AN INVESTIGATION OF THE PROTECTIVE ACTION OF THE E_2 PROSTAGLANDINS ON THE GASTRIC MUCOSA

BY

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ABSTRACT

The prostaglandins are found throughout the body and are therefore thought to have some physiological role. It has been suggested that prostaglandin E_2 is concerned with the maintenance of gastric mucosal integrity.

In a previous study of the gastric effects of the E_2 prostaglandins, natural prostaglandin E_2 (PGE_2), 15-methyl prostaglandin E_2 (15M), and 16.16-dimethyl prostaglandin E_2 (16DM), it was observed that all three agents appeared to increase mucus production and that 16DM appeared to stimulate a non-acid secretion in basal Heidenhain pouches. These properties of the E_2 prostaglandins have been studied in detail. (Experiments 1 and 2)

It has previously been shown that 15M can prevent the damaging effect of aspirin and indomethacin on the gastric mucosal barrier. Using a model in which gastric mucosal barrier damage could be produced and sustained, the ability of these agents to reverse established damage was studied. (Experiment 3)

Experiment 1: The effect of topical and intravenous E_2 prostaglandins on gastric mucus production was studied in rats by measuring both the amount of mucus shed into a small volume of normal saline in the closed stomach and the amount of mucus bound to the mucosa, over a three hour period. Mucus was measured indirectly by measuring the binding of Alcian Blue. All prostaglandins caused a significant increase in the mucus found in solution, but not in that bound to the mucosa.

Experiment 2: The effect of topical and intravenous administration of the three E_2 prostaglandins on non-parietal cell secretion was measured in perfused canine Heidenhain pouches. The pouches were perfused with a non-acid solution. The increase in volume was measured using polyethylene glycol as a volume marker and the HCO_3^- content by the method of back titration. 16DM was found to cause a significant increase in the volume, and the fluid secreted contained Na^+, Cl^- and HCO_3^-. In the absence of acid it is suggested that this represents stimulation of non-parietal cells.

Experiment 3: The reversal of aspirin induced damage to the gastric mucosal barrier was demonstrated in perfused canine Heidenhain pouches. The pouches were perfused for two hours with aspirin to produce gastric mucosal barrier damage, the aspirin was then withdrawn and the pouch perfused with acid alone. The effects of topical and intravenous prostaglandins and intravenous metiamide were tested during this latter period when established barrier damage existed. Intravenous PGE_2 and 15M reversed the damage, but topical prostaglandins and intravenous metiamide did not.

It is concluded that the E_2 prostaglandins have a secretory effect on the basal gastric mucosa, causing an increase in mucus production and in non-parietal cell secretion. This previously unrecognised stimulation of active secretion has lead to the misinterpretation of permeability data for 16DM. These secretory effects may have some protective action. Intravenous PGE_2 and 15M can reverse established gastric mucosal barrier damage in the dog. This indicates the possibility of a therapeutic role for these agents in the management of conditions associated with disruption of the gastric mucosal barrier.

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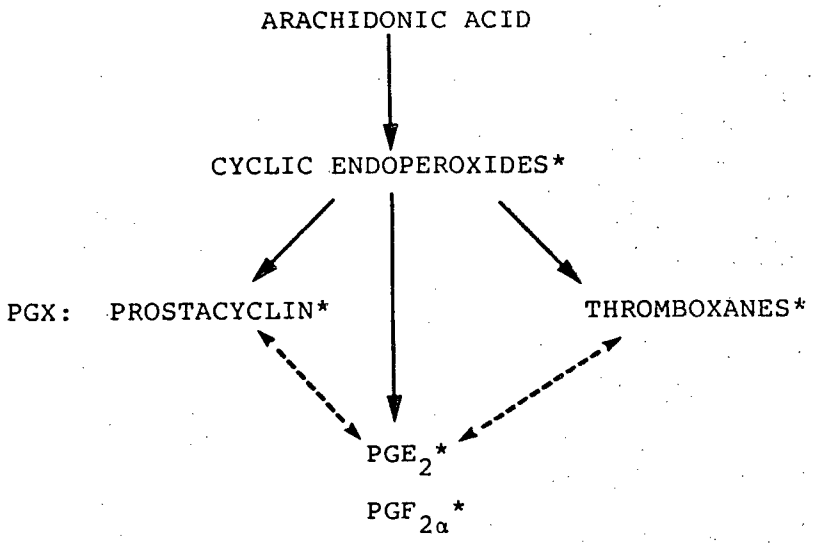
INTRODUCTION

In 1933 Goldblatt[1] discovered an acidic lipid soluble, smooth muscle stimulating compound in seminal fluid. This was the first description of a prostaglandin and yet it went largely unnoticed. There followed a period of inactivity in the study of this compound due mainly to the lack of suit able methods for accurate qualitative and quantitative measurement of its active components. The current high level of interest in the prostaglandins dates back to the 1960's when these compounds were isolated in crystalline form[2] and their chemical structures elucidated.[3] In the past seventeen years a vast amount of work has been done on these substances and much information is available regarding their pharmacological effects.

The prostaglandins are widely distributed in the tissues of the body and are known to have a variety of actions. Major effects include contraction of the uterus[4] and the longitudinal muscle of the gut,[5] the inhibition of circular muscle in the gut[6] and vasodilatation,[7] the inhibition of gastric acid secretion[8] and the accumulation of large volumes of fluid in the small intestine.[9] Their wide distribution and the variety of their effects has led to the assumption that they have a physiological role. This role, if such a role exists, has not yet been determined and there is now evidence that the prostaglandins may be simply metabolites of other active agents such as the endoperoxides or one step on a metabolic pathway from arachi donic acid to prostacyclin, a highly potent agent recently isolated from vascular tissue.[10] (Figure 1) There is no evidence that the prostaglandins or their active precursors are stored in the tissues, biosynthesis seems to take place as required, but the stimulus for this is unknown. The agents

PROSTAGLANDIN SYNTHESIS



* Pharmacologically Active Substances

FIGURE 1: Illustrates the relationship of prostaglandin E_2 to arachidonic acid and other metabolically related active agents.

are thought to be produced at the site of their action and they are known to have an extremely short half life. It is possible that the reason why the prostaglandins have been the agents most investigated is that they are more stable than their active precusors and have therefore been measured, identified and synthesized and have become widely available for laboratory experimentation.

Although the debate regarding their physiological action continues the prostaglandins have been shown to have many pharmacological actions and they are currently in use or under investigation as therapeutic agents in a number of fields.

Prostaglandin E_2 is used for the induction of labour[11] and for the termination of pregnanacy in the first and second trimester.[11,12] It also is used to maintain a patent ductus arteriosus in neonates with major congenital cardiac abnormalities who require the maintenance of a left to right shunt.[13] Controlled clinical trials are currently underway to study its value in erosive gastritis[14] and the value of its orally active methyl analogues in the treatment of peptic ulceration.[15,16]

REVIEW OF THE LITERATURE

The E_2 Prostaglandins and the Stomach

Prostaglandin-like material has been extracted from most portions of the gastrointestinal tract,[17] but there has been no formal characterization of this material. It is reported that E and F prostaglandins are the main types occurring in the gut and the material extracted from the mucosa and submucosa of the stomach is almost certainly PGE_2.[18] The presence of a PGE_2-like material in the gastric mucosa has stimulated considerable interest in the effect of this naturally occurring prostaglandin on gastric function. PGE_2 is a potent inhibitor of gastric acid secretion when given intravenously[8,19] but is almost inactive when given orally.[20] Analogues have been prepared in an attempt to overcome this disadvantage and at the present time two such analogues have been widely investigated, 15M and 16DM. The differences between these two analogues and the parent compound are illustrated in Figure 2.

The term "the E_2 postaglandins" is used to include natural PGE_2 and its two methyl analogues and as the experimental work in this thesis is concerned with certain actions of these agents on the gastric mucosa current knowledge of their gastric effects is reviewed.

The E_2 prostaglandins have two important actions in the stomach:

1. Inhibition of gastric acid secretion.
2. Protection of the mucosa.

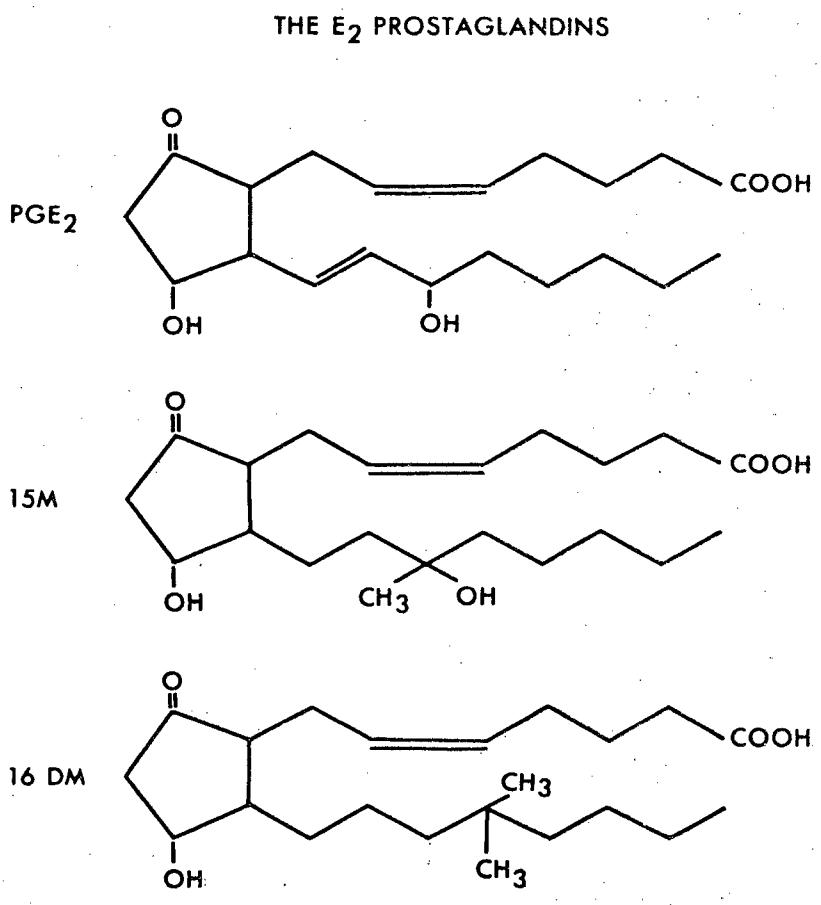


FIGURE 2: Illustrates the similarity in structure of prostaglandin E_2 and its methyl analogues 15-methyl prostaglandin E_2 and 16.16-dimethyl prostaglandin E_2.

1. Inhibition of Gastric Acid Secretion

The accumulated evidence for inhibition of acid secretion by PGE_2 and the closely related PGE_1 is very extensive and includes experiments in man and a variety of animals under basal and stimulated conditions. As PGE_1 was discovered before PGE_2 it has been more extensively studied, but no important differences in the inhibitory actions of the two compounds have been found.

Animal Studies

When given parenterally PGE_1 can inhibit gastric acid secretion in dogs stimulated by a variety of agents including, histamine, pentagastrin, food, 2-deoxy-D-glucose, reserpine and carbachol.[21] Parentral PGE_2 can inhibit histamine stimulated secretion.[22] Basal secretion in rats can also be inhibited by parenteral PGE_2.[22] When given orally in high doses (0.05-5 mg) in an isosmotic buffer (Na_2HPO_4, pH 7.4) PGE_2 can inhibit gastric acid secretion of pylorus ligated rats,[22] but it is ineffective by this route in dogs.

In contrast 15M and 16DM can inhibit gastric acid secretion when given orally as well as parenterally to dogs and have a similar effect in rats.[22,23] These agents have been found to be much more potent than the parent compound and to exert activity for a much longer duration. The ED_{50} (dose inhibiting acid output by 50%) in dogs for PGE_2, 15M and 16DM are 10, 0.3 and 0.1 μg/Kg respectively with a single intravenous injection.[22] Oral 16DM is 2.8 times as potent as 15M.

There is evidence to suggest that the mode of action of the methyl analogues is different when the different routes of administration are used. When applied topically to the mucosa these agents appear to exert a local antisecretory effect rather than a systemic effect secondary to absorption. Experiments on dogs with two Heidenhain pouches demonstrated that local instillation of 16DM to one pouch produced total inhibition of stimulated acid secretion but was without effect in the other. At higher doses a less potent systemic effect could be demonstrated on the second pouch. The systemic action was less potent and of shorter duration than the local action.[24]

Human Studies

Intravenous PGE_2 will inhibit basal[25] and pentagastrin stimulated[19] acid secretion but oral administration is without effect.[20] 15M on the other hand given orally to healthy subjects inhibited pentagastrin stimulated acid output for several hours, the ED_{50} being approximately 1 µg/Kg.[20] 16DM was antisecretory in healthy volunteers when administered both orally and intravenously[26] and both analogues were particularly potent in inhibiting food induced gastric secretion both in healthy volunteers and duodenal ulcer patients.[27]

2. Protection of the Mucosa

The evidence for this action comes under two headings:

A. Protection resulting from inhibition of acid secretion (antiulcer effect).

B. Protection independent of acid secretory inhibition.

A. Protection Resulting From Inhibition of Acid Secretion

As the E_2 prostaglandins have been demonstrated to have powerful antisecretory properties interest has arisen in their possible use for the prevention and cure of gastroduodenal ulcers.

PGE_2 or its methyl analogues given either subcutaneously or orally to rats have been shown to inhibit gastric ulcers produced in a variety of ways including:

a) Shay ulcers produced by pylorus ligation.[22]

b) Steroid induced ulcers.[22]

c) Ulcers produced by oral and intraperitoneal administration of nonsteroidal anti-inflammatory agents, such as aspirin[28] and indomethacin.[29]

PGE_2 or 16DM given either orally or subcutaneously inhibited in a dose dependent manner the development of duodenal ulcers produced in rats by:

a) A single subcutaneous injection of histamine.[30]

b) A constant subcutaneous infusion of histamine and carbachol for 24 hours.[22]

The doses of the prostaglandins used in these experiments were in the acid secretory inhibiting range and similar protective effects have been described for other inhbitors of acid secretion, such as the H_2-receptor antagonists,[31,32] used in comparable inhibitory doses.

B. Protection Independent of Acid Secretory Inhibition

Recent studies suggest that the prostaglandins may also have a protective action unrelated to acid secretory inhibition. Robert has demonstrated that alcohol induced erosive lesions of the glandular portion of the fasted rat stomach can be completely prevented by the prior administration of 16.16-dimethyl PGA_2 and 15-methyl $PGF_{2\beta}$ which are prostaglandins that do not inhibit acid secretion. Also 16DM administered subcutaneously in a dose 100 times less than the threshold dose inhibiting acid secretion in the rat will also prevent the development of these lesions.[33] It is quite clear therefore that the prostaglandins can exert a protective effect upon the gastric mucosa which is independent of acid secretory inhibition.

A possible mechanism for this protective effect is tightening of the gastric mucosal barrier.

The E_2 Prostaglandins and the Gastric Mucosal Barrier

In 1964 Davenport[34] demonstrated that the apparent inhibition of acid secretion caused by the topical application of eugenol to the gastric mucosa of a Heidenhain pouch was in fact due to back diffusion of hydrogen ion from the gastric lumen to the interstitial fluid of the mucosa. He subsequently postulated that the effect of barrier damaging agents, such as aspirin, on the gastric mucosa was the result of the back diffusion of hydrogen ion. The high concentration of hydrogen ion in the interstitial fluid damaged the mucosal cells and the capillaries and caused a release of histamine which led to vasodilatation and the secretion of more hydrogen ion. This sequence of events led to the shedding of mucosal cells, bleeding from the mucosal capillaries and the typical changes of erosive gastritis.[35]

This work led to the revival of the concept of the gastric mucosal barrier.[36] Under normal circumstances the concentration of hydrogen ion and sodium ion are unevenly distributed on either side of the gastric mucosa. There is a high concentration of hydrogen ion in the lumen and a high concentration of sodium in the interstitial fluid. Despite the concentration gradients that therefore exist for these ions there is little movement of hydrogen ion out of the lumen or sodium ion into the lumen. The mucosa is almost impermeable to the diffusion of these ions down their negative concentration gradients. This relative impermeability of the mucosa is referred to as the gastric mucosal barrier. The small movement

of ions that does occur under normal circumstances can be measured and represents the basal level of the gastric mucosal permeability. Agents, such as aspirin, increase the permeability of the mucosa and the measured movement of ions. A significant increase in gastric mucosal permeability above the basal level indicates disruption of the gastric mucosal barrier.

