unstable when heated. Hot solvent extraction also destroyed its activity.

The anti-ulcerogenic activity of banana was not limited to a single variety of banana. It was found in both the West Indies (sample E and E-1) and the Indian samples of banana (samples A, B

and C). The Indian and the West Indies banana samples Were different varieties. It is very likely that the anti-ulcerogenic activity of banana depends on the harvesting time from the tree. Ideally banana should be harvested at various times during its growth cycle at a single site to correlate its pharmacological activity with the biochemical change atripening . However Sanyal(1981 , personal communication) found that the Indian samples at Varnasai were most active when the banana was collected between October and February. It would be ideal for further isolation of the active ingredient to work with one site of samples after determining the most active period of banana cycle.

3. Identity of the anti-ulcerogenic agent in banana:

Although the active agent was not isolated or identified,it was a polar substance(s), extractable by ethanol and water and inactivated by heating above 70 C.

The extraction of the anti-ulcerogenic activity of banana with 95% ethanol and fractionation by the procedure outlined in plan 2 (Chapter 3, section 3.3) resulted in a 500 fold purification of banana activity . The anti-ulcerogenic activity of banana is due to one or more substance(s) that gave a positive reaction with ninhydrin,its molecular weight was similar to sucrose (370) and therefore too high to be an amino-acid. The anti-ulcerogenic agent(s) did not absorb in U.V but were adsorbed by charcoal

4. Mode of the anti-ulcerogenic activity of banana:

The anti-ulcerogenic activity of banana was due to a very potent ingredient which both healed and also protected animals against ulceration. The anti-ulcerogenic activity of banana was not due to a buffering or neutralizing effect since:

a) Banana samples showed different activity to heal and protect animals against ulceration which was not related to their buffering power.

b) Small fractions of banana such as fraction 13-2 were potent in healing ulceration induced by aspirin.

c) Banana fraction 13-2 had a potent anti-ulcerogenic action by both intra-peritoneal and oral routes.

d) The anti-ulcerogenic principle(s) of banana were thermolabile.

The anti-ulcerogenic activity of banana was due to substance(s) acting systematically since the intra-peritoneal treatment by fraction 13 of banana increased the healing rates in rats with aspirin-induced ulceration. The healing of ulceration was due to the proliferation of the mucosa cells. This cellular proliferation increased the thickness of the gastric mucosa as shown by gastric mucosal measurements such as thickness and weight. These results were supported by the finding that DNA increased in the mucosa after treatment with

banana. A suitable model for investigating healing in ulcerated rats would be the measurement of the incorporation of radioactive thymidine after treatment with banana or banana extract. Such a model would be more sensitive, less time consuming and more economical than the one used in the present study. Banana treatment was characterised by an increase in mucosal weight, mucosal thickness, cellular proliferation and an acceleration in the healing of ulcers. No association between its healing properties and changes in gastric acid secretion was found. The constancy in gastric acid secretion may indicate that the cellular proliferation was selective since parietal cells are much slower to proliferate than mucous cells (MacDonald, 1976). Therefore it would be worthwhile to use auto-radiography to identify the type of cells stimulated to divide by banana (e.g. using C^{14}-thymidine incorporation). The histological studies on sections obtained from stomach treated with banana showed that necrosis and oedema were decreased with an increase in mucus secretion (an increase in AB-P.A.S.). However such observation of mucus increase was supported in animals treated with banana by biochemical analysis of mucus constituents. A good dose response with banana was found, correlating healing properties of banana to a parallel increase in mucus content.

5. 5-Hydroxytryptamine (5-HT):

The anti-ulcerogenic activity of banana was not found to be due to the 5-HT content of banana as reported by Professor Sanyal et al., (1961), for several reasons:

a) The skin of banana is richer in 5-HT than banana pulp. However, sample E_1 (skin and banana pulp) was not different to sample E (banana pulp only) in terms of anti-ulcerogenic activity.

b) The 5-HT content of banana increases with ripening. However, the anti-ulcerogenic activity of banana is lost in the ripe-fruit.

c) The 5-HT content of banana sample (A) was 0.5 $\mu g\ g^{-1}$ of banana and the same content in 5-HT was also found in banana sample (G). However, banana sample (A) was very active and banana sample (G) was inactive in healing ulceration induced by aspirin.

d) 5-HT at a concentration of 1.5 mg kg^{-1}, did not improve the healing of ulceration induced by aspirin. The histology showed that 5-HT induces vasoconstriction in the vascular system and oedema was present in the tissue where there was a high degree of necrosis.

Although the histochemistry of stomachs treated with 5-HT showed that 5-HT increased the P.A.S. staining on the tissue, the observation does not correlate with the increase in mucus determined by biochemical analysis of the mucus constituents. The P.A.S. staining was diffused over all the tissue associated with necrosis and oedema. Therefore it appears that the increase of P.A.S. staining activity in response to 5-HT treatment was due to the accumulation of fluid containing mucin in the tissue. This correlates with the observed fluidity of the surface mucosa and the reflux of bile salts from the duodenum to the gastric lumen which contributes a pathological factor in peptic ulceration.

6. Cimetidine:

The inhibition of aspirin-induced ulceration by cimetidine treatment demonstrated the importance of gastric acid secretion in the pathophysiology of peptic ulceration. The prevention of ulceration by cimetidine was correlated with the absence of necrosis in the histology sections. However cimetidine did not heal ulceration as banana did over two days treatment. No increase in mucosal weight, mucosal thickness or mucus production was observed by

either pre-treatment or post-treatment with cimetidine. Therefore there is no correlation between the anti-ulcerogenic activity of banana and that of cimetidine.

7. Prostaglandin E-2 (PGE-2)

In the present study PGE-2 was found to be more active than cimetidine in preventing aspirin-induced ulceration in rats. The increase in activity observed with PGE-2 may be due to factors other than simply an inhibition of gastric acid secretion i.e. probably a cytoprotective effect as first reported by Robert (1973).

Unlike banana PGE-2 did not increase mucus secretion, mucosal weight, or mucosal thickness. Therefore it is unlikely that the activity observed with banana in the present series of experiments was due to an indirect effect via prostaglandins. However, bearing in mind that the model of PGE-2 investigation in the animal test was based on the banana model, it may be worth while to investigate the effects of PGE-2 on mucus secretion using shorter times than the time selected in the present investigation.

8. Dibutyryl 3', 5'-cyclic adenosine monophosphate (dib-cAMP)

The prophylactic effect of dib-cAMP in preventing ulceration induced by aspirin was obscure and is difficult to explain with the present data. Dib-cAMP caused no variation in gastric acid secretion or mucus secretion. Further investigation on the effect of dib-cAMP on mucus secretion or bicarbonate secretion using a shorter time than in the present studies are indeed necessary to understand more about the prophylactic effect of dib-cAMP in preventing ulceration.

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