A variety of agents are known to break the gastric mucosal barrier, the important ones being aspirin,[37,38] indomethacin,[38] bile salts[39] and alcohol,[37,40] and all these agents cause erosive and hemorrhagic lesion of the gastric mucosa. Hemorrhagic lesions associated with stress have also been shown to be associated with disruption of the gastric mucosal barrier.[41] Disruption of the gastric mucosal barrier and the back diffusion of hydrogen ion are thought to be major factors in the pathogenesis of acute hemorrhagic lesion of the gastric mucosa.[42]

The gastric mucosal barrier is a physiological concept rather than a true anatomical entity, and it is not known how the various barrier breakers produce their effect on the barrier. It has been suggested that the barrier is formed by the tight junctions that exist between the lateral borders of the mucosal cells and that barrier damage is associated with separation of the tight junctions which allows the passage of ions between the cells. There is electron microscopic evidence that when dithiothreitol, a barrier breaker that also causes plasma shedding, is applied to the mucosa protein is lost by passing between adjacent mucosal cells and their disrupted tight junctions.[43] It is probable that ionic diffusion occurs by the same route.

The possible role of prostaglandins in maintaining the gastric mucosal barrier was raised after Vane et al demonstrated that certain nonsteroidal anti-inflammatory agents, in particular aspirin[44] and indomethacin,[45] could inhibit the synthesis of prostaglandins. This in vitro effect has since been demonstrated in vivo,[46] and it has been suggested that these two agents produce damage to the gastric mucosal barrier by the inhibition of endogenous prostaglandin synthesis, and therefore that the prostaglandins are responsible for the maintenance of gastric mucosal integrity.

Support for this concept was provided by the experiments of Cohen.[47] He studied gastric mucosal permeability in canine Heidenhain pouches and demonstrated a highly significant increase when aspirin in a concentration of 20 mM and indomethacin in a concentration 2 mM were added to the acid perfusate. In subsequent experiments he added 15M in a dose of 5 µg/Kg to the acid and aspirin and acid and indomethacin perfusates and was able to demonstrate that in the presence of this prostaglandin aspirin and indomethacin did not cause damage to the gastric mucosal barrier. The dose of 15M was the ED_{50} for acid secretory inhibition, but this same effect was achieved with a dose as low as 1.25 µg/Kg. Cohen also recorded in this experiment that when 15M was applied to the mucosa in the absence of the barrier breakers it decreased gastric mucosal permeability below the control level, suggesting that exogenous prostaglandin could not only prevent damage to the gastric mucosal barrier but could also tighten the gastric mucosal barrier.

Further studies of the permeability effects of the E_2 prostaglandins have been reported and have produced conflicting data. O'Brien and Carter[48]

studied the effect of 16DM both topically and intravenously on the canine Heidenhain pouch. They found that 16DM applied topically to the mucosa in a concentration of 15 μg/ml broke the gastric mucosal barrier, but did not produce this damaging effect when given systemically. Bolton and Cohen[49] subsequently studied the permeability effects of all three E_2 prostaglandins administered topically and systemically. They were unable to confirm Cohen's previous finding that 15M tightened the gastric mucosal barrier when applied topically. Using the same dose, and larger doses up to ten times the original dose, they found no significant alteration in gastric mucosal permeability. However they did find that 15M tightened the barrier when given intravenously, an effect also produced by intravenous PGE_2. They also confirmed O'Brien and Carter's data demonstrating disruption of the gastric mucosal barrier by topical 16DM with a concentration as low as 1 μg/ml and also found that it had the same effect systemically if given by constant intravenous infusion as opposed to the single bolus injection used by O'Brien and Carter.

The apparent detrimental effect of 16DM is hard to reconcile with the fact that it is a potent protector of the gastric mucosa.

PURPOSE OF THE STUDY

Evidence has been presented that the prostaglandins have a protective action on the gastric mucosa, an effect which is unrelated to acid secretory inhibition and whose mechanism is uncertain.

As a result of previous observations certain aspects of the gastric effects of the E_2 prostaglandins have been investigated in greater depth.

1. Mucus production.
2. Non-parietal cell secretion.
3. The effect of E_2 prostaglandins on established gastric mucosal barrier damage.

1. Mucus Production

When studying the permeability effects of the E_2 prostaglandins in canine Heidenhain pouches[49] it was noticed that in the experiments in which prostaglandins were used the amount of stringy mucus appearing in the perfusate was greater than in the control experiments. It seemed probable that the prostaglandins were stimulating mucus production and an attempt has been made to quantify this effect. There have been two previous references to the effect of prostaglandins on gastric mucus production. Robert et al[50] when measuring the effect of prosta glandin E_1 on gastric secretion in the rat noted a reduction in mucus output as measured by fucose, hexosamine and sialiac acid concentrations. The dose at which these observation were made was 0.5 µg/Kg/minute.

At larger doses the marked inhibition or overall gastric secretion produced volumes of juice too small for biochemical measurement of the components of mucus. These findings by no means exclude the possibility that at a higher dose stimulation of mucus was produced but was masked by the acid inhibitory effects of the prostaglandin. It has also been reported that 15M increased gastric mucus formation in patients with either gastric ulcers or gastritis. This was assessed visually and histologically, but no measurements were made.[51]

Mucus is a difficult component of gastric secretion to study because of the problem of recovery. Mucus is found in the stomach in two forms, that adherent to the mucosa called barrier mucus and that in the gastric juice, referred to as soluble or free mucus. Not all the mucus in the gastric juice is in solution, and therefore the term free mucus is more accurate. Most studies of the action of various secretory agents such as histamine, histalog and pentagastrin have measured only the free fraction, and because of the problems of recovery and measurement have largely ignored the barrier fraction. A study of mucus production should ideally include measurement of both fractions.

Because of the problems of recovery of barrier mucus any form of chronic pouch preparation has severe limitations, and it was because of this that the rat was chosen for this study despite the fact that the initial observations were made in canine Heidenhain pouches.

Two methods have been described for the measurement of barrier

mucus in the rat. One involves assessment by weight[52] and the other uses a technique of binding to a histologic dye, Alcian Blue.[53] The latter method was chosen because Alcian Blue can also be used to measure the free fraction of mucus. It not only binds to glycoprotein, the main constituent of barrier mucus, but also binds to soluble mucopolysaccharides,[54] the main constituent of the soluble fraction, to form insoluble dye-mucus complexes.

This method allows for the determination of the effect of the prostaglandins on both fractions of mucus as well as on the total amount of mucus produced. In order to assess the magnitude of the secretory action of the prostaglandins, studies were undertaken using another agent known to stimulate mucus production, namely histamine. Unfortunately the other agents that stimulate mucus production all stimulate gastric acid secretion and so the conditions in the two sets of experiments could not be identical. Mucus secretion during acid stimulation was measured as a convenient parameter for comparison.

2. Non-Parietal Cell Secretion

Topical and intravenous 16DM has been demonstrated to damage the gastric mucosal barrier in perfused canine Heidenhain pouches, an action which would indicate a disruptive rather than a protective effect on the gastric mucosa as damage to the gastric mucosal barrier is associated with damage to mucosal cells and bleeding. In direct contrast to this finding it has been demonstrated that 16DM can protect the gastric mucosa from damage firstly by its antiulcer effect when used in doses which inhibit acid secretion and secondly by its protective effect in doses 100 times less than the acid inhibitory dose.

This discrepancy has been studied by re-examining the effect of 16DM in the canine Heidenhain pouch.

In previous studies of gastric mucosal permeability,[49] which were performed by perfusing a nonsecretory canine Heidenhain pouch with a solution containing H^+, Na^+, Cl^- and PEG, the volume of any fluid produced by the pouch could be assessed at the end of the three hour period of perfusion by subtracting the expected final volume (initial volume - sampling volume) from the measured final volume, calculated from the PEG concentration.

The pouches produced some fluid even during the control perfusions, but it was noted that in the presence of 16DM the volume of fluid produced was greatly increased. Similar increases were not observed with the other two prostaglandins.

There are two possible explanations for the cause of this fluid production. Agents which damage the gastric mucosal barrier also cause a production of fluid by the pouch, so it is possible that the increase in fluid production occurred by the same mechanism of an increase in gastric mucosal permeability and the subsequent damaging effect of the hydrogen ion on the mucosa. Doubt is cast upon this explanation by the fact that the degree of damage produced by 16DM, assessed by the increase in gastric mucosal permeability, was small compared with known barrier breakers, yet the volume of fluid produced was greater. A second explanation is that 16DM stimulated active secretion by nonparietal cells in the mucosa. Such an effect could account for the volume of fluid produced, and active secretion of this sort in a system in which calculation of the permeability factor is based on the assumption that the pouch is entirely nonsecretory could lead to discrepancies in the permeability data.

If the first explanation is correct increased production of fluid would only occur in the presence of exogenous acid which could diffuse into the mucosa as a result of the increase in permeability. In the absence of acid there should be no increased production of fluid. On the other hand if the fluid were due to active secretion then it would occur in the presence or absence of acid. Perfusion experiments were therefore performed in canine Heidenhain pouches in which the effect of topical and intravenous E_2 prostaglandins was studied when the perfusate contained no acid. The solution used was isosmotic, and contained Li^+, Na^+, Cl^- and PEG. Lithium behaves in the same way as H^+ in respect of permeability[55] and can be used as an

indicator of permeability in the absence of H^+. Sodium would not be an accurate indicator of permeability if nonparietal cell secretion were occurring as the secreted fluid would contain sodium ions. PEG is a volume marker, and the absence of acid allowed the HCO_3^- content of the perfusate to be measured.

The production of fluid containing Na^+, Cl^- and HCO_3^- in response to 16DM in the absence of exogenous acid would indicate direct stimulation of nonparietal cells.

3. The Effect of the E_2 Prostaglandins on Established Gastric Mucosal Barrier Damage

The inhibitory effect of the E_2 prostaglandins on acid secretion and the proven antiulcer effect in experimental animals has led to their study in man as therapeutic agents in peptic ucleration. PGE_2 is ineffective when given orally, but the two methyl analogues, 15M and 16DM, are highly potent inhibitors by the oral route. Controlled trials have demonstrated that 15M will reduce both the severity of the pain[16] and the size of the ulcer[15] in patients with gastric and duodenal ulcers, significantly better then a placebo. However the agent is not currently in general use, and it seems likely that the recent introduction of the H_2-receptor antagonists on to the European market will greatly reduce interest in the prostaglandins unless serious side effect of the H_2-receptor antagonists emerge. However, the prostaglandins may find a role in the treatment of acute gastric mucosal lesions as their known gastric effects are particularly

appropriate to this condition. The pathogenesis of acute gastric mucosal lesions is complex but two factors are of particular importance, the presence of acid and disruption of the gastric mucosal barrier.[42] The medical management of bleeding from acute gastric mucosal lesions has changed little over the past 30 years and is aimed primarily at reducing the amount of gastric acid present by neutralization and more recently by inhibition. An agent which combined acid inhibition with the ability to tighten the gastric mucosal barrier would theoretically be of great value.[56]

It has previously been demonstrated that 15M can prevent damage to the gastric mucosal barrier by aspirin and indomethacin, and this could indicate a possible prophylactic role. Before suggesting a therapeutic role it would be necessary to demonstrate that the prostaglandins could also reverse gastric mucosal barrier damage once it was established. An attempt has been made to do this using the perfused canine Heidenhain pouch.

Initially it was necessary to design a model in which gastric mucosal barrier damage could be produced and in which the damage would persist after withdrawal of the damaging agent, a situation analagous to that encountered clinically. This was done by perfusing the pouch for two hours with an acid and aspirin solution and then perfusing with acid alone for the third hour. The persistence of damage during the third hour would provide the model on which the effect of the prostaglandins could be tested by adding them to the perfusate and giving them intravenously during the third hour. As evidence has been

presented that the protective effect of the prostaglandins is not related to acid secretory inhbition it was decided to investigate simultaneously the effect of another type of antisecretory agent, an H_2-receptor antagonist, on established gastric mucosal barrier damage. The drug metiamide was used. This agent in solution acts as a buffer and is not suitable for addition to acid perfusates, and therefore only its intravenous effects were studied.

STUDY OF THE EFFECTS OF THE E_2 PROSTAGLANDINS ON GASTRIC MUCUS PRODUCTION IN THE RAT (EXPERIMENT 1)

Material and Methods

(i) Preparation of the Animals: The study was carried out in male Wistar rats weighing approximately 300 gm. After an 18 hour fast a laparotomy was performed under ether anaesthesia, and the esophagus ligated. This was done to prevent saliva contaminating the gastric contents. Vagotomy was accomplished by including the vagi in the esophageal ligature. A needle was passed from the duodenum through the pylorus, and 4 ml. of the test solution were injected into the stomach, the needle withdrawn and the pylorus ligated. After laparotomy, the rats were allowed to recover and then sacrificed at three hours.

Group I: The effect of Topical Application of the Prostaglandins: The test solution consisted of 150 mM sodium chloride in the controls and 150 mM sodium chloride plus the prostaglandin in the experimental groups. 15M and 16DM were added in a concentration of 10 µg/ml and PGE_2 in a concentration of 100 µg/ml. These concentrations are smaller than the concentrations used in experiments to demonstrate the antiulcer properties of these agents and represent doses of 0.13 mg/Kg for 15M and 16DM and 1.3 mg/Kg for PGE_2 which are less than the ED_{50} for inhibition of acid secretion in pylorus ligated rats. There were initially 10 rats in the control group and in each of the three experimental groups, one rat in the PGE_2 group died during the course of the experiment.

Group II: The Effect of Intravenous Administration of Prostaglandins:

The test solution was 150 mM sodium chloride in both control and experimental groups. A fine polyethylene cannula was placed cephalad in the inferior vena cava and brought to the surface through the posterior abdominal wall for the repeated administration of the prostaglandins. The prostaglandins were given by bolus intravenous injection through the cannula. Three injections were given: the first when the test solution was placed in the stomach, and then at one and two hours afterwards. Each bolus dose of 15M and 16DM was 0.6 µg/Kg and each dose of PGE_2 0.6 mg/Kg. These doses are below the ED_{50} for inhibition of acid secretion in pylorus ligated rats when these agents are given subcutaneously. There were initially five rats in the control and each of the three experimental groups, one rat in the control group died, and in one rat in the 15M group the cannula became dislodged.

Group III: The Effect of Subcutaneous Administration of Histamine di-HCl:

The preparation of the animals was the same as for the other groups. The histamine was given by intermittent subcutaneous injection in 1 ml of normal saline. Three subcutaneous injections were given: the first when the test solution was placed in the stomach, and then at one and two hours afterwards. Each bolus dose was 6 mg/Kg. The control animals received normal saline. There were five rats in the control and experimental group.

(ii) Collection of Samples: At the completion of the experiment the rats were sacrificed. The stomachs were removed, opened down the lesser curvature and the contents collected. The gastric contents were homogenised in a Dounce homogeniser and the volume recorded.

Analysis

1. Hydrogen Ion: The hydrogen ion concentration was measured by titration against 0.1 N sodium hydroxide in an automatic titrimeter (Radiometer Copenhagen).

2. Mucus: The mucus content was estimated by measuring the amount of Alcian Blue bound to the whole stomach and to a sample of the gastric contents. The Alcian Blue used was Alcian Blue GX8.

A) Free Mucus: The dye binding in the gastric contents was measured by the method of Piper et al.[57] They studied a variety of conditions which could influence the Alcian Blue binding properties of gastric juice mucoproteins and reached the following conclusions:

a) Concentration of Alcian Blue: The concentration was varied between 2 and 100 mg/100 ml and maximum precipitation was obtained at 40 mg/100 ml.

b) pH of the Reaction Mixture: Maximum precipitation of the dye was obtained at pH 6.

c) Temperature of the Reaction Mixture: No difference was found between binding at 20°C and 37° C.

d) Time of Incubation: It was found that dye binding increased with time, the amount of dye precipitated at the end of the 24 hours was almost double that at one hour.

e) Ionic Strength of the Reaction Mixture: Varying the molarity of sodium chloride was found to have no effect on dye binding.

The optimal conditions outlined here were used in these experiments.

One millilitre of the homogenised gastric contents was mixed with 3.3 ml. of McIlvaines citrate phosphate buffer and 0.2 ml. of Alcian Blue of concentration 10 mg/ml and made to 5 ml. with distilled water. The Alcian Blue solution was freshly prepared each day. The concentration of Alcian Blue in the reaction mixture was 40 mg/100 ml and the pH 5.8. The reaction mixture was incubated at 20°C for 24 hours and then centrifuged at 2500 revolutions per minute for 10 minutes. The concentration of Alcian Blue in the supernatent fraction was estimated spectrophotometrically at 615 nm and compared with that in a tube containing the identical reagents except that the gastric juice was replaced by buffer. Dye binding was expressed as the amount of Alcian Blue in milligrams precipitated from the reaction mixture during incubation.

The standard curve for Alcian Blue in this reaction mixture is shown in Figure 3. The curve was constructed

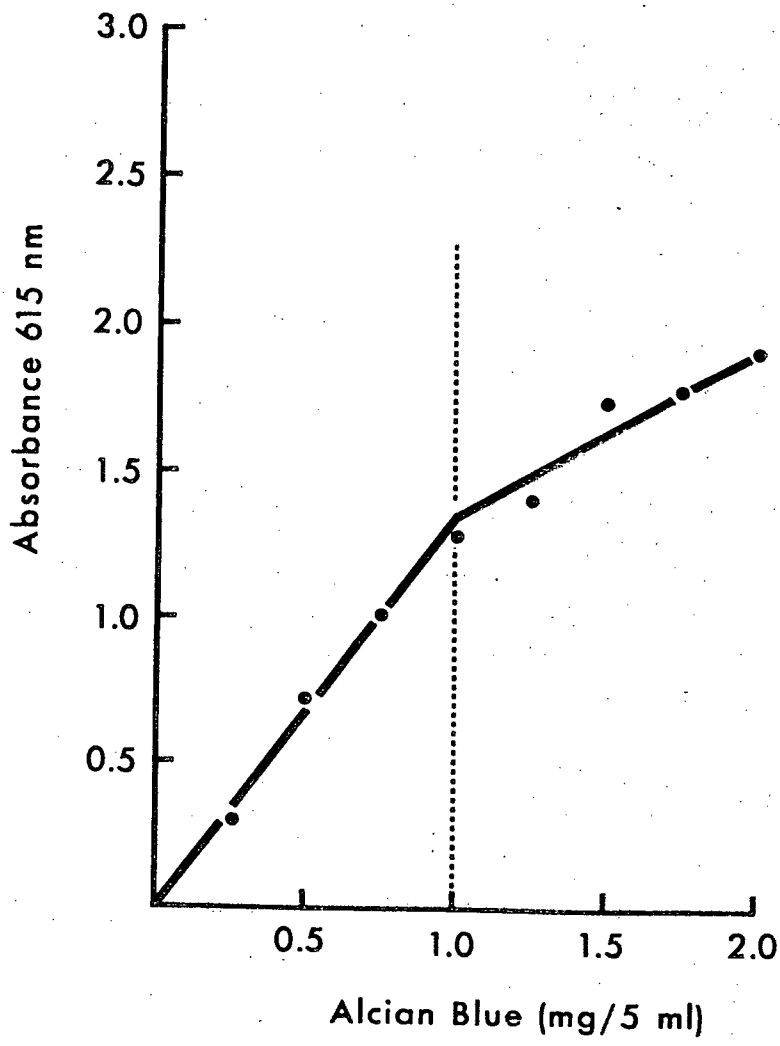


FIGURE 3: The standard curve for Alcian Blue prepared in the reaction mixture used for the measurement of free mucus.

from triplicate assays of each concentration tested and can be seen to be biphasic. One millilitre of of the supernatant of each sample was diluted with one millilitre of buffer prior to measuring the absorbance so that all the absorbance readings fell on the first part of the curve.

An error could occur if Alcian Blue bound to plasma proteins. There is always a small leak of plasma proteins into the gastric lumen and this may be increased by an increase in mucosal blood flow. Studies were performed in which plasma from a rat was used to replaced the gastric contents in the reaction mixture and the binding measured.

B) Barrier Mucus: This was estimated by a modification of the method of Corne et al.[53] The everted stomach was incubated in 25 ml. of McIlvaines citrate phosphate buffer containing Alcian Blue in a concentration of 40 mg/100 ml, for two hours. The stomach was then removed and the solution centrifuged at 2500 revolutions per minute for 10 minutes and the concentration of Alcian Blue estimated spectrophotometrically. Dye binding was expressed as the amount of Alcian Blue in milligrams precipitated from the solution during incubation.

The standard curve for Alcian Blue in McIlvaines citrate phosphate buffer is shown in Figure 4. It was constructed in the same way as the other standard curve and is similarly biphasic. The supernatant samples were also diluted with buffer to bring the absorbance readings into the first part of the curve.

Corne demonstrated by histological examination of the rat stomach stained for two hours with Alcian Blue that there was no penetration of the dye into the mucosal tissue.

(iv) Statistical Analysis: The results were assessed using Student's t-test for paired values and applying the Hotelling correction.

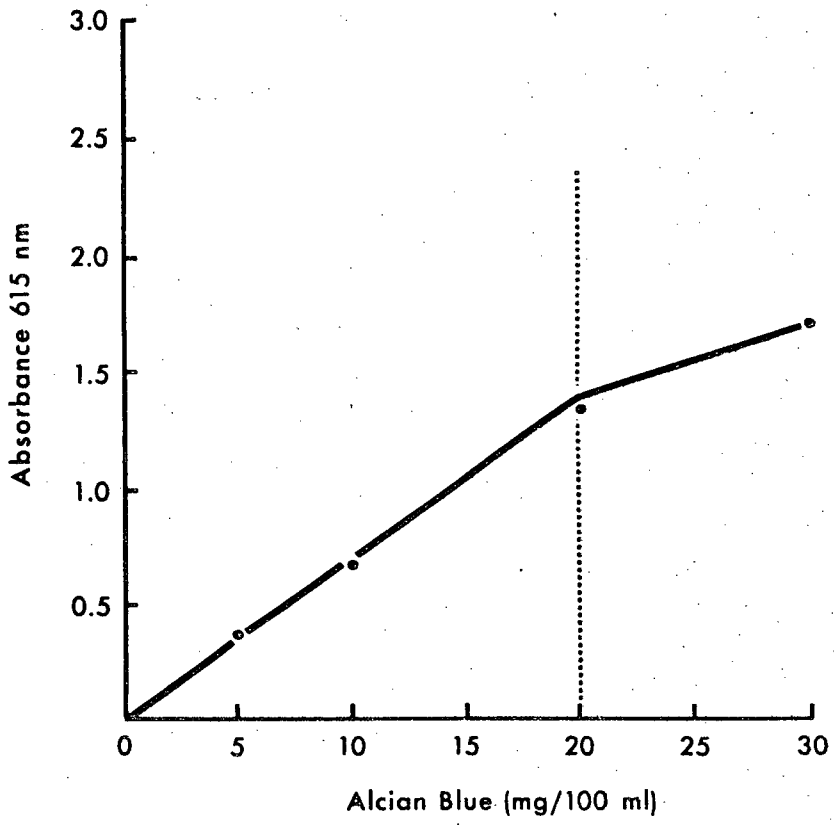


FIGURE 4: The standard curve for Alcian Blue used in the measurement of bound mucus.

Results

The results of the topical application of the prostaglandins are tabulated in Table I and those for intravenous administration are tabulated in Table II.

Prostaglandin E_2 had the same effect on mucus production when given by either route. As can be seen from Table I and II it had no effect on barrier mucus as measured by Alcian Blue bound to the gastric mucosa but caused a highly significant increase in mucus in the gastric contents ($p < 0.01$) and the total amount of mucus produced ($p < 0.01$). 16DM had a similar effect when applied topically (Table I), having no effect on barrier mucus but causing a highly significant increase in free ($p < 0.01$) and total ($p < 0.01$) mucus. Given intravenously (Table II) the effect was less marked. There was a significant increase in free mucus ($p < 0.05$) but the overall increase was not significant. 15M given intravenously caused the largest increase in free mucus ($p < 0.01$) and consequently in the total mucus produced ($p < 0.01$) but was without effect on barrier mucus. Given topically (Table I) it caused a reduction in barrier mucus ($p < 0.01$) and a corresponding increase in free mucus ($p < 0.01$) but without a significant increase in the total amount.

The alteration in the volume and acid output produced by the three agents is shown in the Tables. Four millilitres was the volume of test solution instilled into the stomach at the onset of the experiments. Topical application of the prostaglandins caused an increase in volume of between 1 and 3 ml., being greatest with PGE_2 and least with 16DM.

EXPERIMENT	NO.	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
CONTROL	10	4.05 ±0.03	70.4 ±10.2	0.80 ±0.06	0.57 ±0.09	1.37 ±0.11
15M 10 µg/ml	10	5.45** ±0.27	23.1** ±5.9	0.45** ±0.08	1.44** ±0.19	1.89 ±0.25
16DM 10 µg/ml	10	5.07** ±0.08	20.4** ±3.2	0.67 ±0.13	1.66** ±0.30	2.33** ±0.35
PGE_2 100^2 µg/ml	9	6.82** ±0.41	12.7** ±5.82	0.71 ±0.19	2.16** ±0.505	2.88** ±0.55

** = $p < 0.01$

TABLE I: Effect of topical E_2 prostaglandins on Alcian Blue binding, volume secreted and acid output in the closed rat stomach over 3 hours (Mean ± S.E.).

EXPERIMENT	NO.	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
CONTROL	4	3.88 ±0.12	40.75 ±7.78	0.71 ±0.04	1.60 ±0.18	2.31 ±0.22
15M 0.3 µg/Kg/Hr	4	4.33 ±0.02	82.4* ±13.2	0.98 ±0.03	4.72** ±0.11	5.82** ±0.04
16DM 0.3 µg/Kg/Hr	5	4.04 ±0.02	45.1 ±7.7	0.61 ±0.05	2.82* ±0.46	3.42 ±0.49
PGE_2 0.3 mg/Kg/Hr	5	4.30 ±0.05	36.1 ±2.07	0.97 ±0.12	3.74** ±0.47	4.70** ±0.54

* = $p < 0.05$ ** = $p < 0.01$

TABLE II: Effect of intravenous E_2 prostaglandins on Alcian Blue binding, volume secreted and acid output in the closed rat stomach over 3 hours (Mean ± S.E.).

All these increases were highly significant ($p < 0.01$). Intravenous administration however did not cause a significant increase in the volume of fluid in the stomach. Topical application of each prostaglandin caused a highly significant ($p < 0.01$) reduction in acid output. When given intra venously 16DM and PGE_2 had not effect on acid output, but 15M caused an increase in acid output that just reached significance ($p < 0.05$).

Table III shows the results of repeated subcutaneous injections of histamine. Histamine produced a significant increase ($p < 0.05$) in volume secreted, but the acid output was variable between the individual animals at this dose and the increase in acid output was not significant. There was a significant ($p < 0.05$) reduction in Alcian Blue bound to the mucosa, but no alteration in that bound to the gastric contents, or the total amount bound. Ulcers did not develop in any of the stomachs.

Table IV shows the degree of binding of Alcian Blue that occurred in rat plasma. In two specimens of pooled plasma 0.14 mg. Alcian Blue was bound per ml. of plasma.

EXPERIMENT	NO.	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
CONTROL	5	4.14 ±0.01	147.34 ±23.38	0.51 ±0.11	0.45 ±0.06	0.96 ±0.16
HISTAMINE S.C. 6 mg/Kg/Hr	5	4.80* ±0.34	246.84 ±95.03	0.26* ±0.04	0.49 ±0.06	0.75 ±0.09

* = $p < 0.05$

TABLE III: Effect of subcutaneous histamine on Alcian Blue binding, volume secreted and acid output in the closed rat stomach over 3 hours (Mean ± S.E.).

	PLASMA A	PLASMA B	BLANK
Absorbance	1.0	1.0	1.1
mg AB/5 ml.	0.75	0.75	0.82
Correction for Dilution	1.50	1.50	1.64
mg. AB ppt.	0.14	0.14	

TABLE IV: Alcian Blue binding by rat plasma.

A STUDY OF THE EFFECT OF THE E_2 PROSTAGLANDINS ON NONPARIETAL CELL SECRETION IN CANINE HEIDENHAIN POUCHES (EXPERIMENT 2)

Material and Methods

(i) Preparation of the Animals: The study was carried out in a total of six female mongrel dogs weighing approximately 20 Kg., four dogs being used in each set of experiments. Each dog underwent a laparotomy at which an antrectomy and gastroduodenal anastomosis were performed and a Heidenhain pouch prepared. The pouch opened to the surface through a wide bore metal cannula. The dogs were allowed four weeks to recover from surgery before any tests were performed.

(ii) Characteristics of the Model: In this model the Heidenhain pouch should not secrete acid except in response to an exogenous stimulus. Each dog was tested to demonstrate firstly that the pouch could respond to an intramuscular dose of pentagastrin by acid secretion, and secondly that following three hours perfusion with normal saline no acid was secreted into the perfusate.

(iii) The Perfusion System: Perfusion studies were performed using a system illustrated schematically in Figure 5. The pouches filled by gravity infusion from a reservoir set at the height of the pouch, to avoid distension. The perfusate entered at the bottom and left from the top and thereby attained maximum contact with the mucosa. The peristaltic pump was placed on the withdrawal line to return the

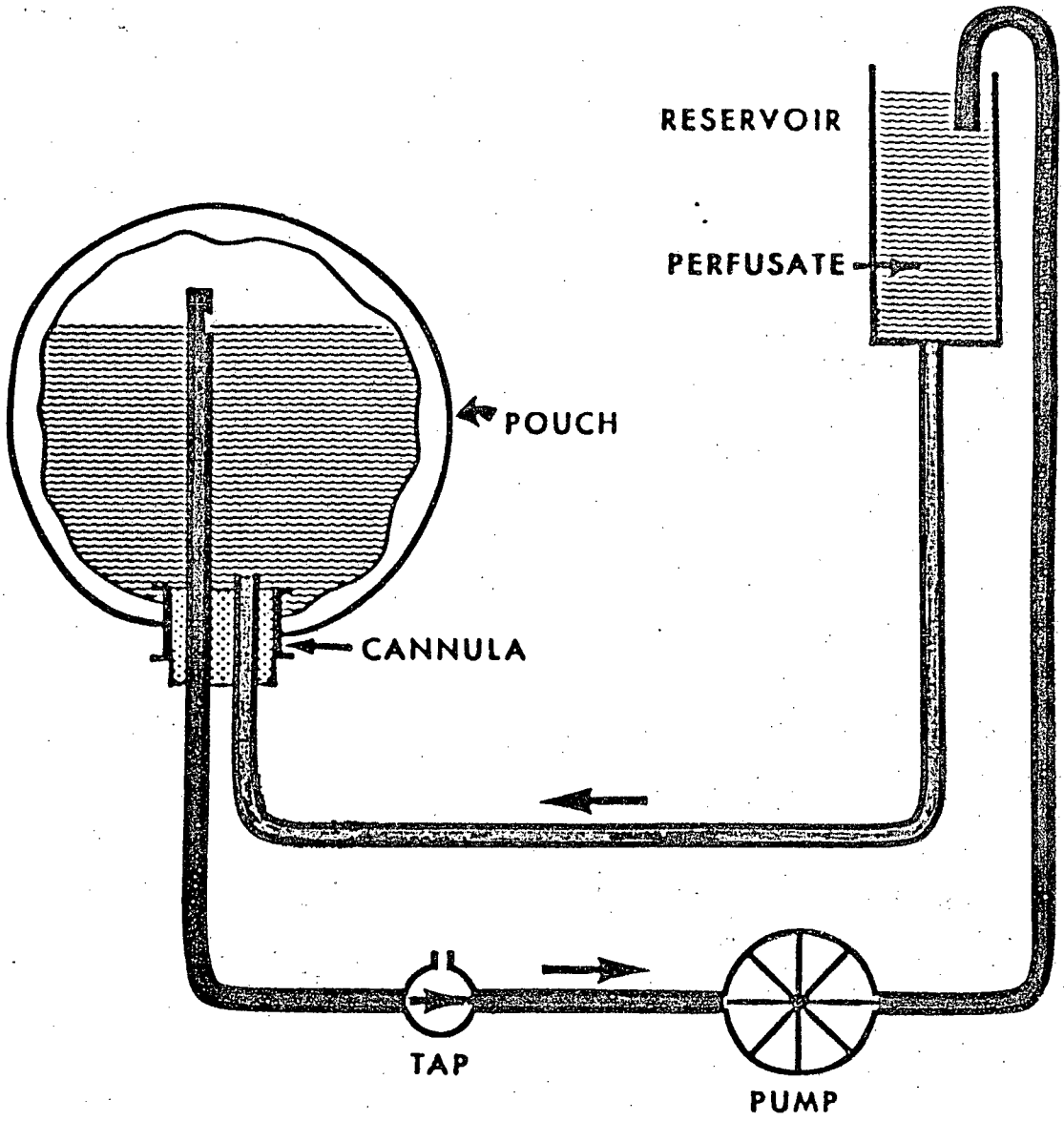


FIGURE 5: Illustrates the perfusion system used in the canine Heidenhain pouches.

fluid to the reservoir and a sampling tap allowed regular samples to be taken. Prior to each experiment the dogs were fasted for 18 hours and during the experiment stood comfortably supported on a Pavlov table. The pouches were perfused for three hours with 60 ml. of a solution that contained 5 mEq/L lithium, 5 gm/L poly ethylene glycol (PEG) as a volume marker and was made isosmotic at 300 mOsm/L with NaCl. Samples (3.5 ml.) were taken at 30 minute intervals and were analysed for Na^+, Li^+, K^+, Cl^-, HCO_3^- and PEG.

(iv) Analysis

1) Sodium, Lithium and Potassium: These ions were analysed by flame photometry using the Corning Flame Photometer.

2) Chloride: This ion was measured on a Corning chloride meter.

3) Bicarbonate: This was measured by the method of back titration. One milliliter of the sample was added to 0.5 ml. of 0.1 N HCl, heated to drive off the CO_2, diluted with 10 ml. distilled water and titrated against 0.1 N sodium hydroxide using an automatic titrimeter (Radiometer Copenhagen).

4) PEG: The PEG concentration was measured by the method of Malawer and Powell[58] which is a modification of the turbimetric method of analysis. The turbimetric method is based on the creation of an oil-in-water emulsion of the water soluble PEG when trichloroacetic acid (TCA) is added. In this method described by Hydén[59] the emulsion lacks stability and the plateau of maximum turbidity is short lived so that precise timing is required from the addition of the TCA reagent until

the optical density is read. The instability in Hydén's method results from the fact that in the concentration used the droplets coalesce. Coalescence can be prevented by the addition of a emulsifying agent which can enter the oil-water interface and produce a film around the droplets. Gum arabic is such an agent. The addition of gum arabic is the basis of Malawer and Powell's modification and gives a stable and prolonged peak of maximum turbidity which makes precise timing unnecessary and provides a linear PEG concentration - optical density relationship over the range of PEG concentration used in this experiment which was 500-300 mg/100 ml (Figure 6).

Group I: The Effect of Topical Application of the Prostaglandins: In the control studies the pouches were perfused with the lithium/saline/PEG solution alone. In the experimental studies the prostaglandins were added to the perfusate in the following concentrations: 15M and 16DM, 10 µg/ml; PGE_2, 100 µg/ml.

Group II: The Effect of Intravenous Administration of Prostaglandins: In these studies the pouches were perfused exclusively with the lithium/saline/PEG solution. In the control studies 25 ml. normal saline was given intravenously each hour. In the experimental studies the prostaglandins were administered in 75 ml. normal saline at a rate of 0.6 µg/Kg/hour for 15M and 16DM and 0.6 mg/Kg/hour for PGE_2.

These concentrations and infusion rates correspond to those used in previous studies of the permeability effects. The control experiments and each of the prostaglandin experiments were performed twice in each dog.

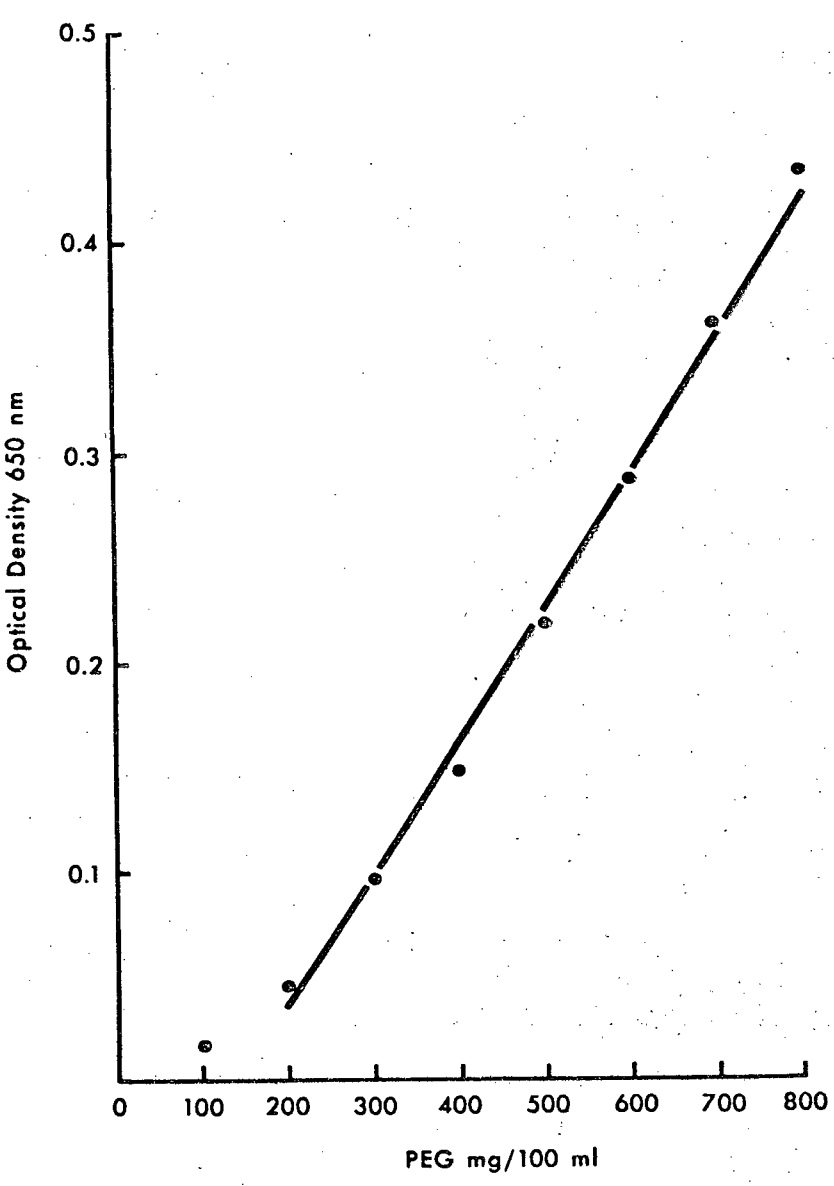


FIGURE 6: A representative standard curve for polyethylene glycol.

Calculation of Data: Net ion fluxes (NIF) and permeability factors (K) were calculated for each ion from the following equations:

$$Vo = Vi(PEGi)/(PEGo)$$

$$Vl = (Vo - SV)(PEGo/PEGi)$$

$$Vj = (Vj\text{-}i - SV)(PEGj/PEGj\text{-}1)$$

$$NIF = VjCj - (Vj\text{-}1 - SV)\,Cj\text{-}1$$

where V = volume of perfusate

C = concentration of ion

PEG = concentration of PEG

SV = sample volume

i = instilled solution

o = zero time solution.

For each ion six 30-minute flux determinations were made, and the NIF for the experiment was taken as the mean of the last five values.

Permeability factors for each ion were calculated from the formula:

$$K\ (ml/30\ minute) = \frac{\text{net ion flux } (\mu eq/30\ minutes)}{1/2(Cj - Cj\text{-}1)\ (\mu eq/ml)}$$

Six permeability factors were calculated for each ion corresponding to the six 30-minute periods. The permeability factor for the experiment was taken as the mean of the last five values.[55] For each concentration or dose of each prostaglandins eight sets of NIF and K values were available for each ion. The results are expressed as the mean and SE of these eight values.

Statistical Analysis: The statistical significance of any difference between the control and experimental values was assessed using Student's t-test for paired values.

Results

The volume secreted and the bicarbonate output for the topical application of the prostaglandins is shown in Table V and for intravenous administration in Table VI.

When given by either route 16DM caused a highly significant ($p < 0.005$) increase in the volume secreted over the three hour period, in each case being almost double the amount produced in the control experiments.

15M and PGE_2 given by either route did not cause a significant alteration in the volume of fluid produced. In both groups the increase in fluid produced by 16DM was associated with a highly significant ($p < 0.005$) increase in bicarbonate secretion over the three hour period. When applied topically 15M caused a highly significant ($p < 0.01$) increase in bicarbonate output but had no effect when given intravenously. PGE_2 had no effect on bicarbonate secretion when given by either route.

The effect of topical application of the three prostaglandins on the four ions measured is shown in Tables VII and VIII, Table VII showing the net ion flux (NIF) for each ion and Table VIII the permeability factor (K).

16DM caused a highly significant increase ($p < 0.0005$) in the NIF of Na^+ and Cl^- but did not alter the Li^+ flux. These alterations in NIF are also reflected in the changes in the permeability factors of these ions and the permeability factor for K^+ was also significantly increased ($p < 0.05$). 15M and PGE_2 were without effect on NIF and permeability factors of all the ions.

	NO.	VOLUME PRODUCED ml/3 hours	HCO_3^- µEq/3 hours
CONTROL	8	18.16 ±3.31	121.99 ±31.96
15M 10 µg/ml	8	18.42 ±1.39	231.15* ±23.93
16DM 10 µg/ml	8	37.68** ±3.42	483.37** ±110.99
PGE_2 100 µg/ml	8	22.30 ±2.65	55.95 ±22.79

* = $p < 0.01$ ** = $p < 0.005$

TABLE V: Effect of topical E_2 prostaglandins on volume and bicarbonate secretion from Heidenhain pouch (Mean ± S.E.).

	NO.	VOLUME PRODUCED ml/3 hours	HCO_3^- µEq/3 hours
CONTROL	8	13.43 ±2.01	65.91 ±18.52
15M 16,16 DM 0.6 µg/Kg/Hr	8	11.91 ±1.01	30.13 ±10.11
16DM 0.6 µg/Kg/Hr	8	24.47** ±1.60	317.78** ±66.27
PGE_2 0.6 mg/Kg/Hr	8	12.10 ±1.22	62.37 ±31.88

** = $p < 0.005$

TABLE VI: Effect of intravenous E_2 prostaglandins on volume and bicarbonate secretion from Heidenhain pouch (Mean ± S.E.)

	NO.	Cl^-	Li^+	Na^+	K^+
CONTROL	8	119.60 ±56.30	-3.22 ±0.80	161.74 ±58.05	26.94 ±2.47
15M 10 µg/ml	8	125.50 ±29.84	-1.46 ±1.08	229.79 ±28.77	21.09 ±2.79
16DM 10 µg/ml	8	553.28** ±87.38	-0.90 ±1.56	666.42** ±87.57	33.48 ±3.27
PGE_2 100 µg/ml	8	236.48 ±82.85	-3.14 ±1.15	267.76 ±54.22	30.72 ±6.37

** = $p < 0.0005$

TABLE VII: Effect of topical E_2 prostaglandins on net ion fluxes (µeq/30 minutes) from Heidenhain pouches (Mean ± S.E.)

	NO.	Cl^-	Li^+	Na^+	K^+
CONTROL	8	0.80 ±0.38	-0.82 ±0.17	1.08 ±0.38	14.88 ±1.73
15M 10 µg/ml	8	0.85 ±0.17	-0.40 ±0.28	1.65 ±0.20	15.86 ±0.80
16DM 10 µg/ml	8	4.02** ±0.64	-0.49 ±0.53	4.76** ±0.61	19.06* ±1.36
PGE_2 100 µg/ml	8	1.50 ±0.50	-0.93 ±0.34	1.86 ±0.37	18.53 ±1.84

* = $p < 0.05$ ** = $p < 0.0005$

TABLE VIII: Effect of topical E_2 prostaglandin on permeability factors K (ml/minute) (Mean ± S.E.).

The effect of intravenous administration of the prostaglandins is shown in Tables IX and X, Table IX showing the NIF for each ion and Table X the K factors.

The effect of 16DM on Na^+ and Cl^- is similar intravenously as when applied topically causing a higly significant ($p < 0.0005$) increase in NIF, and in addition a significant ($p < 0.05$) increase in NIF of K^+. However the NIF of Li^+ was significantly ($p < 0.05$) reduced.

15M caused a reduction in the NIF of Na^+ and Cl^-, there being a net loss of these ions from the pouch during the perfusion period. The reduction in Na^+ flux was significant ($p < 0.05$). 15M did not affect the Li^+ or K^+ fluxes. Prostaglandin E_2 was without effect on the NIF of all the ions.

Similar alterations were produced in the permeability factors. 16DM caused a highly significant ($p < 0.0005$) increase in $K_{Na}+$ and $K_{Cl}-$ and a significant ($p < 0.05$) reduction in $K_{Li}+$. The increase in K_K+ was not significant. With 15M the reductions in $K_{Na}+$ and $K_{Cl}-$ were significant ($p < 0.05$), but 15M was without effect on $K_{Li}+$ and K_k+. PGE_2 was without effect on the permeability factors.

	NO.	Cl^-	Li^+	Na^+	K^+
CONTROL	8	31.74 ±34.55	-5.25 ±1.02	49.91 ±37.20	17.16 ±1.61
15M 0.6 µg/Kg/Hr	8	-31.78 ±19.12	-4.85 ±0.86	-40.35* ±18.03	17.34 ±3.76
16DM 0.6 µg/Kg/Hr	8	363.21** ±41.28	-2.48* ±0.74	421.85** ±66.79	24.98* ±1.85
PGE_2 0.6 mg/Kg/Hr	8	39.66 ±28.46	-4.66 ±0.88	30.73 ±27.80	20.34 ±2.09

* = $p < 0.05$ ** = $p < 0.0005$

TABLE IX: Effect of intravenous E_2 prostaglandins on net ion fluxes (µeq/30 minutes) from Heidenhain pouches (Mean ± S.E.)

	NO.	Cl^-	Li^+	Na^+	K^+
CONTROL	8	0.23 ±0.24	-1.39 ±0.28	0.32 ±0.26	14.76 ±1.21
15M 0.6 µg/Kg/Hr	8	-0.22* ±0.10	-1.30 ±0.22	-0.29* ±0.10	13.11 ±2.38
16DM 0.6 µg/Kg/Hr	8	2.55** ±0.28	-0.73* ±0.17	3.02** ±0.43	17.27 ±1.70
PGE_2 0.6 µg/Kg/Hr	8	0.28 ±0.20	-1.19 ±0.22	0.22 ±0.17	16.29 ±1.03

* = $p < 0.05$ ** = $p < 0.0005$

TABLE X: Effect of intravenous E_2 prostaglandins on permeability factors K (ml/minute) (Mean ± S.E.)

THE EFFECT OF E_2 PROSTAGLANDINS ON THE REVERSAL OF ESTABLISHED GASTRIC MUCOSAL BARRIER DAMAGE (EXPERIMENT 3)

1. Preparation and Validation of a Suitable Model

Material and Methods: The experiments were carried out on five antrectomized dogs with Heidenhain pouches. The perfusion system used was as described and illustrated in the previous chapter but various modifications in the method were introduced. The three hour perfusion period was divided into three separate one hour periods, samples were taken at 10 minute intervals and the perfusing solution was changed at the end of each hour. In this way it was possible to calculate the permeability factor for each hour. The results for the first two hours were averaged.

Control experiments were performed in which the pouches were perfused for each of the three one hour periods with an acid/saline solution containing 120 mEq/L hydrogen ion, 5 gm/L PEG as a volume marker and made isosmotic at 300 mOsm/L with sodium chloride. In subsequent experiments damage of the gastric mucosal barrier was produced by perfusing the pouches during the first two hours (Damage Period) with acid saline solutions containing 20 mM aspirin. During the third hour (Recovery Period) only an acid/saline perfusate was used. (Figure 7)

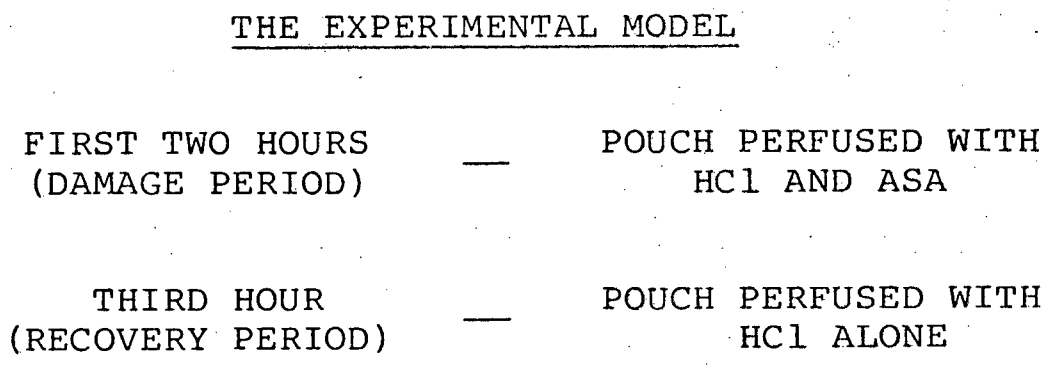


FIGURE 7: Illustration of the experimental model used to test the effect of various drugs on established gastric mucosal barrier damage.

Analysis: The following analyses were carried out on each 10-minute sample, by the methods previously described.

1) Hydrogen Ion
2) Sodium and Potassium
3) Chloride
4) Polyethylene Glycol

Calculation of Data: The results are expressed as permeability factors (K) for each ion which were calculated from each experiment from the regression line of the log of the concentration of the ion against time. The slope of the regression line is multiplied by the average volume of the perfusate to give the K values.

Results: The addition of 20 mM aspirin to the perfusate caused a significant ($p < 0.005$) increase in $K_{H}+$ and $K_{Na}+$ during the Damage Period, indicating damage to the gastric mucosal barrier (Table XI). In the Recovery Period when only an acid saline perfusate was used the pouches that had been previously perfused with the acid/saline and aspirin exhibited evidence of continuing gastric mucosal barrier damage as both $K_{H}+$ and $K_{Na}+$ remained significantly ($p < 0.05$) elevated above the control levels. (Table 11) In the pouches exposed to aspirin there was no significant difference between the $K_{H}+$ and $K_{Na}+$ values in the Damage and Recovery Periods.

Conclusions: This model provides a period of one hour in which gastric mucosal barrier damage persists despite withdrawal of the damaging agent. In subsequent experiments this model was used.

DAMAGE PERIOD

EXPERIMENT	PERFUSING SOLUTION	NO	NIF µeq/minute Na^+	NIF µeq/minute H^+	K Na^+	K H^+
CONTROL	ACID SALINE	20	14.39 ±1.47	-2.49 ±1.44	0.30 ±0.03	-0.13 ±0.01
DAMAGE	ACID SALINE + 20 mM ASA	20	22.50** ±2.50	-16.64** ±2.54	0.42** ±0.30	-0.29** ±0.04

RECOVERY PERIOD

CONTROL	ACID SALINE	10	14.27 ±2.08	-0.56 ±2.53	0.27 ±0.03	-0.12 ±0.02
DAMAGE	ACID SALINE	10	20.30 ±3.28	-9.20* ±3.51	0.41* ±0.05	-0.20* ±0.04

* = $p < 0.05$ ** = $p < 0.005$

TABLE XI: Effect of 20 mM aspirin during the Damage Period on net ion flux (NIF µeq/minute) and permeability factor (K) for Na^+ and H^+ (Mean ± S.E.).

2. Study of the Effects of PGE_2, 15M and Metiamide on Established Gastric Mucosal Barrier Damage

Material and Methods: Using the model just described and validated experiments were performed to study the effect of topical PGE_2 and 15M and intravenous PGE_2, 15M and metiamide.

Group I: The Effect of Topical Applications of the Prostaglandins: PGE_2 and 15M were added to the acid saline perfusate during the Recovery Period, PGE_2 in a concentration of 100 µg/ml and 15M in a concentration of 10 µg/ml.

Group II: The Effect of Intravenous Administration of the Prostaglandins: PGE_2 and 15M were given by intravenous infusion in 25 ml. normal saline during the Recovery Period - PGE_2 at a rate of 0.3 mg/Kg/hour, 15M at a rate of 0.6 µg/Kg/hour.

Group III: The Effect of Intravenous Administration of Metiamide: Metiamide was given at a rate of 3 mg/Kg/hour. This dose of metiamide is above the ED_{50} for the inhibition of stimulated acid secretion in a canine Heidenhain pouch[60] and is comparable in its antisecretory effect with the doses of the prostaglandins used.

Results

In Table XII the amount of the damage produced during the Damage Period in all the experiments is shown in terms of permeability factors K_H and K_{Na} and the net ion flux of H^+ and Na^+.

The experiments in which the effect of topical 15M was tested K_H+, $K_{Na}+$, and H^+ flux in the Damage Period were less than those in the validation experiments, indicating that the degree of damage was of a lesser degree. In the experiments in which the agents were given intravenously the H^+ flux was significantly ($p < 0.005$) reduced in the 15M experiment and the Na^+ flux ($p < 0.05$) in the metiamide experiment, but there was no difference in the permeability factors, and it is therefore considered that the amount of damage carried over into the Recovery Period was comparable in all the experimental groups, except that for topical 15M.

Table XII shows the effect of topical PGE_2 and 15M on K_H+ and $K_{Na}+$ values and NIF during the Recovery Period. There was no signifi cant difference between the K values or the NIF when no treatment was given and when PGE_2 was applied topically. Topical 15M did not cause a significant reduction in K_H+ or the Na^+ flux, but $K_{Na}+$ was reduced ($p < 0.005$) as was the H^+ flux ($p < 0.05$). However as there was no difference between the $K_{Na}+$ or H^+ flux values in the Damage and Recovery Period for the topical 15M experiments, the low values in the Recovery Period can not be taken to indicate any reversal of damage.

DAMAGE PERIOD

EXPERIMENT	NO.	NIF µeq/minute Na^+	NIF µeq/minute H^+	K Na^+	K H^+
No Treatment	20	22.50 ±2.50	-16.64 ±2.54	0.42 ±0.03	-0.29 ±0.04
Top. PGE_2	18	19.28 ±1.85	-11.35 ±1.83	0.35 ±0.04	-0.25 ±0.01
Top. 15M	16	17.08 ±2.49	-7.17** ±1.86	0.24** ±0.03	-0.21* ±0.02
I.V. PGE_2	24	21.20 ±1.62	-13.44 ±2.72	0.43 ±0.01	-0.27 ±0.03
I.V. 15M	18	20.44 ±2.42	-4.63** ±3.53	0.35 ±0.05	-0.23 ±0.03
I.V. Metiamide	12	17.09* ±1.60	-11.53 ±1.80	0.36 ±0.03	-0.22 ±0.02

* = $p < 0.05$ ** = $p < 0.005$

TABLE XII: Comparison of the alteration in NIF (µeq/minute) and K occurring in the Damage Period in all the groups in which aspirin damage was produced (Mean ± S.E.).

PERFUSING SOLUTION		TREATMENT DURING		RECOVERY PERIOD			
				NIF µeq/minute		K	
DAMAGE	RECOVERY	R.P.	NO.	Na^+	H^+	Na^+	H^+
Acid Saline + ASA 20 mM	Acid Saline	Nil	10	20.30 ±3.28	-9.20 ±3.51	0.41 ±0.05	-0.21 ±0.04
Acid Saline + ASA 20 mM	Acid Saline	Top. PGE_2 100 µg/ml	10	23.80 ±3.69	-3.41 ±1.86	0.34 ±0.06	-0.22 ±0.03
Acid Saline + ASA 20 mM	Acid Saline	Top. 15M 10 µg/ml	8	17.68 ±2.95	-0.39* ±3.48	0.22** ±0.03	-0.17 ±0.03

* = $p < 0.05$ ** = $p < 0.005$

TABLE XIII Effect of treatment with topical PGE_2 and 15M on established GMB damage. NIF and K (Mean ± S.E.).

Table XIV shows the effect of the three intravenously administered agents on K_H^+ and K_{Na}^+ in the Recovery Period. PGE_2 caused a highly significant ($p < 0.005$) reduction in K_H^+ and K_{Na}^+ during this period and 15M a significant reduction ($p < 0.025$). Metiamide on the other hand did not alter the permeability factors, there being no significant difference between K_H^+ and K_{Na}^+ when treatment with metiamide was compared with no treatment. The data for the NIF of Na^+ supports the permeability factor data, there being a significant ($p < 0.025$) reduction with PGE_2 and 15M but no change with metiamide. The NIF of H^+ was reduced by PGE_2 and 15M but these values did not quite reach significance. Metiamide did not alter the NIF of H^+.

PERFUSING SOLUTION		TREATMENT DURING		RECOVERY PERIOD			
				NIF µeq/minute		K	
DAMAGE	RECOVERY	R.P.	NO.	Na^+	H^+	Na^+	H^+
Acid Saline + ASA 20 mM	Acid Saline	Nil	10	20.30 ±3.28	-9.20 ±3.51	0.41 ±0.05	-0.21 ±0.04
Acid Saline + ASA 20mM	Acid Saline	I.V. PGE_2 0.3 mg/Kg/Hr	12	10.52* ±1.58	-2.14 ±1.88	0.23** ±0.04	-0.10** ±0.01
Acid Saline + ASA 20 mM	Acid Saline	I.V. 15M 0.6 µg/Kg/Hr	9	12.13* ±1.98	-1.23 ±3.90	0.23* ±0.05	-0.10* ±0.03
Acid Saline + ASA 20 mM	Acid Saline	IV Metiamide 3 mg/Kg/Hr	6	16.95 ±1.75	-5.97 ±1.39	0.37 ±0.03	-0.17 ±0.02

* = $p < 0.025$ ** = $p < 0.005$

TABLE XIV: Effect of treatment with intravenous PGE_2, 15M and Metiamide on established GMB damage. NIF and K (Mean ± S.E.).

DISCUSSION

The E_2 prostaglandins have been shown capable of preventing damage to the gastric mucosa by a mechanism which is unknown but which is not related to their action as inhibitors of acid secretion.

Because of the potency of the E_2 prostaglandins as inhibitors of acid secretion, it was not until these agents were studied in basal pouch preparations that it was appreciated that they also had a secretory action on other cells in the gastric mucosa. These effects on mucus and nonparietal cell secretion have been studied as both effects could be regarded as protective in nature and may play a part in the protective effect of the prostaglandins. This secretory action may also be responsible for the misinterpretation of some permeability data.

Most previous studies have measured only one component of gastric mucus. The majority have measured free mucus, this being the easiest to collect and measure, but at least two studies have measured barrier mucus. In this study an attempt was made to measure the effect of the prostaglandins in both fractions.

The results indicate that when given topically and intravenously the E_2 prostaglandins cause an increase in the free fraction of mucus but are without effect on the barrier fraction, but there was some variation with the individual agents. Prostaglandin E_2 produced the same effects both topically and intravenously being without effect on the barrier mucus but causing an increase in the free fraction and an overall increase in mucus

production. This same pattern was shown by topical 16DM and intravenous 15M. Intravenous 16DM caused a similar trend but the total amount of mucus produced was not siginficantly greater than the controls. The only results which did not fit this pattern were those with topical 15M in which there was no increase in the total amount of mucus produced, but there was a significant fall in barrier mucus and a corresponding increase in free mucus suggesting that this agent stripped mucus from the mucosa so increasing the free fraction without stimulating mucus production. An attempt was made to compare the stimulant effect of the prostaglandins with another stimulant of gastric mucus (histamine), but this was not satisfactory because of the different effects of the stimulants on acid secretion. A combination of pyloric ligation and parenteral histamine is ulcergenic in the rat, and it was felt that in the presence of muosal ulceration Alcian Blue would bind to the areas of mucosal destruction, so giving falsely high values for barrier mucus. Unfortunately the dose of histamine chosen did not produce a significant increase in acid output, but it did not cause ulcers. Histamine caused a reduction in barrier mucus but no alteration in free mucus or the total amount produced. The increase in free mucus produced by the prostaglandins was greater than the effect produced by histamine but as the dose of histamine was not maximal - it was not possible to draw any conclusion regarding the magnitude of the response evoked by the prostaglandins. As dose-response studies with the prostaglandins were not performed the maximal mucus response to prostaglandin is not known.

Because the role of gastric mucus is unclear the interpretation of this data is difficult. Gastric mucus is freely permeable to hydrogen ion and has only minimal buffering capacity, and it plays no part in the

gastric mucosal barrier or in the neutralization of intraluminal acid. It probably acts as a lubricant on the gastric mucosa and prevents minor degrees of mechanical damage to the underlying cells. It is barrier mucus therefore which is probably the important fraction while free mucus probably represents denatured and shed barrier muucs. If freshly secreted mucus first appears as barrier mucus adherent to the mucosa, and then as a result of the action of acid, pepsin and local enzymes is broken down and shed as free mucus, one might expect an increase in the barrier fraction in response to a stimulant of mucus secretion. If however the barrier fraction stays more or less constant then an increase in the free mucus fraction would indicate that existing barrier mucus had been shed and replaced by freshly secreted mucus.

The prostaglandins did not increase barrier mucus and all except 15M caused no change in this fraction but an overall increase in mucus produc tion. The replacement of existing barrier mucus by freshly secreted mucus which would perform its function more efficiently could be regarded as protective, at least as far as minor mechanical trauma is concerned. The reason for the reduction in barrier mucus caused by topical 15M is not clear and may represent an inaccuracy in the method.

The method of measurement used in this study is probably less accurate than many of the biochemical methods of analysis, but has the advantage that both fractions can be measured. It was chosen for this reason and its simplicity, on the understanding that if the prostaglandins were confirmed as stimulants of mucus production then other more sophisticated techniques could be applied to the problem in the future.

The other important finding in this study was that when applied topically to the mucosa all three prostaglandins caused an increase in volume despite a fall in acid output. In these experiments it was impos sible to measure bicarbonate secretion, but there was indirect evidence that the fluid secreted was alkaline. PGE_2 applied topically is a poor inhibitor of acid secretion and yet in a dose well below the ED_{50} it caused a greater reduction in acid output than its more potent methyl analogues. It also caused the largest volume increase and therefore the apparent reduction in acid output and the unexpected potency could be due to neutra lization of the small amount of acid secreted in these vagotomised rats by the additional secretion of an alkaline gastric juice.

There are at least three possible explanations for these observed effects: damage to the gastric mucosal barrier, an increase in mucosal blood flow or stimulation of nonparietal and mucus secreting cells.

Damage to the gastric mucosal barrier could cause an increase in the fluid transudate into the gastric lumen and an apparent reduction in acid ouput due to back diffusion. However of the prostaglandins studied only 16DM has been suspected of breaking the gastric mucosal barrier and other experiments in this thesis refute this suggestion.

The effect of alteration in blood flow may be more important. The origin of nonparietal cell secretion remains uncertain. Is it a plasma transudate containing Na^+, HCO_3^- and protein,[61,62] or do nonparietal cells actively secrete HCO_3^- [63] and mucus in response to specific stimuli? If the former were true an increase in mucosal blood flow accompanied by an increase

in hydrostatic pressure could lead to an increased transudation of fluid and protein. Alcian Blue has been shown not to bind significantly to plasma protein and therefore the measured increase in Alcian Blue binding in the gastric contents is not solely due to an increase in the plasma protein in the gastric lumen. Also it is to be expected that the vasoactive properites of those agents would be more apparent with intravenous rather than topical administration. The increases in mucus production following intravenous administration occured in the absence of any volume changes and so must be independent of the secretion of alkaline juice. This evidence strongly suggests that in the rat these agents stimulate mucus and nonparietal cell secretion.

Further evidence that the increase in fluid in the rat stomachs was due to nonparietal cell secretion was gained from the second series of experiments, but these experiments, performed in dogs, indicate that there may be some species variation in response to the individual agents. In the dogs only 16DM stimulated nonparietal cell secretion but did so when given by both the topical and intravenous routes.

In the dog experiments the effect of the three E_2 prostaglandins on permeability and nonparietal cell secretion was studied in the presence of a pouch perfusate that was acid free. In this system only 16DM caused an increase in the fluid produced by the pouches, but this amounted to almost double the control volume. Associated with this was a significant increase in the amount of bicarbonate secreted and in the influx of Na^+ and Cl^-. Using topical 16DM the total influx of cation in μEq/30 minutes (Na^+ and K^+) was approximately 700 μEq. The total influx of anion in μEq/30 minutes was

553 μEq Cl^- plus one sixth of the bicarbonate output over the three hours, i.e. 80 μEq/30 minutes, a total of 633 μEq/30 minutes. Using intravenous 16DM the corresponding figures are for cation 445 μEq/30 minutes and for anion 416 μEq/30 minutes. Minor degrees of pouch distension could lead to the secretion of H^+ and the resulting neutralization of bicarbonate could account for the anion deficit.

The evidence suggests that 16DM stimulates nonparietal cell secretion in the dog, the fluid produced being rich in Na^+ and Cl^- and containing some bicarbonate.

15M and PGE_2 both topically and intravenously had no effect on fluid production and PGE_2 and intravenous 15M were without effect on bicarbonate secretion. Topical 15M however caused a significant increase in bicarbonate secretion. This result is not readily explained but the other values may be underestimates as a result of distension and partial neutralization.

The effect of the prostaglandins on gastric mucosal permeability was measured by studying the movement of Li^+. The net efflux of Li^+ from the Heidenhain pouch of antrectomized dogs has been studied by Chung et al[55] in relation to the net efflux of hydrogen. In the presence of various barrier breakers it was found that the ratio $K_{Li}+/K_H+$ remained unchanged and it was concluded that $K_{Li}+$ is a useful indirect measure of mucosal permeability. The accuracy of the relationship $K_{Li}+/K_H+$ has been questioned in human studies where a variety of uncontrolled factors may interfere, but in the closed perfusion system of a Heidenhain pouch the relationship is valid and has been confirmed by extensive studies in this laboratory.

All three prostaglandins applied topically and 15M and prostaglandin E_2 given intravenously were without effect on net ion flux of Li^+ and $K_{Li}+$, whereas the intravenous administration of 16DM caused a significant reduction in NIF of Li^+ and $K_{Li}+$. This indicates that 16DM tightens the gastric mucosal barrier. This finding is contrary to that previously reported but is more in keeping with the other recorded effects of 16DM.

O'Brien and Carter[48] reported an increase in permeability in canine Heidenhain pouches exposed to 16DM by directly measuring the loss of H^+ from the pouch. They used a system of repeated instillations rather than the continuous perfusion system used in these experiments. In their experi mental system each experiment consisted of six 30-minute periods. Periods 1 and 2 were controls and Period 3 the test period; Periods 4, 5 and 6 were further control periods. The net ion flux of H^+ in Period 3 is compared with those in Periods 1 and 2, and from their data (Table XV) it can be seen that the NIF of H^+ in Periods 1 and 2 prior to the application of 16DM was abnormally low. Had the value been in the same range as in their control experiments there would have been no significant difference. No signficance can be attached to the Na^+ flux data in view of the stimulation of nonparietal cell secretion. Their claim that 16DM increases gastric mucosal permeability is based on questionable data.

However this finding did receive support from the work of Bolton and Cohen,[49] in which they claimed that not only did topical 16DM break the gastric mucosal barrier but the same effect was produced by continuous infusion of the agent. The method by which their data was calculated did not allow for the possibility of active nonparietal cell secretion, and this

EXPERIMENTS	H^+ BACK DIFFUSION (MEAN AND S.E.)			NET Na^+ OUTPUT (MEAN AND S.E.)		
	PERIODS 1 AND 2 n = 12	PERIOD 3 n = 6	P VALUE	PERIODS 1 AND 2 n = 12	PERIOD 2 n = 6	P VALUE
CONTROL (basal solution)	-81 ± 46	-85 ± 57	0.47	190 ± 57	134 ± 41	0.40
16 DM 300 µg/20 ml in pouch (basal solution)	-13 ± 19	187 ± 76	>0.001	132 ± 61	781 ± 64	>0.001

TABLE XV: Comparision of ionic fluxes between control and test periods (from O'Brien PE, Carter DC, Gut 16: 437-442, 1975.

has probably led to erroneous conclusions. Mucosal permeability (K) was determined by measuring the slope of the regression line for the log of the concentration of each ion against time, and multiplying this by the average volume of the perfusate during the experiment. This method of calculation is valid only if the average volumes in the groups under comparison are the same. If in one group the volume increase is much larger then the concen tration of the various ions will change not only as a result of movement across the mucosa but also due to a dilutional factor. This effect probably accounts for the finding of Bolton and Cohen and their data is currently under re-evaluation by the method used in this study, which has already been described. In this method NIF and K are calculated for each 30 minute interval taking into account the volume changes over that period.

Two conclusions can be drawn from this study. Firstly that 16DM stimulates a nonparietal cell secretion containing Na^+, Cl^- and HCO_3^- and secondly that 16DM does not break the gastric mucosal barrier. The production of an increased amount of fluid by the pouches in the absence of H^+ indicates that this cannot be the result of back diffusion and damage, and therefore must came from some other source. The fact that the fluid contains bicarbonate suggests that it is the result of nonparietal cell secretion and confirms what was suspected from the rat studies, although there appears to be some species variation. The Li^+ data confirms that 16DM does not break the barrier and in fact suggests that it may tighten it.

It has been demonstrated that despite their acid inhibitory action the E_2 prostaglandins can stimulate certain gastric mucosal cells leading to

the production of mucus and nonparietal cell secretion. It is possible that these effects contribute significantly to the protective action of the prostaglandins. Although the amount of HCO_3^- secretion is small and not enough to cause significant neutralization of acid, the exact role of the mucus remains uncertain. The protective properties of the prostaglandins demonstrated by Robert were for stronger local irritants, a situation where increased mucus production would be beneficial.

The other importance of these two studies lies in the demonstration that these agents, and in particular 16DM, while inhibiting acid secretion have other secretory effects which have been masked by the potency of acid inhibition. While these effects may not be important physiologically or even pharmacologically, failure to recognize them has led to misinterpretation of the effect of 16DM on the gastric mucosal barrier. Although 16DM is the most potent prostaglandin both as an inhibitor of acid secretion and as a protective agent, it has been considered dangerous for clinical evaluation because of its apparent damaging effect on the gastric mucosal barrier. On the basis of these studies this view of 16DM would appear to be unwarranted and it may soon become available for clinical evaluation.

In the third study the ability of PGE_2 and 15M to reverse established barrier damage was studied and compared with the effect of metiamide. 16DM was not studied because at the time this study was planned 16DM was considered a barrier breaker.

The results have been calculated as NIF and permeability factors for Na^+ and H^+. The calculation of (K) from the computer calculated slope of

the regression line for the log of the concentration of the ions is more accurate than the net ion flux calculated from the first and last values, as it involves the use of a line based on seven points. As there is no significant difference between the volumes of fluid produced by the pouches in the five treatment groups and the groups with which they are compared, (Table XVI) and active secretion does not occur with the agents used, this method of calculation is considered valid. The conclusions are based on the (K) results - the NIF data is used for support.

After demonstrating the validity of the model it was necessary to compare the degree of damage in the Damage Period in all the groups as this can be used as an indication of the degree of damage present in the Recovery Period to which the various therapeutic agents are applied. K_H+ and $K_{Na}+$ in the Damage Period prior to treatment with topical 15M were significantly lower than in the untreated group indicating that the degree of damage produced was less. In the other four treatment groups the degree of damage was comparable with the untreated groups.

Topical PGE_2 had no effect on K_H+ and $K_{Na}+$ indicating no effect on the damaged barrier. Topical 15M caused a significant reduction in $K_{Na}+$ but as the degree of damage was insufficient no conclusion can be drawn. It is likely that this agent had no effect when applied topically, but further tests in which comparable degrees of damage were first produced would be necessary to establish this conclusively. Given intravenously, both PGE_2 and 15M caused a significant reduction in $K_{Na}+$ and K_H+ indicating that in the presence of existing gastric mucosal damage these agents had a beneficial effect. The permeability factors measured during the recovery period on

EXPERIMENT	VOLUMES PRODUCED (ml/1 hour) DAMAGE PERIOD	RECOVERY PERIOD
CONTROL	13.28 ± 0.96	13.18 ± 1.62
ASA	13.63 ± 1.28	13.37 ± 1.83
TOP. PGE_2	15.22 ± 0.86	17.72 ± 1.77
TOP. 15M	16.85 ± 1.46	18.40 ± 2.92
I.V. PGE_2	14.30 ± 0.89	11.20 ± 0.67
I.V. 15M	16.45 ± 1.23	13.10 ± 0.94
I.V. METIAMIDE	12.99 ± 0.85	13.94 ± 1.65

TABLE XVI: Volumes produced by the pouches in the various experimental groups (Mean ± S.E.)

treatment with the two agents were the same as in the original control experiments when the pouches were not exposed to aspirin. Therefore in the presence of existing gastric mucosal barrier damage these agents were able to restore gastric mucosal permeability to normal levels. This effect was not produced by metiamide.

The failure of metiamide to influence gastric mucosal permeability under these circumstances indicates that this effect of the prostaglandins, which could be regarded as therapeutic rather than simply protective is independent of acid inhibition. The acid inhibitory potential of the batch of metiamide used was confirmed (Appendix 1).

A reduction in mucosal blood flow could reduce ionic fluxes and account for the findings in the Recovery Period. However studies in the dog[64,65] show that the reduction in mucosal blood flow associated with the inhibition of stimulated acid secretion by PGE_1 is the result and not the cause of acid inhibition, and studies in rats[66] indicate that under basal conditions PGE_2 has a vasodilator effect on the gastric mucosa. The effect of 15M on basal blood flow has not been studied.

There is evidence that aspirin[67] and indomethacin[68] can damage the sodium pump leading to the accumulation of intracellular sodium and water. Such an effect could ultimately lead to cell lysis. 16DM has the opposite effect stimulating the sodium pump.[68]

It is possible that an increase in gastric mucosal permeability results from the interference with the normal homeostasis of mucosal cells and that

in these experiments biochemical but not structural damage was induced by aspirin and then reversed by the infusion of PGE_2 and 15M assuming these agents have the same cellular effects as 16DM. The rapid restoration of cellular homeostasis would return the mucosal epithelium to normal and lead to a return to normal permeability. If this explanation is correct one would expect 16DM to have the same effect. Now that the confusion over the permeability effect of 16DM has been clarified this could be studied.

Such a mechanism would explain why the protective action of the prosta glandins is unrelated to acid secretory inhibition. The evidence provided here of the reversal of barrier damage in addition to the known acid inhibitory action of the prostaglandins could make them ideal therapeutic agents in acute gastric mucosal lesions. It is interesting to note that despite early reports of the value of the H_2 receptor antagonists in erosive gastritis,[69] their value in stress induced upper gastrointestinal tract haemorrhage has recently been questioned.[70]

CONCLUSIONS

The conclusions that can be drawn from the first experiment are that the three E_2 prostaglandins applied topically to the rat stomach stimulate a non parietal cell secretion and 16DM and PGE_2 stimulate mucus production leading to an increase in the free component of mucus, but have no effect on the barrier fraction. Given intravenously all three agents cause an increase in free mucus but without an increase on the barrier fraction. Nonparietal cell secretion was not stimulated by intravenous administration.

The second experiment indicated a species variation in the effect of the E_2 prostaglandins on nonparietal cell secretion. In the dogs 16DM both topically and intravenously caused an increase in the fluid secreted which contained sodium, chloride and bicarbonate. PGE_2 and 15M did not stimulate nonparietal cell bicarbonate secretion in the dog.

The final experiment demonstrates that the intravenous administration of PGE_2 and 15M can reverse established gastric mucosal barrier damage. This property is not exhibited by topical application of the prostaglandins, and it is not possessed by metiamide.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.1	107	0.70	0.32	1.02
2	4.0	68	0.81	0.94	1.75
3	3.8	76	0.92	0.89	1.81
4	4.0	68	0.70	0.83	1.53
5	4.3	60	0.92	0.78	1.70
6	4.0	24	1.17	0.48	1.65
7	4.1	131	0.88	0.37	1.25
8	4.0	88	0.44	0.71	1.15
9	4.1	41	0.88	0.12	1.00
10	4.1	41	0.59	0.24	0.83
MEAN ± S.E.	4.05 ±0.03	70.4 ±10.2	0.80 ±0.06	0.57 ±0.09	1.37 ±0.11

TABLE XVII: Experiment 1: Topical prostaglandins: Controls

RATS	VOLUME in ml.	ACID OUTPUT µeq H^+/3 Hr	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	6.4	45.0	0.16	1.16	1.32
2	6.2	6.2	0.16	1.29	1.45
3	4.2	0	0.22	1.09	1.31
4	6.2	18.6	0.38	1.13	1.51
5	6.6	52.8	0.54	1.72	2.26
6	5.3	42.0	0.88	2.99	3.87
7	4.8	19.0	0.88	1.14	2.02
8	4.5	27.0	0.44	1.87	2.31
9	5.1	20.0	0.51	1.06	1.57
10	5.2	0	0.37	0.93	1.30
MEAN ± S.E.	5.45 ±0.27	23.1 ±5.9	0.45 ±0.08	1.44 ±0.19	1.89 ±0.25

TABLE XVIII: Experiment 1: Topical prostaglandins: 15M 10 µg/ml

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	5.4	5.4	1.35	0.14	1.49
2	5.2	16.0	0.49	1.79	2.28
3	5.0	8.0	0.81	1.95	2.76
4	5.4	38.0	1.25	3.36	4.61
5	5.3	16.0	1.03	2.75	3.78
6	5.0	20.0	0.37	1.19	1.56
7	4.8	24.0	0.37	1.14	1.51
8	5.1	20.0	0.37	1.66	2.03
9	4.8	34.0	0.44	0.57	1.01
10	4.6	23.0	0.22	2.05	2.67
MEAN ± S.E.	5.07 ±0.08	20.44 ±3.22	0.67 ±0.13	1.66 ±0.30	2.33 ±0.35

TABLE XIX: Experiment 1: Topical Prostaglandins: 16DM 10 µg/ml.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	7.9	40.0	0.88	1.54	2.42
2	6.9	41.0	0.32	0.16	0.48
3	8.3	0	0.16	4.74	4.90
4	8.3	0	1.76	1.29	3.05
5	7.2	0	1.60	4.11	5.71
6	5.8	11.6	0.44	3.10	3.54
7	5.2	0	0.22	2.05	2.27
8	6.7	0	0.66	1.59	2.25
9	DIED DURING THE COURSE OF EXPERIMENT				
10	5.0	22.0	0.44	0.89	1.33
MEAN ± S.E.	6.82 ±0.41	12.7 ±5.82	0.72 ±0.19	2.16 ±0.51	2.88 ±0.55

TABLE XX: Experiment 1: Topical Prostaglandins: PGE_2 100 µg/ml

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.0	64.0	0.60	1.28	1.88
2		DIED DURING COURSE OF EXPERIMENT			
3	3.5	35.0	0.70	1.93	2.63
4	4.0	32.0	0.85	1.92	2.77
5	4.0	32.0	0.70	1.28	1.98
MEAN ± S.E.	3.88 ±0.12	40.75 ±7.78	0.71 ±0.04	1.60 ±0.18	2.31 ±0.22

TABLE XXI: Experiment 1: Intravenous Prostaglandins: Controls

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.4	70.4	1.05	4.62	5.67
2	4.3	112.0	1.00	4.82	5.82
3		INTRAVENOUS CANNULA DISLODGED			
4	4.3	95.0	0.90	4.97	5.87
5	4.2	52.0	0.95	4.95	5.90
MEAN ± S.E.	4.22 ±0.02	82.4 ±13.24	0.98 ±0.03	4.72 ±0.11	5.82 ±0.04

TABLE XXII: Experiment 1: Intravenous Prostaglandins: 15M 0.6 µg/Kg/Hr.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.1	16.4	0.57	1.48	2.05
2	4.0	56.0	0.70	3.83	4.53
3	4.1	45.1	0.44	2.06	2.50
4	4.0	60.0	0.60	3.74	4.34
5	4.0	48.0	0.72	2.97	3.69
MEAN ± S.E.	4.04 ±0.02	45.1 ±7.7	0.61 ±0.05	2.82 ±0.46	3.42 ±0.49

TABLE XXIII: Experiment 1: Intravenous Prostaglandins: 16DM 0.6 µg/Kg/Hr.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.3	30.1	1.20	3.20	4.40
2	4.4	35.2	1.33	5.20	6.53
3	4.3	43.0	0.76	2.40	3.16
4	4.4	35.2	0.85	3.77	4.62
5	4.1	36.9	0.70	4.12	4.82
MEAN ± S.E.	4.3 ±0.05	36.1 ±2.07	0.97 ±0.12	3.74 ±0.47	4.70 ±0.54

TABLE XXIV: Experiment 1: Intravenous Prostaglandins: PGE_2 0.6 mg/Kg/Hr.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
1	4.1	164.1	0.60	0.37	0.97
2	4.0	96.0	0.15	0.32	0.47
3	4.1	176.3	0.40	0.39	0.79
4	4.3	90.3	0.60	0.60	1.20
5	4.2	210.0	0.80	0.59	1.39
MEAN ± S.E.	4.14 ±0.01	147.34 ±23.38	0.51 ±0.11	0.45 ±0.06	0.96 ±0.16

TABLE XXV: Experiment 1: Subcutaneous Histamine: Controls.

RATS	VOLUME in ml.	ACID OUTPUT $\mu eq\ H^+/3\ Hr$	ALCIAN BLUE BOUND (in mg.) MUCOSA	CONTENTS	TOTAL
6	5.2	343.2	0.30	0.40	0.70
7	4.3	116.1	0.40	0.69	1.09
8	4.3	64.5	0.20	0.56	0.76
9	4.2	134.4	0.20	0.42	0.62
10	6.0	576.0	0.20	0.36	0.56
MEAN ± S.E.	4.80 ±0.34	246.84 ±95.03	0.26 ±0.04	0.49 ±0.06	0.75 ±0.09

TABLE XXVI: Experiment 1: Subcutaneous Histamine: Histamine 6 mg/Kg/Hr.

	BICARBONATE SECRETED µeq/3 Hr				
EXPERIMENT	DOG 3	DOG 13	DOG 16	DOG 23	MEAN ± S.E.
CONTROL	183.73	0	161.73	227.84	121.99 ± 31.96
		0	70.73		
		212.4	119.28		
15M 10 µg/ml	224.0	167.25	247.73	111.64	231.15 ± 23.93
	222.75	254.05	305.36	316.47	
16DM 10 µg/ml	960.57	115.80	735.50	396.45	483.37 ± 110.99
	821.25	332.65	330.98	173.78	
PGE_2 100 µg/ml	178.59	0	55.89	118.32	55.95 ± 22.79
	0	65.07	29.71	0	

TABLE XXVII: Experiment 2: Topical Prostaglandin: Bicarbonate Secretion µeq/3 Hr.

	VOLUME PRODUCE (in ml/3 Hr)				
EXPERIMENT	DOG 3	DOG 13	DOG 16	DOG 23	MEAN ± S.E.
CONTROL	38.09	20.56	18.41	21.46	18.16 ± 3.31
		13.84	11.65		
		6.98	14.32		
15M 10 µg/ml	20.50	20.25	19.55	20.32	18.42 ± 1.39
	14.00	10.69	20.02	22.04	
16DM 10 µg/ml	38.39	22.40	38.05	43.79	37.68 ± 3.42
	55.75	31.03	38.05	34.01	
PGE_2 100 µg/ml	23.04	7.88	23.91	35.31	22.30 ± 2.65
	24.03	20.17	20.39	23.69	

TABLE XXVIII: Experiment 2: Topical Prostaglandins: Volume Secreted ml/3 Hr.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
13	157.48	0.98	0.21	0.02	262.68	1.70	30.56	11.37
	-96.61	-0.61	-5.49	-1.30	-25.15	-0.16	18.45	13.05
	49.53	0.31	-4.60	-1.05	83.58	0.59	29.48	19.79
16	55.22	0.34	-5.53	-1.53	57.34	0.38	17.24	10.11
	149.52	0.98	-3.35	-9.83	176.74	1.14	33.50	20.19
	9.59	0.07	-4.89	-1.22	9.42	0.007	20.50	8.13
23	203.09	1.31	-0.73	-0.19	262.14	1.69	33.70	15.94
3	435.67	3.09	-1.40	-0.46	467.22	3.30	32.09	20.51
MEAN	119.60	0.80	-3.22	-0.82	161.74	1.08	26.94	14.88
± S.E.	±56.30	±0.38	±0.80	±0.17	±58.08	±0.38	±2.47	±1.73

TABLE XXIX: Experiment 2: Topical Prostaglandins: Permeability Data: Controls.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
13	1.00	0.09	1.31	0.35	173.66	1.25	14.59	14.81
	87.17	0.58	-2.03	-0.53	137.50	1.01	20.19	13.84
16	200.60	1.33	-1.41	-0.37	201.81	1.47	19.37	16.28
	190.97	1.34	-0.88	-0.16	238.47	1.77	20.05	13.51
3	246.49	1.57	3.97	0.99	384.85	2.73	16.52	18.09
	38.45	0.22	-2.44	-0.64	154.27	1.10	12.25	13.53
23	101.16	0.67	-5.33	-1.47	263.48	1.79	35.41	19.45
	138.22	1.02	-4.94	-1.40	284.32	2.12	30.36	17.43
MEAN	125.50	0.85	-1.46	-0.40	229.79	1.65	21.09	15.80
± S.E.	±29.84	±0.17	±1.08	±0.28	±28.77	±0.20	±2.79	±0.80

TABLE XXX: Experiment 2: Topical Prostaglandin: Permeability Data: 15M 10 µg/ml

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
13	268.97	1.86	7.53	1.86	344.43	2.45	23.42	12.84
	255.09	1.86	-1.57	-0.38	358.39	2.54	22.58	17.22
16	729.64	5.45	-6.63	-3.03	850.81	6.11	49.92	19.50
	290.54	2.09	-3.65	-1.18	465.53	3.34	26.81	15.78
3	703.91	5.04	-4.05	-1.85	950.60	6.72	32.56	19.02
	872.21	6.43	-2.17	-0.48	927.97	6.53	34.82	20.58
23	734.99	5.33	1.10	0.26	750.87	5.50	40.50	25.33
	570.91	4.11	2.24	0.81	682.83	4.90	37.28	22.21
MEAN	553.28	4.02	-0.90	-0.49	666.42	4.76	33.48	19.06
± S.E.	±87.38	±0.64	±1.56	±0.53	±87.57	±0.61	±3.27	±1.36

TABLE XXXI: Experiment 2: Topical Prostaglandins: Permeability Data: 16DM 10 µg/ml.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
13	238.91	1.49	-0.66	-0.23	263.25	1.76	35.57	18.97
	-136.70	-0.86	-6.48	-1.54	-62.63	-0.43	15.40	10.15
16	184.78	1.18	-4.99	-1.53	208.86	1.43	27.71	15.41
	367.55	2.39	-1.09	-0.20	366.41	2.55	27.37	21.03
3	22.07	0.18	-0.62	-0.16	303.97	2.08	20.97	21.08
	647.92	3.91	1.55	0.35	458.23	3.21	18.09	21.83
23	230.81	1.61	-5.95	-1.77	267.69	1.86	28.28	13.41
	336.50	2.13	-6.94	-2.36	336.31	2.46	72.40	26.39
MEAN	236.48	1.50	-3.14	-0.93	267.76	1.86	30.72	18.53
± S.E.	±82.85	±0.50	±1.15	±0.34	±54.22	±0.37	±6.37	±1.84

TABLE XXXII: Experiment 2: Topical Prostaglandin: Permeability Data: PGE_2 100 µg/ml.

	BICARBONATE SECRETION µeq/3 Hr				
EXPERIMENT	DOG 4	DOG 12	DOG 16	DOG 23	MEAN ± S.E.
CONTROL	24.44	92.14	115.46	0	65.91 ± 18.52
	137.73	88.26	69.29	0	
15M	0	75.10	44.99	0	30.13 ± 10.11
0.6 µg/Kg/Hr	0	47.35	52.41	22.12	
16DM	462.84	580.59	113.18	289.15	317.78 ± 66.27
0.6 µg/Kg/Hr	363.48	418.11	314.90	0	
PGE_2	45.23	0	86.14	272.1	62.37 ± 31.88
0.6 mg/Kg/Hr	47.44	0	48.05	0	

TABLE XXXIII: Experiment 2: Intravenous Prostaglandin: Bicarbonate Secretion µeq/3 Hr.

	VOLUME PRODUCE (in ml/3 Hr)				
EXPERIMENT	DOG 4	DOG 12	DOG 16	DOG 23	MEAN ± S.E.
CONTROL	13.37	11.20	22.23	8.47	13.43 ± 2.01
	10.41	8.63	10.69	22.50	
15M	8.87	13.90	9.49	14.16	11.91 ± 1.01
10 µg/ml	11.59	11.85	16.91	8.94	
16DM	25.08	24.23	17.77	19.28	24.47 ± 1.60
0.6 µg/Kg/Hr	30.63	29.01	27.48	22.33	
PGE_2	11.94	12.25	7.57	18.92	12.10 ± 1.22
0.6 mg/Kg/Hr	9.77	14.34	12.55	9.48	

TABLE XXXIV: Experiment 2: Intravenous Prostaglandin: Volume secreted ml/3 Hr.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
16	-50.47	-0.36	-3.09	-0.81	-18.05	-0.13	14.06	10.59
	128.57	0.96	-3.21	-0.81	145.01	1.15	24.10	14.57
23	71.17	0.49	-5.88	-1.74	103.63	0.73	20.39	21.16
	128.53	0.92	-2.41	-0.66	167.47	1.22	11.81	12.92
4	-86.11	-0.60	-7.70	-2.00	-86.79	-0.61	15.02	14.58
	101.63	0.71	-3.01	-0.78	109.79	0.79	22.74	16.87
12	-108.39	-0.77	-10.81	-2.96	-107.74	-0.79	13.70	11.00
	69.00	0.49	-5.94	-1.43	30.01	0.23	15.50	16.43
MEAN	31.74	0.23	-5.25	-1.39	42.91	0.32	17.16	14.76
± S.E.	±34.55	±0.25	±0.28	±37.20	±37.20	±0.26	±1.61	±1.21

TABLE XXXV: Experiment 2: Intravenous Prostaglandins: Permeability Data: Controls.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
4	-42.11	-0.30	-3.46	-0.88	-58.37	-0.42	11.71	14.67
	-7.57	-0.06	-4.38	-1.19	-75.33	-0.55	12.15	14.81
23	-67.78	-0.46	-9.04	-2.45	-63.29	-0.44	28.16	19.96
	-4.52	-0.05	-5.75	-1.69	20.20	0.12	15.66	5.95
16	71.01	0.52	-3.40	-0.92	49.99	0.37	27.70	15.11
	-114.45	-0.81	-7.71	-2.00	-105.21	-0.75	32.36	25.20
12	-41.20	-0.30	-3.04	-0.83	-40.02	-0.30	4.36	5.42
	-47.68	-0.34	-2.04	-0.51	-50.84	-0.36	6.67	6.82
MEAN	-31.78	-0.22	-4.85	-1.30	-40.35	-0.29	17.34	13.11
± S.E.	±19.12	±0.10	±0.86	±0.22	±18.03	±0.10	±3.76	±2.38

TABLE XXXVI: Experiment 2: Intravenous Prostaglandins: Permeability Data: 15M 0.6 µg/Kg/Hr.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
12	433.92	3.06	-4.85	-1.46	519.54	3.75	19.67	16.98
	515.99	3.61	-2.22	-0.57	644.89	4.34	23.46	20.14
23	271.77	1.94	-2.85	-0.83	330.32	2.35	27.19	22.85
	217.28	1.52	-3.96	-1.12	257.10	1.83	20.78	9.56
4	481.15	3.42	-0.76	-0.35	567.71	4.12	22.93	17.22
	394.88	2.74	-2.17	-0.64	531.32	3.71	24.69	18.49
16	213.79	1.49	-4.55	-1.11	74.03	0.73	24.52	10.95
	376.97	2.64	1.52	0.22	449.92	3.34	36.61	22.00
MEAN	363.21	2.55	-2.48	-0.73	421.85	3.02	24.98	17.27
± S.E.	±41.28	±0.28	±0.74	±0.17	±66.79	±0.43	±1.85	±1.70

TABLE XXXVII: Experiemnt 2: Intravenous Prostaglandins: Permeability Data: 16DM 0.6 µg/Kg/Hr.

	Cl^-		Li^+		Na^+		K^+	
DOG	NIF	K	NIF	K	NIF	K	NIF	K
4	-12.75	-0.10	-4.77	-1.24	-8.98	-0.06	21.76	19.69
	-44.81	-0.31	-6.22	-1.66	-52.79	-0.39	21.71	17.32
12	68.90	0.47	-4.66	-1.16	40.98	0.30	22.14	18.09
	70.68	0.51	-3.62	-0.90	11.10	0.10	21.37	17.50
23	176.29	1.27	-4.62	-1.20	212.77	1.52	31.07	17.44
	-54.44	-0.37	-9.68	-2.44	0.76	0.10	19.48	16.63
16	5.17	0.03	-1.34	-0.32	23.66	0.17	13.65	10.96
	109.27	0.76	-2.44	-0.66	18.41	0.14	11.56	12.71
MEAN	39.66	0.28	-4.66	-1.19	30.73	0.22	20.34	16.29
±.S.E.	±28.46	±0.20	±0.88	±0.22	±27.80	±0.17	±2.09	±1.03

TABLE XXXVIII: Experiment 2: Intravenous Prostaglandins: Permeability Data: PGE_2 0.6 µg/Kg/Hr.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	13.38	-1.51	0.30	-0.11	17.45	15.81	11.77	0.23	-0.12	20.40
	3.28	-5.24	0.08	-0.06	8.03					
	11.58	-2.47	0.27	-0.11	9.60	10.74	2.40	0.23	-0.07	10.64
	9.21	-5.50	0.22	-0.10	9.23					
15	16.11	-7.89	0.37	-0.16	11.85	13.19	7.18	0.22	-0.10	14.83
	13.69	-8.23	0.32	-0.14	11.26					
	6.29	2.45	0.14	-0.05	11.87	6.24	-5.84	0.18	-0.08	7.53
	10.65	6.78	0.19	-0.09	15.15					
21	25.04	-9.08	0.54	-0.23	13.06	16.49	1.88	0.35	-0.02	12.58
	10.60	-3.90	0.23	-0.13	11.07					
	22.87	-7.75	0.48	-0.21	12.72	23.39	-4.31	0.46	-0.02	13.39
	19.01	3.42	0.31	-0.15	15.09					
22	25.67	-5.91	0.51	-0.22	16.81	25.71	5.3	0.38	-0.23	23.42
	27.20	16.04	0.41	-0.19	27.20					
	14.29	-2.52	0.33	-0.13	11.92	14.81	-4.59	0.25	-0.15	9.92
	17.23	2.13	0.37	-0.15	14.04					
17	12.71	-3.79	0.24	-0.17	17.74	5.25	-16.48	0.19	-0.14	8.36
	9.52	-13.12	0.26	-0.16	8.87					
	8.41	-2.95	0.20	-0.07	9.10	11.12	-3.00	0.27	-0.11	10.79
	11.16	-0.79	0.25	-0.09	13.72					
MEAN	14.39	-2.49	0.30	-0.13	13.28	14.27	-0.56	0.27	-0.12	13.18
± S.E.	±1.47	±1.44	±0.03	±0.01	±0.96	±2.08	±2.53	±0.03	±0.02	±1.62

TABLE XXXIX: Experiment 3: Permeability Data And Volumes For Each Period: Controls.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	12.49	-8.70	0.29	-0.17	10.96	13.51	-7.63	0.35	-0.13	8.94
	12.16	-9.00	0.30	-0.16	8.68					
	14.58	2.75	0.20	-0.17	19.87	14.88	0.21	0.26	-0.13	12.88
	11.19	-4.29	0.20	-0.14	15.17					
15	14.81	-0.78	0.32	-0.17	17.58	15.40	-12.59	0.40	-0.17	10.76
	12.80	-9.99	0.31	-0.16	8.03					
	11.78	-15.35	0.31	-0.17	12.13	10.41	-5.07	0.26	-0.12	10.76
	13.90	-14.30	0.37	-0.18	13.38					
21	28.67	-28.37	0.57	-0.41	14.62	29.96	-30.35	0.65	-0.36	13.66
	35.02	-16.90	0.60	-0.43	25.86					
	37.13	-27.74	0.64	-0.49	9.10	43.81	-21.36	0.64	-0.46	19.73
	49.39	-37.82	0.71	-0.61	18.73					
22	27.89	-16.22	0.55	-0.39	21.69	23.17	10.66	0.43	-0.19	26.81
	20.45	-21.16	0.50	-0.26	9.73					
	28.97	-33.07	0.52	-0.44	12.29	25.10	-8.14	0.41	-0.24	12.70
	34.46	-34.50	0.59	-0.50	10.38					
17	25.60	-4.79	0.42	-0.31	19.85	12.94	-9.62	0.34	-0.13	7.37
	12.14	-12.77	0.29	-0.20	10.15					
	32.92	-18.41	0.27	-0.19	12.57	13.88	-8.19	0.36	-0.13	10.08
	13.68	-20.42	0.36	-0.20	10.97					
MEAN	22.50	-16.64	0.42	-0.29	13.63	20.30	-9.20	0.41	-0.21	13.37
± S.E.	±2.50	±2.54	±0.03	±0.04	±1.28	±3.28	±3.51	±0.05	±0.04	±1.83

TABLE XL: Experiment 3: Permeability Data And Volumes for Each Period: Aspirin Damage.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	7.76	1.62	0.08	-0.14	14.55	13.68	5.49	0.26	-0.10	17.31
15	31.97	-1.23	0.60	-0.30	21.43	25.91	3.01	0.43	-0.18	18.01
	20.52	-7.84	0.47	-0.21	12.59					
	13.40	-4.60	0.20	-0.17	13.60	9.22	-1.81	0.12	-0.06	8.26
	18.25	-8.36	0.27	-0.20	17.69					
	18.10	-6.40	0.35	-0.23	18.43	29.26	-4.08	0.48	-0.22	20.86
	14.26	-17.55	0.33	-0.25	8.71					
21	36.51	-26.57	0.77	-0.44	17.11	37.32	0.45	0.61	-0.29	23.08
	13.92	-16.34	0.27	-0.21	12.93	1.88	-11.52	-0.01	-0.15	10.54
	21.89	-6.22	0.31	-0.23	20.51					
22	3.74	-8.54	0.05	-0.24	16.16	25.18	-10.79	0.44	-0.29	15.09
	14.24	-27.62	0.41	-0.29	8.96					
17	23.09	-14.32	0.25	-0.29	19.37	32.63	0.13	0.25	-0.29	27.62
	16.73	-6.57	0.26	-0.18	13.86					
	26.35	-11.12	0.34	-0.32	17.29	28.38	-5.45	0.31	-0.26	18.23
	23.92	-11.16	0.35	-0.25	16.32					
	20.77	-15.65	0.43	-0.31	12.29	34.62	-0.62	0.47	-0.35	18.24
	21.66	-15.94	0.44	-0.30	12.34					
MEAN	19.28	-11.35	0.35	-0.25	15.22	23.80	-3.41	0.34	-0.22	17.74
± S.E.	±1.85	±1.83	±0.04	±0.01	±0.86	±3.69	±1.86	±0.06	±0.30	±1.77

TABLE XLI: Permeability Data And Volumes for Each Period: Topical PGE_2 10 µg/ml.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	13.86	-8.98	0.21	-0.17	13.72	17.82	6.44	0.22	-0.13	18.84
	6.89	-9.12	0.13	-0.11	13.94					
	11.21	0.64	0.12	-0.15	13.92	9.27	3.11	0.11	-0.08	12.05
	8.59	-13.63	0.19	-0.14	9.90					
15	37.36	-10.24	0.53	-0.37	23.56	24.82	-12.80	0.36	-0.27	17.63
	10.92	-20.68	0.23	-0.19	12.72					
	34.15	-3.40	0.39	-0.36	30.29	18.11	-11.71	0.24	-0.24	18.99
	23.26	-11.63	0.34	-0.26	24.34					
21	16.05	9.88	0.08	-0.15	18.24	15.12	-2.97	0.18	-0.17	17.37
	7.85	-2.19	0.09	-0.11	12.90					
17	29.65	-13.56	0.45	-0.34	21.18	33.63	16.58	0.29	-0.25	37.54
	11.28	-4.57	0.17	-0.12	20.57					
	4.87	-5.29	0.04	-0.13	9.82	14.47	3.77	0.22	-0.09	12.79
	19.04	0.51	0.26	-0.18	14.98					
	24.52	-15.94	0.34	-0.36	18.66	7.82	-5.59	0.13	-0.09	11.96
	13.84	-6.59	0.24	-0.13	10.80					
MEAN ± S.E.	17.08 ±2.49	-7.14 ±1.86	0.24 ±0.03	-0.21 ±0.02	16.85 ±1.46	17.63 ±2.95	-0.39 ±3.48	0.22 ±0.03	-0.17 ±0.03	18.40 ±2.93

TABLE XLII: Experiment 3: Permeability Data And Volumes For Each Period: Topical 15M 10 µg/ml.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	16.85	13.87	0.22	-0.13	18.41	0.82	6.71	0.06	-0.03	11.35
	13.63	0.74	0.22	-0.16	15.36					
	13.63	-0.60	0.34	-0.09	11.16	0.70	4.00	-0.05	-0.04	8.77
	11.12	3.14	0.19	-0.15	14.48					
	11.84	4.16	0.22	-0.11	13.61	7.99	1.24	0.18	-0.06	10.52
	9.79	-13.81	0.27	-0.16	7.33					
15	17.08	-12.22	0.45	-0.19	9.22	13.00	2.92	0.26	-0.10	11.02
	11.26	-1.77	0.22	-0.12	9.36					
	18.14	-2.04	0.34	-0.22	24.95	7.57	-4.33	0.22	-0.05	10.27
	19.51	-3.50	0.37	-0.20	14.92					
	18.67	-8.48	0.45	-0.19	14.23	8.82	3.29	0.21	-0.04	10.94
	13.93	-10.78	0.35	-0.17	9.21					
21	34.52	-34.63	0.64	-0.53	17.81	16.52	-10.60	0.37	-0.16	9.13
	34.18	-17.80	0.60	-0.40	24.24					
	26.20	-19.20	0.57	-0.35	14.08	15.50	-2.68	0.35	-0.14	16.47
	31.48	-25.17	0.69	-0.41	16.69					
	31.23	-35.03	0.64	-0.48	12.11	12.90	1.67	0.25	-0.09	11.29
	24.60	-14.31	0.46	-0.29	14.15					
22	25.17	-26.13	0.61	-0.38	10.81	14.48	-13.82	0.38	-0.18	14.94
	26.31	-18.70	0.50	-0.34	17.70					
	26.67	-34.33	0.53	-0.50	16.92	12.22	-9.23	0.30	-0.13	8.60
	33.28	-22.23	0.63	-0.39	14.31					
	18.47	-16.42	0.40	-0.31	12.38	15.89	-5.00	0.36	-0.19	11.20
	21.28	-27.48	0.55	-0.35	9.87					
MEAN	21.20	-13.44	0.43	-0.27	14.30	10.52	-2.14	0.23	-0.10	11.20
	±1.62	±2.72	±0.01	±0.03	±0.89	±1.58	±1.88	±0.04	±0.01	±0.67

TABLE XLIII: Experiment 3: Permeability Data and Volumes For Each Period: I.V. PGE_2 0.3 mg/Kg/Hr.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	3.96	-10.23	0.11	-0.08	5.45	9.49	11.78	0.08	-0.03	14.25
	5.82	-0.27	0.06	-0.08	11.58					
	13.46	10.99	0.18	-0.11	18.72	8.98	8.84	0.16	-0.03	12.31
	12.71	2.85	0.13	-0.14	15.78					
	18.07	11.55	0.26	-0.14	21.27	10.77	-0.35	0.24	-0.10	12.21
	9.28	-10.58	0.25	-0.12	10.36					
15	22.57	-10.05	0.33	-0.25	19.29	10.46	2.96	0.21	-0.05	13.59
	15.50	13.10	0.17	-0.07	18.01					
	16.72	-1.55	0.37	-0.18	17.04	9.09	-13.73	0.31	-0.10	6.61
	19.85	2.24	0.40	-0.18	16.97					
21	19.67	-23.44	0.36	-0.31	8.05	18.24	-9.82	0.26	-0.24	13.17
	14.74	0.21	0.17	-0.14	13.38					
	32.32	-1.74	0.58	-0.25	20.81	2.47	12.92	-0.06	-0.01	13.94
	29.00	24.87	0.25	-0.14	26.27					
	39.13	-13.03	0.65	-0.45	23.31	18.73	-20.96	0.48	-0.23	14.64
	31.25	-21.54	0.60	-0.39	16.28					
22	26.92	-32.24	0.64	-0.45	16.02	21.01	-2.96	0.37	-0.22	17.20
	37.11	-24.63	0.74	-0.47	17.57					
MEAN	20.44	-4.63	0.35	-0.23	16.45	12.13	-1.23	0.23	-0.10	13.10
± S.E.	±2.42	±3.53	±0.05	±0.03	±1.23	±1.98	±3.90	±0.05	±0.03	±0.94

TABLE XLIV: Experiment 3: Permeability Data And Volumes For For Each Period: I.V. 15M 0.6 µg/Kg/Hr.

	DAMAGE PERIOD					RECOVERY PERIOD				
	NIF µeq/min		K			NIF µeq/min		K		
DOG	Na^+	H^+	Na^+	H^+	Volume ml/Hr	Na^+	H^+	Na^+	H^+	Volume ml/Hr
14	16.30	3.93	0.29	-0.16	20.24	16.00	-8.73	0.37	-0.18	15.80
	11.44	-11.07	0.28	-0.15	11.52					
	9.39	-16.56	0.24	-0.18	11.41	14.96	-10.24	0.33	-0.15	12.94
	17.73	-9.26	0.39	-0.19	11.66					
15	12.67	-9.21	0.25	-0.18	14.27	14.41	-6.62	0.33	-0.15	13.42
	18.80	-12.91	0.44	-0.20	11.47					
	14.10	-16.10	0.29	-0.24	12.28	15.11	-4.59	0.32	-0.12	10.02
	17.55	-12.32	0.38	-0.23	10.12					
17	30.29	-12.37	0.51	-0.35	16.96	31.82	-0.55	0.53	-0.26	21.03
	22.94	-22.32	0.53	-0.34	9.87					
	14.97	-8.00	0.35	-0.17	13.33	15.65	-5.11	0.32	-0.16	10.48
	19.00	-12.30	0.43	0.23	12.80					
MEAN	17.09	-11.53	0.36	-0.22	12.99	16.95	-5.97	0.37	-0.17	13.94
± S.E.	±1.60	±1.80	±0.30	±0.02	±0.85	±1.75	±1.39	±0.03	±0.02	±1.65

TABLE XLV: Experiment 3: Permeability Data And Volumes For Each Pouch: Metiamide 3 mg/Kg/Hr.

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APPENDIX

1. Confirmation of the Effectiveness of the Metiamide used in Experiment 3

An experiment was performed in two dogs to establish that the batch of metiamide used was effective as an inhibitor of acid secretion. An infusion of histamine di-HCl, 50 µg/Kg was given to two dogs over 105 minutes, the mean 15 minute acid outputs are recored as the solid line in Figure 8. In a subsequent experiment the same dogs were subjected to the same infusion of histamine but metiamide 3 mg/Kg/hour was infused from 60-120 minutes. The broken line indicates the mean 15 minute acid output in this experiment.

The batch of metiamide was considered normal in its antisecretory effect.

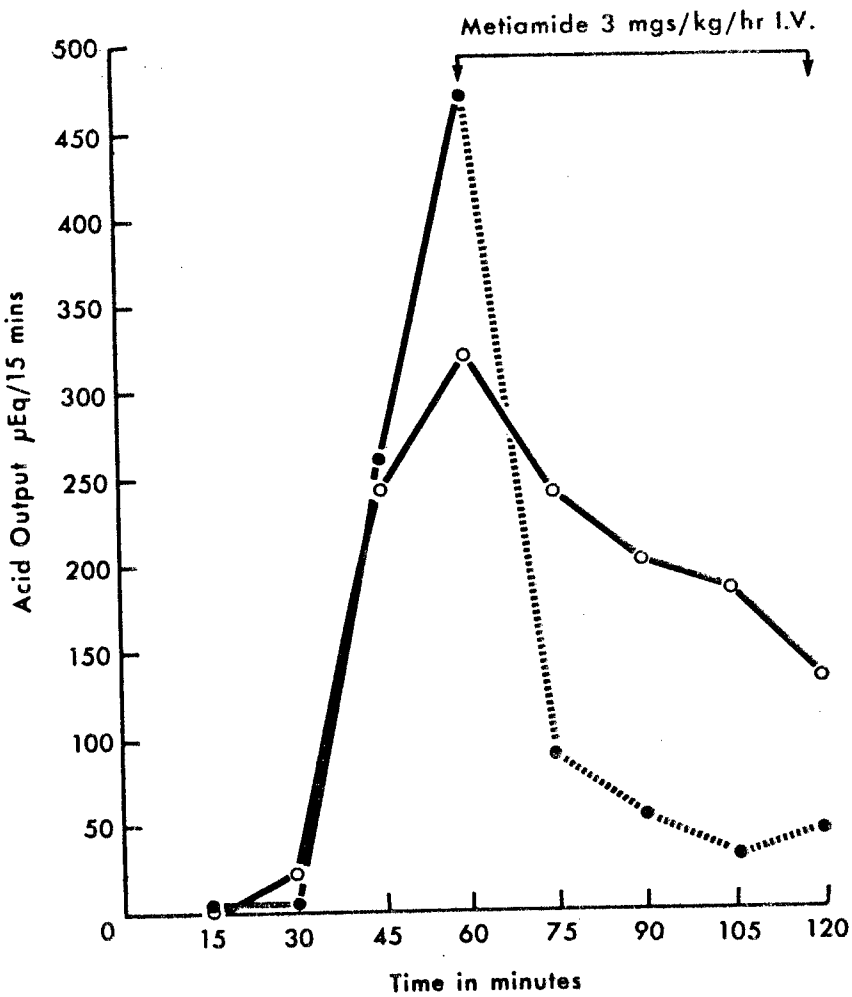

FIGURE 8: Illustrates the inhibitory effect of intravenous metiamide on histamine stimulated acid output from a Heidenhain pouch. In both experiments a constant infusion of histamine di-HCl 50 µg/Kg/Hr was given from 15 to 120 minutes. The dotted line indicates the effect of metiamide 3 mgs/Kg/Hr from 60 to 120 minutes.

2. Effect of Dilution of Alcian Blue Binding

An experiment was performed in which the effect of serial dilutions of 1 ml. of gastric juice on Alcian Blue binding was studied. The results (Table XLVI) illustrate that the differing volumes of gastric juice are not likely to have introduced a significant error.

DILUTIONS	ALCIAN BLUE BOUND mg/ml
1 ml.	0.14
2 ml.	0.07
4 ml.	0.04
8 ml.	0.01

TABLE XLVI: Effect of serial dilutions of 1 ml. of gastric juice on Alcian Blue binding.